KIDNEY FUNCTION

in

THE OVINE FOETUS

by

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SUMMARY

Catheters were inserted into the femoral artery and vein, urinary bladder and amniotic sac of foetal sheep and samples were collected daily during the last 35 days of gestation. Analysis of these samples revealed changes in the composition of foetal urine that were significantly correlated with foetal age. A series of experiments involving the administration of hormones and diuretic chemicals was then carried out in an attempt to determine the cause of the changes in urine composition. Kidney morphology was also examined.

Foetal glomerular filtration rate (GFR) was found to increase progressively and mature foetuses were able to increase GFR by as much as 80%. The diuretic experiments confirmed the existence of active Na^+ reabsorption in the foetal nephrons and the reabsorption appeared to be linked to K^+ secretion. The Na^+ reabsorption was also accompanied by passive water reabsorption. Carbonic anhydrase activity in the foetal nephrons and its involvement in Na^+ - H^+ exchange, was also demonstrated. With respect to maturational changes, evidence was obtained that the capacity for Na^+ reabsorption in the loops of Henle increases with gestational age, as well as distal Na^+ - K^+ and Na^+ - H^+ exchange. The hormone administration experiments indicated that during the last 35 days of pregnancy, aldosterone promotes Na^+ retention and that cortisol has a mild natriuretic and diuretic effect. Also dexamethasone administered alone, or in combination with metyrapone, produced changes in urine output and composition that were consistent with the existence of pituitary-adrenal interactions in the foetus, 11β hydroxylase activity in the foetal adrenals and responsiveness of the foetal kidneys to 11-desoxycorticosteroids. Other hormones including progesterone, ADH and angiotensin produced variable

effects on urine composition, but ADH and angiotensin reduced urine output, presumably by increasing renal vascular resistance. These hormone experiments were of a preliminary nature and further work will be necessary to confirm the observations made. Nevertheless on the basis of these experiments and the observed relationship between endogenous hormone levels and normal urinary parameters; it is suggested that increasing plasma renin activity plus a greater capacity for Na^+ reabsorption was responsible for the decrease in urinary Na^+ concentration that occurred prior to the 145th day of pregnancy. However, the preparturient increase in urinary Na^+ concentration and Na^+ excretion rate is thought to have been due to a rise in GFR induced by an increase in plasma cortisol concentration. Because of Na^+ - K^+ exchange in the foetal nephrons, these factors also appear to have influenced urinary K^+ concentration during gestation. The urinary concentrations and excretion rates of uric acid and urea were related to the gradual increase in GFR that occurs prior to day 145 and to the exponential increase in GFR during the last five days of pregnancy.

The ability of the foetal kidney to compensate for disruptions to normal fluid and electrolyte balance within the foetus was also studied. This ability was apparent in foetuses older than 115 days, irrespective of whether the disruptions were the result of direct manipulation of foetal body fluids or indirect disruptions consequent upon changes in the composition of maternal body fluids or amniotic fluid. The renal mechanisms involved in these homeostatic activities included both changes in GFR and changes in the reabsorptive activity of the renal tubules. The relative involvement of each appeared to be a function of foetal age and the nature of the imbalance in the internal environment of the foetus.

DECLARATION

I hereby declare that the work presented in this thesis has been carried out by myself, except where otherwise stated, and that this dissertation has not been submitted in full, or in part, in any previous application for a degree.

B.J. PUDNEY

A C K N O W L E D G M E N T S

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ABBREVIATIONS USED

Cl^-	chloride
Cr	creatinine
H^+	hydrogen ion
HCO_3^-	bicarbonate
Hg	mercury
K^+	potassium
Na^+	sodium
UA	uric acid
$[Cl^-]$	chloride concentration
[Cr]	creatinine concentration
$[H^+]$	hydrogen ion concentration
$[K^+]$	potassium concentration
$[Na^+]$	sodium concentration
[UA]	uric acid concentration
[urea]	urea concentration
CrCl	creatinine clearance
CrER	creatinine excretion rate
KER	potassium excretion rate
NaER	sodium excretion rate
UAER	uric acid excretion rate
urea ER	urea excretion rate
ACTH	adreno-cortico trophic hormone
ADH	antidiuretic hormone (vasopressin)
AVP	arginine vasopressin
Cort.	cortisol
E_1	oestrone
$E_2 17 \propto$	oestradiol-17 $\propto$

$E_2 17\beta$	oestradiol-17β
$Na^+ - K^+$ ATPase	sodium-potassium activated, adenosine triphosphatase
NEFA	non-esterified fatty acids
PRA	plasma renin activity
PRC	plasma renin concentration
prog.	progesterone
17∝ HP	17∝ -hydroxyprogesterone
20∝ HP	20∝ -hydroxypregn-4-en-3-one
20∝ HSD	20∝ -hydroxy-steroid-oxido-reductase
AF	amniotic fluid
CBG	corticosteroid binding globulin
CPB	competitive protein binding
GFR	glomerular filtration rate
iv	intra-venous
MAP	mean arterial pressure
OD	outside diameter
PAH	para-ammino-hippuric acid
PCV	packed cell volume (haematocrit)
PV	polyvinyl
RAS	renin angiotensin system
RPF	renal plasma flow
SE	standard error
TBW	total body water
v/v	volume to volume
Kg	kilogram
gm	gram
mg	milligram
µg	microgram
ng	nanogram

l or L	litre
ml	millilitre
µ	micron
mµ	millimicron
Å	angstrom unit
mEq	milli-equivalent
µEq	micro-equivalent
mU	milli-unit
µU	micro-unit
IU	international unit
µC	micro-curie
mm	millimetre
mOsm	milli-osmole
mV	milli-volt
M	molar
min	minute
hr	hour

The objective of this research was to examine the functional development of the foetal kidney during gestation. The sheep was chosen as the subject of this research because it is particularly amenable to the surgical intervention necessary in studying foetal kidney function in vivo.

At the time this study was commenced, much of the information relating to foetal kidney function concerned acute experiments carried out on sheep foetuses. However, a few reports of renal studies using chronically catheterised sheep foetuses had appeared in the literature and the importance of chronic preparations, as a means of conducting long-term physiological and biochemical studies on unstressed foetuses was beginning to be recognised. Important pioneering studies in this area were carried out by D.P. Alexander and her colleagues during the 1950's (Alexander et al 1958 a,b.). However, their early preparations were acute and involved exteriorisation of the foetus and it was not until the mid-1960's that the technical difficulties of establishing in utero preparations with chronically catheterised foetuses were overcome (Meschia, 1965).

In 1972, Mellor, Williams and Matheson published a paper describing a technique for the chronic catheterisation of the foetal sheep bladder and since that time these authors have employed their technique in a variety of renal studies. The technique described by Mellor et al is similar to the technique developed independently for use in the present work, although Mellor et al did not include vascular cannulae in their preparation. As will be seen in section 3.0, the preparation used in the present research included the establishment of indwelling catheters in the femoral artery and vein of the foetus and at the time this preparation was developed no other had been described

in which foetal fluids, foetal urine and foetal blood could be collected simultaneously.

Once this experimental preparation could be reliably established, the first step in the project was to determine normal values for the various parameters selected as indices of kidney function. These included urinary Na^+, K^+, pH uric acid, urea and creatinine concentration plus flow rate and glomerular filtration rate (GFR). From this data certain trends in kidney activity during foetal life were established with particularly dramatic changes apparent during the last 10-15 days of gestation. Thus the next step was to attempt to relate these trends to anatomical or functional changes in the developing kidney or to other developments in foetal physiology.

Since foetal GFR was found to be significantly correlated with foetal age, the characteristics of glomerular filtration were studied in more detail. Changes in total glomerular surface area were considered as were the ultra-structural features of the filtration barrier in foetuses of various ages. In addition, the influence of foetal arterial blood pressure and renal vascular resistance on glomerular filtration was examined. Finally, since the GFR of foetuses is reputedly more variable than that of adults, foetal GFR was studied during drug-induced diuresis.

The function of the renal tubules was studied in two ways. Firstly, the fractional reabsorption of water and electrolytes was assessed throughout gestation. Secondly, foetuses of varying maturity were treated with a range of common diuretic agents. It was anticipated that the second approach would prove useful for a number of reasons. Since the pharmacological action of these drugs often involves the inhibition of transport mechanisms in the renal tubules and since, in many cases, the nature and site of this inhibition is relatively well established; it was felt that the diuretics could be used to indicate

the existence of similar mechanisms in the tubules of the foetal kidney. Secondly, should the diuretics induce a response, the magnitude of the response may indicate the degree of maturity of the particular mechanism inhibited. In effect, the use of these diuretics enabled a comparison to be made between the function of the renal tubules in foetal sheep and the function of renal tubules in adult mammals.

Next, in a series of preliminary experiments, hormonal influences on foetal kidney function were examined and particular attention was paid to the involvement of the foetal pituitary-adrenal axis. The degree of 17α & 11β hydroxylase activity in foetal adrenals at various stages of gestation is currently in dispute, hence the involvement of foetal mineralo-corticoids in regulating electrolyte metabolism in the foetus is unclear (Wintour et al, 1975; Thomas et al, 1976).

This question was studied by treating foetuses with cortico-steroids, corticotrophin and a cortico-static agent. It was also examined by relating endogenous cortico-steroid levels to urine composition in untreated foetuses. In addition, the responsiveness of the foetal kidney to vasopressin was tested and the involvement of the renin-angiotensin system in foetal water and electrolyte homeostasis assessed. The latter was achieved by infusing angiotensin II and by measuring plasma renin activity throughout the last third of gestation.

Finally the homeostatic capacity of the foetal kidney was examined to determine its ability to regulate the internal environment of the foetusthroughout gestation (Gellhorn & Flexner, 1942; Hellman et al, 1948; Stanier, 1965). The renal response of the sheep foetus to stimulii such as salt loading and haemorrhage was assessed. In addition, the internal environment of the foetus was manipulated indirectly, by altering the salt concentration and osmolality of maternal plasma, thereby establishing transplacental gradients which disrupt the normal transfer of electrolytes and water across the placenta. The ability

of the foetal kidney to control electrolyte and water excretion, under these circumstances, is of particular interest since it is known that the placenta offers little resistance to the transfer of water. The transfer of the physiologically important cations is only slightly more restricted. Thus any regulatory capacity of the foetal kidney, would enable the foetus to limit changes of its body fluid composition in situations where dietary or environmental conditions had altered the composition of maternal body fluids.

2. *LITERATURE REVIEW*

2.1 The methodology of foetal kidney research

It is only during the last two decades that research into the function of foetal kidneys has made significant progress. Previously, isolated observations and unrelated investigations characterised the meagre intrusion of this subject into the physiological literature.

Leonardo da Vinci, at the commencement of the 16th century, noted that human foetuses produce urine during foetal life (quoted by Needham, 1931). In 1902 Jacque compared the osmolality of amniotic and allantoic fluid in sheep, with that of foetal urine and concluded that urine passes into the allantoic sac via the urachus until about the 90th day of gestation. He further suggested that thereafter the urine passes increasingly into the amniotic sac as the urethra becomes more patent. It was not until 1958 that Alexander et al, provided corroborative evidence for Jacque's theory. In the meantime, a few unrelated studies of foetal kidney function had been published.

Makepeace et al (1931) analysed foetal bladder samples from humans and discovered that human foetuses produce hypotonic urine. About the same time the glomerular excretion of ferrocyanide and the tubular excretion of phenol red was reported in the foetal rabbit, cat, opossum, chick and pig (Gersh 1937). Later, Wells (1946) and Daly et al (1947) demonstrated that the foetal rat kidney produces a diuresis in response to sub-cutaneous injections of urea. In 1947 quantitative observations on phenol sulphophthalein excretion by the foetal rabbit kidney were published (Williamson and Hiatt 1947) and finally, in the early 1950's Kinnumen and Paalanen (1952) examined the excretion of uric acid in human foetuses.

In studying the kidney function of human foetuses, investigators have been particularly limited. They have had to be content with collecting urine from foetuses taken during natural or artificial abortion

or with collecting urine voided soon after delivery. Other approaches have included histological examination of kidneys from the abortus and radiographic study of dye excretion. Obviously the use of abortuses provides no opportunity for the prolonged study of individual foetuses and in the case of foetuses that were naturally aborted, it is likely that their physiology is deranged. The study of newborn infants is also of limited value. The belief that the analysis of samples taken at birth, particularly from premature infants, will provide information concerning in utero kidney function, is questionable. The physiological changes associated with parturition are marked and not consistent with the more quiescent period of foetal life.

Thus it has become apparent that animal experiments must form the main basis for the study of renal function during foetal life. Hopefully, such studies may provide a context for the interpretation of the less extensive human data; although variation between species, in other better researched areas of reproductive physiology, suggests that this hope may not be fulfilled. Nevertheless, attention has now centred on the development of surgical procedures which will make the kidneys of animal foetuses accessible to experimental manipulation.

Intra-uterine foetal surgery has been attempted for over half a century with varying degrees of success. Perhaps the first success was achieved by Mayer in 1918 who transplanted the foetus of a guinea-pig from the uterus to the peritoneal cavity. Soon after, in 1919, Wolff applied a similar technique to rabbits. Foetal surgery on sheep was successfully performed by Barcroft and Barron in 1936 during their studies on foetal respiratory movements and again in 1953 by Schinckel and Ferguson who conducted skin transplants on foetal lambs. However, with respect to foetal kidney function, the most significant era began with the work of Alexander et al in 1958.

Alexander's group pioneered the use of sheep foetuses for quantitative experimentation and their initial work centred on kidney function. It is from this point that we can date the evolution of ordered research on the foetal kidney.

The sheep was chosen as the experimental animal because it is docile, has a relatively long gestation and because conception can be accurately timed. In addition, the pregnant uterus is thin walled with wide intervascular and intercotyledonary spaces, allowing easy access to the foetus. For these reasons, the sheep continues to be the most popular research animal in this field. In their early work, Alexander and her colleagues used exteriorised foetuses with their placental circulation maintained. This technique has since been emulated by other workers (Smith et al, 1966).

Unfortunately, these acute preparations have major deficiencies; the experimenter must contend with surgical and anaesthetic stress and deterioration of the preparation. Heyman (1967) has demonstrated that following exteriorisation of the sheep foetus, placental vascular resistance is increased and umbilical blood flow decreased. What is particularly important, is that these changes in foetal circulation are not accompanied by major change in foetal blood gas levels or pH. Thus experiments in which these blood parameters are used to monitor the physiological normality of the foetus (Smith et al, 1966; Alexander and Nixon, 1961) are suspect.

Many of the artefacts produced in acute preparations have only become obvious by comparison with the results of later chronic experiments. For example, progressive foetal hypoxia, observed in acute experiments using near term foetuses, was found to be an artefact of technique (Meschia et al, 1965; Comline and Silver, 1970 and 1972). Similarly, Alexander et al (1958b), reported that low excretory rates of hypertonic urine are observed more commonly in sheep foetuses close

to term. But Gresham et al (1972) claim, in the light of their chronic studies, that this trend merely represents a more pronounced response of the older foetuses to stress.

There is general agreement amongst experimenters in this field, that a period of days (5-7 days) is required for the foetus to return to normal following surgery. Foetal recovery cannot be complete until the ewe has returned to normal. These facts emphasise the limitations of using acute preparations to study foetal kidney function.

The above indictment of acute studies is not intended to detract from the early work of Alexander and her colleagues. These early studies demonstrated that the foetal kidney was amenable to research and produced results that initiated many subsequent investigations. They not only added to the meagre knowledge of renal function in foetuses, but more significantly they stimulated criticism and innovation and created the starting point for the subsequent development of chronic foetal preparations.

In 1965 Meschia et al described a method for the chronic catheterisation of the umbilical vessels in the sheep conceptus. Since that time the use of indwelling catheters in chronic preparations has become the prime method for studying the physiology of foetal animals. The range of available techniques has been extended and in studies of kidney function, various anatomical approaches to the foetal urinary tract have been used. These include catheterisation of the foetal urethra (Buddingh et al 1969), bladder (Mellor et al 1972), urachus (Gresham et al 1972) and ureter. Some workers emphasise the importance of placing catheters into the foetal fluid sacs to enable the foetal urine to be recirculated in a normal manner. Buddingh and his associates claim that failure to do so will not only disrupt normal urine flow rate and composition, but will prohibit the maintenance of the preparation. On the other hand, Gresham et al (1972) have reported that the continuous drainage of foetal urine for as long as 18 days, does not

effect foetal viability. Further, in those foetuses where the urine had been drained, all urinary parameters were comparable with those in foetuses where the urine was recirculated. However, one clear effect of continuous urine damage is a marked reduction in the volume of allantoic and amniotic fluid. Thus, despite Gresham's assurances, it is desirable, from the point of view of maximising physiological normality to recirculate urine.

Although a great deal of information regarding the function of foetal kidneys has been obtained using chronic preparations, the use of these preparations has certain limitations and difficulties. The controversy regarding the necessity of recirculating urine indicates that there is still uncertainty with respect to the proper use of chronic models. Also, the different methods used for collecting foetal urine could have subtle influences on the results obtained; as could other factors such as partial urinary obstruction due to the catheter and leakage of urine through uncannulated channels. More importantly, if chronic experiments are to offer real advantages over acute studies and the reliability of the results assured, there are certain practices that must be adhered to. Firstly, care must be taken to adapt the pre-operative animals to pens and handling. Secondly, sufficient time for recovery should be allowed after surgery before experiments commence and, finally, sterility should be maintained without excessive use of antibiotics.

2.2 Foetal kidney development

The remainder of this review will summarise the results of much of the research that has been conducted on foetal kidney function. It is, however, not a complete review of all such work, as the emphasis has been placed on those studies of particular relevance to the present work.

2.2.1 Morphological development

The anatomical and ultrastructural aspects of renal development have been reviewed by Vernier and Smith (1968). Therefore, only a brief summary of this aspect of renal development will be presented.

In most vertebrates, the kidney develops through three stages; pronephros, mesonephros, and metanephros. In humans, the existence of the pronephros is short-lived and it is replaced in the 4mm. embryo by the mesonephros. The size and persistence of the mesonephros varies between species; in some, e.g. man and rat, it degenerates rapidly, while in others, including the sheep, it persists until relatively late in gestation. In the sheep, the mesonephros has almost completely degenerated by the 55th. day of gestation (Davies and Davies 1950) and in fact, metanephric development begins even earlier.

The embryological sequence of renal development has been studied in humans. It was found that the caudal end of the mesonephric duct develops into the ureter, renal pelvis and collecting duct, while the nephrogenic cord of the embryo gives rise to the tubular system. Ultimately, the collecting duct and tubules come into apposition and a glomerulus forms in the blind end of the tubule. This completes the development of the nephron in the metanephric kidney. Despite chronological variations in the metanephric development of different species it is probable that the embryological sequence is similar.

Once the metanephric kidney is established, maturational changes continue, but the details of these changes are not clear. Histological examinations imply that the tubular elements of the nephron but not the glomerulii are well developed at term, although there is some evidence that the kidney tubules continue to lengthen after birth. In an early study, Gruenwald and Popper (1940) concluded that the tall columnar epithelium of the glomerular capsule in human foetuses would limit

filtration until the epithelium was shed at birth. A multitude of functional studies have since shown that filtration does occur in foetal life and that, in fact, the foetus often produces a higher urine flow, per unit of body weight, than does the adult (Alexander et al, 1958b). Nevertheless, much of the detail of glomerular development remains controversial. Until recently, even the origin of the vascular elements of the glomerulus was not clear. One view was that the glomerular capillaries develop in place, along with the tubular elements of the glomerulus. Another view was that the developing glomerulus is penetrated by a branch of the primitive renal artery which ramifies to produce the network of glomerular capillaries. Microdissection studies combined with dye injections support the latter view (Osathondh and Potter, 1963).

Electron microscope studies of foetal glomerulii in humans, have revealed changes in ultrastructure that appear to be of functional significance. Vernier and Birch-Andersen (1962) have noted that the endothelial cells of the glomerulii become progressively thinner as gestation proceeds and that increasing numbers of fenestrae appear. These endothelial fenestrae or pores, are crossed by a thin membrane and are a typical feature of the capillary endothelium in adult glomerulii. It was also observed that the epithelial cells develop more foot-processes as gestation continues and that in the space between the endothelial and epithelial cells, a basement membrane appears which becomes denser and broader as term approaches. At term, the basement membrane is approximately $1000\overset{o}{A}$ in width and it continues to widen after birth.

It is assumed that the development of increasing numbers of endothelial fenestrae and epithelial cell foot-processes would facilitate glomerular filtration, however this filtration probably becomes more selective as the basement membrane develops. The permeability characteristics of foetal glomerulii have been evaluated using ferritin and it

appears that at least for that substance, the basement membrane is a filtration barrier. More recently, Graham and Karnovsky (1966) have demonstrated that the membrane which crosses the channels between the epithelial cell foot-processes also acts as a filtration barrier.

Vernier and Birch-Andersen (1962) have also shown that in humans, anatomical development of the kidney continues after birth, since the glomerulii increase in diameter from about 100μ at birth to about 200μ at 20 years of age. With respect to the renal tubules, Fetterman et al (1965) have reported that in full-term human foetuses, 20% of the nephrons have loops of Henle which are still within the renal cortex. After birth they found that the tubules increased in length and size, thereby providing a greater surface area for the normal reabsorptive and secretory functions of kidney tubules. It has been suggested that this development would increase the capacity of the developing kidney to act as a homeostatic organ. It may also be that the normal hypotonicity of foetal urine reflects the limited reabsorptive area available to the foetus and the inability to establish renal medullary solute gradients, before elongation of the tubules.

The development of the foetal urinary tract apart from the foetal kidney, merits discussion. The development of the lower urinary tract is similar in human and sheep foetuses. (Tanagho 1972). In the sheep foetus the uro-genital sinus separates from the cloaca and differentiates into the urinary bladder and the urethra (Tanagho 1972). The bladder extends to the umbilicus and opens directly into the allantois during the first two thirds of gestation. By about the 75th day of gestation the urethra is well formed but it remains packed with epithelial cells and is not yet patent. It is not until about the 90th day of gestation that urine is passed via the urethra into the amniotic sac. Also about this time, the bladder begins to descend caudally, separating from the umbilicus and pulling on the attachment of the allantois such that the urachus is

gradually obliterated.

Tanagho (1972) has shown that when partial urethral obstruction is surgically induced in foetuses at about the 75th day of gestation, there is no resultant pathological effect. This is because the opening of the bladder through its apex to the allantois acts as a safety outlet and protects the upper urinary tract. If however, a similar obstruction is introduced after the 90th day, pathological changes do occur. These experiments confirm the relative importance of the urachus and urethra before and after the 75th day of gestation in the ovine foetus.

The ultrastructure of the urinary bladder epithelium in foetuses ranging in age from 50 to 141 days has been studied (France et al, 1974). Of particular interest were the tight intercellular junctions which bind the epithelial cells together to form a barrier to the free diffusion of solutes across the epithelium. There was no visible change in the general ultrastructure of the epithelium nor in the nature of the tight junctions that would indicate that the permeability of the foetal bladder alters during gestation.

2.2.2 Functional development

The urine flow per unit time, from an individual foetus varies considerably, in fact, the smaller the test period, the greater the variation in flow will appear. Nevertheless, the output of foetal urine is usually high and as Alexander et al (1958 a,b.) observed the foetal sheep produces a higher urine flow per unit body weight than the basal urine flow of the ewe. In an acute study, Alexander et al (1958 a,b.) recorded a flow rate of 1.9ml/min/Kg in a 61 day-old foetus. With more mature foetuses the flow rate was found to decrease progressively, reaching a minimum of 0.04ml/min/Kg in a 142 day-old foetus. Bernstine (1970) in discussing urine flow rate, commented that a value of 0.64ml/min quoted by Alexander et al (1958 a,b.) for a 117 day-old foetus was

abnormally high since a sustained flow rate of that magnitude would yield a 24 hour urine volume of 921ml. Certainly, this is an above average rate of urine flow, but experience in the present work indicates that it is not abnormal. In a larger series of acute experiments, Smith et al (1966) recorded an average flow rate of 0.053 $\pm$ 0.012ml/min/Kg in near term foetal sheep. In other work, using chronically catheterised foetuses in which 24 hour urine collections were made, an average flow rate of 11.4 $\pm$ 8.0ml/hr was calculated for the period between 108 and 125 days of gestation and 14.3 $\pm$ 9.7ml/hr for the period between 126 and 143 days.

In general, it appears that a urine flow rate of between 0.15 and 0.3ml/min is average for mature sheep foetuses although in most reports occasional values below this range are quoted, particularly for near term foetuses. In other species, Rahill and Subramanian (1973) found that for canine foetuses within 10 days of birth, the average urine flow rate was 0.18 $\pm$ 0.08ml/min (n=11) while Chez and Smith (1964) recorded a flow rate of 0.03ml/min in a full-term rhesus monkey. With human foetuses measurement of urine production rates can now be made using ultrasonic techniques. Wladimiroff and Campbell (1974) applied these techniques on 92 antenatal patients and estimated that the average rate of foetal urine production was 9.6ml/hr at 30 weeks and 27.3ml/hr at 40 weeks. The latter figure is surprisingly high, particularly since direct measurements of urine production in premature infants within 48 hours of delivery, yield values of between 3.0 and 4.5ml/hr. (Wilkinson, 1973).

With respect to the composition of foetal urine, most studies have confirmed Alexander et al's (1958 a,b.) observation that foetal urine is hypotonic in relation to foetal plasma. Alexander et al also found that the percentage reabsorption of filtered electrolytes increases as gestation proceeds. On the basis of these observations, Alexander et al

proposed that the onset of glomerular filtration precedes the development of the overall reabsorptive capacity of the renal tubules and that this lack of tubular reabsorption results in the formation of large volumes of hypotonic urine. Alexander further suggested that as the renal tubules extend and mature, the reabsorptive capacity develops and the urine becomes increasingly hypertonic. However, in a series of chronic experiments Bernstine (1970), found that the osmolality of foetal urine did not change with increasing gestational age. He found that despite short-term fluctuations in urine osmolality, when urine specimens collected over a period of several hours were pooled, the osmolality changes between successive samples were slight. With respect to the concentration of specific solutes, Bernstine found, as did Alexander et al, that the sodium concentration of foetal urine decreased as term approached. However, the average decrease for the 7 foetuses Bernstine examined was not as large as the difference between the two foetuses Alexander et al studied. Also, the urinary potassium concentrations reported in these two studies are quite different and there is no consensus in either absolute values or gestational trends. In contrast, both studies report increased levels of urinary creatinine in older foetuses although again the concentrations quoted are of a different order.

This lack of consensus between the work of Bernstine (1970) and that of Alexander et al (1958) is obviously due to differences in experimental technique and to variation in sample size. However, although Bernstine examined more animals than Alexander et al, neither study was comprehensive enough to reliably reveal trends in urine composition.

Table 1 summarises the data from these studies and also includes information from reports by Smith et al (1966), Rahill and Subramanian (1973) and Chez and Smith (1964).

TABLE 1: COMPOSITION OF FOETAL URINE

ANIMAL	PARAMETER	GESTATION PERIOD EARLY	MID $\bar{X} \pm SE$	LATE $\bar{X} \pm SE$
SHEEP	Osmolarity (mOsm/L)	239 (n=2)	207 (n=2) 237.4 ± 92.8 (n=7)	116 (n=2) 238.8 ± 83.5 (n=7) 271 ± 38 (n=18)
	[Creatinine] (mg/100ml)	3 (n=2)	6 (n=2) 12.3 ± 11.4 (n=7)	27 (n=2) 19.5 ± 16.5 (n=7) 35.5 ± 12 (n=18)
	[Na+] (mEq/1)	81 (n=2)	71 (n=2) 74.6 ± 40.6 (n=7)	26 (n=2) 60.7 ± 31.5 (n=7)
	[K+] (mEq/1)	4 (n=2)	4 (n=2) 17.7 ± 19.0 (n=7)	8 (n=2) 14.6 ± 9.8 (n=7) 4.8 ± 2.9 (n=18)
	[Urea] (mg/100ml)	102 (n=2)	123 (n=2)	287 (n=2)
	pH			7.75 ± 0.41 (n=7) 6.84 ± 0.44 (n=2)
DOG	Osmolarity (mOsm/L)			346 ± 50 (n=8)
	[Na+] (mEq/1)			99.2 ± 16.5 (n=8)
	[K+] (mEq/1)			15.4 ± 4.6 (n=8)
MONKEY	Osmolarity (mOsm/L)			271 ± 18.5 (n=9)
	[Creatinine] (mg/100ml)			28.4 ± 12.7 (n=9)
	[Na+] (mEq/1)			106 ± 25.3 (n=9)
	[K+] (mEq/1)			7.6 ± 4.3 (n=9)

(See text page 15)

The most complete study of foetal urine composition to date is that of Mellor and Slater (1972b) who catheterised the bladders of 10 sheep foetuses between the 79th and 96th day of gestation and obtained samples of urine and foetal fluids each day until term. They did not, however, include vascular cannulae to sample foetal blood.

As in other studies (Bernstine 1970), Mellor and Slater found that foetal surgery effected the concentration of most urinary solutes. Potassium and urea were particularly effected, both showing marked falls in concentration during the first two days after surgery but increasing gradually thereafter. With respect to gestational trends in the composition of urine, it was found that in unstressed foetuses the average [Na^+] decreased from 60 - 28 m Eq/L in the period between 91 and 130 days of gestation. In comparison, the average [K^+] increased from 1.7 m Eq/L between days 91 and 97, then fell to 2.5 m Eq/L on the 130th day of gestation. During the same interval, the urea concentration of foetal urine remained within a relatively constant range (10.5 mM - 13.4 mM) as did urinary pH (7.24 - 7.30).

In the 14 days immediately prior to birth far more dramatic changes were recorded. The average [Na^+] of foetal urine continued to decrease until 4 days before birth at which point it began to rise from a level of 22.5 mEq/L to reach 47.5 mEq/L during labour. The average [K^+] which had started to rise at day 118 of gestation, continued to increase during the last 14 days of pregnancy, rising from 3.2 mEq/L 14 days before birth to 26.2 mEq/L during parturition. Urea concentration also showed a preparturient rise, although no significant increase was noted until 8 days before birth, at which time the average urea concentration was 8.5mM. Thereafter the urea concentration rose sharply to reach 47.1mM at term. Conversely the mean pH of foetal urine remained between 7.19 and 7.24 until 8 days before birth and then began to decline, reaching a minimum of 6.27 at term. This latter result

confirms the preparturient decrease in urinary pH previously observed by Mellor and Slater (1972a).

Thus, in addition to having analysed the changes in the concentration of urinary solutes from 91 to 130 days of gestation; Mellor and Slater have reported for the first time, that dramatic changes of foetal urine composition occur during the last 14 days of foetal life. The present study corroborates many of these findings and, in addition, the simultaneous collection of foetal plasma in the present work permits a consideration of these changes in terms of the internal environment of the foetus.

Alexander et al (1958 b) were the first to report on the clearance of plasma constituents by the foetal kidney. Again, these were acute studies on ovine foetuses. They found that the clearance rates of non-ionic plasma constituents such as urea, creatinine and fructose were basically the same and that when these values were corrected for foetal body weight, the clearance rates decreased with increasing gestational age. At day 61 of gestation, the clearance rates were 2.4ml/min/Kg while at day 142 they were 0.4ml/min/Kg. In a later study, Alexander and Nixon (1962) attempted to analyse GFR and tubular secretory activity in the ovine foetus by measuring the clearance rate of exogenous inulin and p-amino-hippuric acid (PAH). They found that the clearance of PAH increased with foetal age suggesting a progressive increase in tubular secretory activity. Similarly the clearance of inulin was positively correlated with foetal age implying an increase in GFR as gestation proceeds. Subsequently, Smith et al (1966) who also used exteriorised sheep foetuses, reported that in 120 to 130 day-old foetuses, inulin clearance did not rise, but in 130 to 142 day-old foetuses there was an increase. In near term foetuses, Smith et al (1966) recorded an average inulin clearance rate of 0.75ml/min/Kg which is comparable with Alexander and Nixon's (1962) findings. In this same study, the average rate of

creatine clearance for foetuses aged between 130 and 142 days, was 0.45ml/min/Kg. More recently, Bernstine (1970) carried out a series of experiments on foetal sheep using chronic preparations and found that endogenous creatinine clearance increased from 0.77ml/min at 110 days gestation to 1.26ml/min at 126 days. When these values were corrected for foetal body weight it became apparent that the observed increase was due to enlargement of the kidney.

In reference to the association between inulin clearance and GFR, it should be noted that although early workers used inulin clearance as an estimate of GFR, they did so without valid grounds. Applying this association to foetuses pre-supposes that the permeability characteristics of the renal tubules of foetuses are similar to those of adults. It is only recently (Lockhart and Spitzer, 1974) that any evidence in support of this supposition has been advanced.

During 1970 Smith and Schwartz introduced isotopic methods into the study of renal clearance in foetal animals. They measured the clearance of iothalamate which had been labelled with I^{131}. The advantages of isotope techniques were quickly recognised and Buddingh et al (1971) used inulin, labelled with ^{14}C, to determine inulin clearance. This remains a useful method for studying foetal kidney function.

In a series of experiments Buddingh et al found that inulin-^{14}C clearance remained relatively constant in foetuses aged between 120 and 130 days. However, in foetuses older than 130 days, inulin-^{14}C clearance increased rapidly. Between 120 and 130 days of gestation the inulin-^{14}C clearance, which now can be regarded as a valid estimate of foetal GFR, varied between 0.8 and 1.5ml/min and increased to between 2.9 and 4.0 ml/min on day 140. If these values are corrected for foetal weight, the GFR for foetuses between 120 and 130 days of gestational age is in the range 0.4 to 0.8ml/min/Kg, while on day 140 it is between 1.0 and 1.3ml/min/Kg. Comparing these figures it appears that much of the increase

in filtration observed by Buddingh et al (1971) reflects the quickening enlargement of the foetal kidney near term. However, when this factor is removed, there remains a residual increase in GFR which may be due to functional changes within individual nephrons.

Gresham et al (1972) also used inulin-^{14}C to measure GFR in foetal sheep. In their work, 14 measurements were made in 8 foetuses of between 122 and 134 days of gestational age. The overall average for GFR was 1.07ml/min/Kg while the individual values for foetuses aged between 122 and 130 days varied between 0.86 and 1.08ml/min/Kg. Over the remainder of the age range examined, the values were generally higher, varying between 1.13 and 1.54ml/min/Kg. However, in the 4 day period, between days 130 and 134, the estimates of GFR were variable and there was little association with foetal age. The most recent study of foetal GFR was carried out by Robillard et al (1974). In a series of acute preparations, GFR was measured using sodium iothalamate-^{125}I and it was found that GFR was significantly correlated with foetal age. However, there was no significant increase in GFR expressed as ml/min/Kg of body weight or in ml/min/gm of combined kidney weight.

Apart from studying inulin-^{14}C clearance, Gresham et al also determined the renal clearance of fructose, urea and creatinine. The average clearances (expressed in ml/min/Kg) for fructose, urea and creatinine were 0.59, 0.59 and 1.58 respectively. Over the period of gestation analysed, there was no significant correlation between the individual clearance values for these substances, and foetal age.
The values for GFR reported by Gresham et al (1972) were slightly higher than those reported by Buddingh et al for foetuses of corresponding ages. In both studies there was an overall increase in GFR as the foetal kidney enlarged, presumably due to the proliferation and enlargement of glomerulii. In addition, there appeared to be a smaller increase in GFR that was independent of kidney enlargement. However, the evidence

for these associations is slight and the relationship between GFR and other features of kidney development have been further studied in the present work.

2.3 *Foetal kidney function and the composition of foetal fluids*

As mentioned previously, speculation on the relationship between foetal fluids and foetal urine dates back to Jacque's work in 1902. Despite this long history, the dynamics of foetal fluid circulation and the contribution of foetal urine is poorly understood. However, the fact that the amniotic and allantoic fluids are not static accumulations of fluid is clear. In the guinea-pig it has been established that the entire amniotic fluid is replaced in less than one hour while in humans it is replaced in slightly less than three hours (Bor et al, 1964). Therefore, we know that the amniotic fluid undergoes active circulation and that the nett result of this circulation is to produce long and short term changes in the composition and volume of this fluid. For example, the volume of amniotic fluid in humans increases during gestation and at 18 weeks gestation, the liquor volume increases by 10 - 13ml per day (Abramovich 1970). Similar long term changes of amniotic fluid volume are seen in other species, noteably the rat and the sheep (Malan et al, 1937; Cloete, 1939; and Mellor and Slater, 1971).

Several mechanisms including free diffusion, active transport, ionic gradients and pinocytosis, have been proposed to explain the passage of water and solutes to and from the foetal fluid compartments. Also, a variety of organs and tissues have been implicated in these exchanges. It is now apparent that the foetal kidney is an important contributor to both the allantoic and amniotic fluid but it is by no means the sole contributor.

Alexander et al (1958 a) studied gestational variation in the

composition of foetal fluids and foetal urine. They found that amniotic fluid has osmotic and other characteristics more closely related to those of foetal plasma than to foetal urine. But on the basis of the relative concentration changes they reiterated Jacque's (1902) proposal that in late pregnancy foetal urine enters the amniotic fluid and influences its composition. With respect to allantoic fluid, they noted that its composition resembles that of foetal urine and concluded that this was the major contributor to allantoic fluid.

More recently, Mellor and Slater (1972b, 1973) have used chronically catheterised foetuses to collect samples of foetal urine, amniotic fluid and allantoic fluid. The data obtained from the analysis of these samples supports the hypothesis that foetal urine is only one, of possibly many sources of the foetal fluids in late pregnancy. With respect to specific characteristics of the foetal fluids, Mellor et al concluded that the passage of foetal urine into the foetal fluids decreased their osmolality and decreased the concentration of Na^+, K^+ and Cl^- in both amniotic and allantoic fluid. Conversely, the addition of urine increased the concentration of urea in both foetal fluids. So in general, it seems that the urine of sheep foetuses enters the allantoic sac at a decreasing rate until about the 100th day of gestation and there is an increasing passage of foetal urine into the amniotic sac from about the 80th day. Nevertheless, according to Mellor and Slater (1971, 1973), foetal urine contributes to both fluids until term and the relative contribution to the two fluids remains unchanged after the 110th day of pregnancy.

Despite the obvious importance of the passage of foetal urine into the amniotic fluid, as mentioned, many other mechanisms have been implicated in the dynamics of this fluid. Although Plentl (1966) dismissed the foetal skin as a site of significant exchange of solute between the foetus and amniotic fluid, Lind et al (1972) claimed that,

at least in early pregnancy, the foetal skin is so permeable to water and electrolytes that the amniotic fluid is virtually an extension of the foetal extracellular fluid. Bor et al (1964) found that following the injection of Na^{22} into the amniotic sac there was a high activity in the foetal skin indicating that Na^{+} enters the foetus via the skin. Finally, Mellor and Slater (1971, 1972, 1973) have produced evidence that until about the 130th day of pregnancy, Cl^{-} is transported into the amniotic sac from blood vessels in the amnion and foetal skin. Thus the fact that foetal skin is involved in the circulation of amniotic fluid appears certain, but to what degree it is involved is uncertain, as is the precise mechanism of exchange for the various fluid constituents.

The foetal lungs and respiratory tract are also known to contribute to the amniotic fluid. Adamson et al (1973) estimated that between 100 and 200ml of fluid are secreted daily by the foetal lungs in late gestation. It has been determined that this flow begins at about the 60th day of gestation in sheep (Berton 1969) although the rate of secretion and the composition of this fluid in early pregnancy, is not known. In other experiments, Merlet et al, as cited by Mellor and Slater (1971), reported that in 2 mature foetuses the average flow rate of fluid from the trachea was 8ml/hr. while in near term foetuses, flow rates of 10-30ml/hr. have been recorded (Adams et al, 1967). The $[Na^{+}]$ of this pulmonary fluid resembles that of foetal plasma (Adams et al, 1967).

Other tissues including the amnion and chorion and parts of the foetal gut have been implicated in the exchanges that occur between the foetus and the amniotic fluid (Plentl 1966; Bor et al, 1968; Bourne and Lacy, 1960; Windle et al, 1959 and Pritchard, 1966). However, few quantitive studies have been carried out and consequently the nature of the involvement of these tissues in amniotic fluid dynamics is obscure.

Most research indicates that the formation of allantoic fluid is less complex than the formation of amniotic fluid. Again foetal urine is a major contributor, but there is evidence that the urine flowing into the allantoic sac, via the urachus, has its composition modified by equilibration with maternal and foetal blood. Once in the allantoic sac, the fluid composition is modified further by the active transport of various constituents into and out of the foetus (Mellor 1970; Mellor and Slater, 1971,1972b and 1973). Sodium appears to be actively transported from the allantoic fluid into the foetal blood and K^+ in the reverse direction. Mellor and Slater (1971), have suggested that the transport of these substances may be coupled and is probably influenced by foetal corticosteroids.

2.4 Foetal kidney function and the foetal environment

2.4.1 The homeostatic ability of the foetal kidney

The ability of the foetal kidney to act as a homeostatic organ, could protect the foetus in situations where maternal imbalances would otherwise disrupt the internal environment of the foetus. Therefore, whether or not the foetal kidney possesses a significant homeostatic capacity and, if so, whether or not the mechanisms involved are similar to those of the adult kidney, are questions that have been investigated in the present work.

McCance (1972) has discussed the role of the developing kidney in the maintenance of internal stability. Although his review emphasises the situation in human infants, the conclusions arrived at can serve as a model for considering renal homeostasis in foetuses and neonates of other species. McCance points out that newborn infants have a very limited capacity to excrete water administered in excess (Ames 1953 and Barnett et al, 1952). Also, they tend to reabsorb most of the filtered Na^+ even

when Na^+ salts have been administered in excess. (Theodonius et al, 1971). Finally, McCance notes that newborn humans have a limited ability to excrete H^+ even in conditions of mild non-respiratory acidosis (Hatemi and McCance, 1961).

What is the situation in other species? The neonatal guinea-pig, which at birth is more mature than human infants, does respond to a water load by increasing urine flow (Dicker and Heller, 1951). Conversley, the rat, which is immature at term, shows no diuretic response to excess fluids administered in the immediate post-natal period. The response of newborn dogs to saline loading has been studied by Goldsmith et al (1974) and Kleinman (1975). Both report that pups respond to saline loading, but that the ability to excrete the excess water and Na^+ is more limited than in adult dogs. Each report offers a different reason for this limited response. Goldsmith and his colleagues believe that it is due to the low GFR of the newborn and the inability to adjust the GFR when necessary. However, Kleinman and Hsieh (1974) argued that both pups and adults show an equivalent increase in GFR in response to similar salt loads. They believe the limited response of the young animals is due to increased sodium reabsorption in the distal tubules even though most researchers believe the reabsorptive capacity of neonates is limited. Obviously this is an area that requires further study. The information, regarding the homeostatic ability of the foetal kidney is no more definite.

Bernstine (1970) reported that the intravenous administration of 3L. of 5% dextrose in water to a pregnant dog at term, did not result in a significant increase in foetal urine output; nor was foetal urine flow increased when similar amounts of physiological saline were infused into the bitch. However, when 20ml of physiological saline was administered intravenously to a foetal dog there was a large increase (1000%) in urine output with no significant change in urine

osmolality. This increase in urine flow followed a decrease in plasma osmolality which returned to normal after the diuresis. The increased fluid output by the foetal dog in the 2 hours following saline infusion accounted for about 1/3 of the saline infused and Bernstine concluded that the remainder crossed the placenta into the maternal circulation. In a similar study, Alexander and Nixon (1961) found that the urine output from the sheep foetus could be increased by infusing hypotonic sulphate solution directly into the foetus. Moore et al (1974) have since confirmed this response by infusing hypotonic saline into the foetal lamb.

The effects of varying the plasma concentration of electrolytes in pregnant animals and their foetuses has been studied on a few occasions. Kirksey, Pike and Callan (1962) found that if a sodium deficient diet was fed to pregnant rats there was no significant change in the $[Na^+]$ of foetal plasma and amniotic fluid, despite obvious depletion of the sodium in maternal plasma. In contrast, Winkler et al (1962) reported that the $[Na^+]$ of foetal plasma showed corresponding changes to fluctuations in the $[Na^+]$ of maternal plasma induced by peritoneal dialysis. However, Winkler et al did find that a fall of approximately 15mEq/L in the $[Na^+]$ of maternal plasma caused a smaller reduction, about 6mEq/L, in the plasma $[Na^+]$ of the foetus. Thus it would appear that some mechanism exists that enables the foetus to maintain its plasma Na^+ level relatively constant despite large fluctuations in the $[Na^+]$ of maternal plasma. Whether or not this mechanism involves the foetal kidney cannot be determined from the experiments described.

In other experiments where pregnant rats were fed diets deficient in K^+ and the maternal plasma $[K^+]$ was reduced by half, it was found that the $[K^+]$ of foetal plasma did not change significantly. If, however, maternal hyperkalemia was produced by K^+ infusion, foetal hyperkalemia developed (Dancis and Springer, 1970). Again it may be that

as a result of maternal hypokalemia there is a loss of foetal K^+ across the placenta but that the effect on the $[K^+]$ of foetal plasma is buffered by decreased K^+ excretion by the foetal kidney. Similarly, even though the K^+ infusion produced hyperkalemia in the foetus, there may have been a disproportionate increase in the $[K^+]$ of foetal urine. In the absence of information on the electrolyte concentration of foetal urine the homeostatic ability of the foetal kidney cannot be assessed.

The effect of Na^+ depletion in pregnant sheep was investigated by Phillips and Sundaraman (1966). Sodium depletion was induced by draining parotid saliva for up to 6 days. Single samples of foetal fluids were obtained at the end of the depletion period when the foetus was delivered by caesarian section. In these sodium depleted ewes, the $[Na^+]$ of foetal plasma and amniotic fluid were lower than in control ewes and the volume of allantoic fluid was greater. In view of the relationship between foetal urine composition and the composition of amniotic and allantoic fluid, Phillips and Sundaraman (1966) claim that their findings indicate that, "the sodium deficient foetus, like a sodium deficient adult, responds to the deficiency by restricting sodium losses in the urine and by excreting water". Nevertheless, since foetal urine was not sampled, the evidence is indirect and not conclusive.

More conclusive evidence is available, concerning the ability of the foetal lamb to vary urinary acidification in response to artificially induced metabolic acidosis. Smith and Schwartz (1970), induced acidosis by infusing 0.1 or 0.3M hydrochloric acid into foetuses and in all cases there was a significant increase in the urinary excretion of ammonium phosphate and titratable acid and, accordingly, there was a decrease in urine pH. However, the response obtained was more limited than that which would occur, with similar treatment

in adults. This agrees with the findings of Vaughan et al (1968) who determined that the acid load necessary to induce significant changes in urinary acidification is 3 times greater in the foetal lamb than in the adult sheep. It also agrees with the work of Daniel et al (1975) who found that following lactic acid infusion into foetal lambs (115 - 125 days old), the kidney makes a limited contribution to foetal homeostasis by increasing H^+ excretion and by slightly increasing phosphate and ammonia excretion. Thus, although it is apparent that the foetal lamb can respond to acid loads and prolonged acidosis by increasing H^+ excretion, this compensatory ability is more limited in the foetus than in the adult sheep. Moore et al (1972) infused sodium sulphate solution into foetal lambs resulting in a decrease of foetal urine pH and an increase in the excretion of titratable acid and ammonium ions. They then concluded that urinary acidification occurs by a process of Na^+ - H^+ exchange within the nephron and that any factor which increases Na^+ reabsorption will also increase H^+ secretion.

2.4.2 Placental transfer of water and electrolytes

The limited evidence available suggests that at least in mature foetuses, the kidneys can regulate water and electrolyte excretion in response to variations in plasma electrolyte concentration and osmolality. The most likely cause of such disruption to the foetal environment would be changes in the osmolality and electrolyte concentration of maternal plasma and subsequent equilibration between mother and foetus involving transfer of water and electrolytes across the placenta. Changes in the maternal fluid composition would potentially be amplified in the foetus and in such circumstances the homeostatic ability of the foetal kidney would enable the foetus to limit the disruption of its body fluids. Since the composition of maternal body fluids could be

effected by dietary or other environmental conditions, it is likely that in the absence of a significant placental buffer, the homeostatic ability of the foetal kidney would be essential for the survival of the foetus.

It has been mentioned above that in rats, (Winkler et al, 1962), and sheep (Phillips and Sundaraman, 1966) there is a tendency for the electrolyte concentration of mother and foetus to equilibrate. More precise studies of placental electrolyte transmission, in a variety of species, have been carried out using Na^{24} (Flexnor and Gellhorn, 1942; Flexner and Pohl, 1941 a,b). These studies indicate that at least in rodents, the rate of passage of Na^+ is not rapid as it takes 10 hours for 10% equilibration of Na^+ between maternal and foetal blood. McGaughey et al (1958) have also reported that the placenta offers considerable resistance to the movement of ions. Meschia et al (1958), measured the difference in electrical potential across the placenta of goats and found potentials of 25 to 133 mV between maternal and foetal blood, with the foetus being negative. The potential was higher in early gestation and lower nearer term and the chorion was suggested as the site of origin of the potential. Similar findings have since been reported by Mellor and Slater (1970). The existence of these electrical potentials was given significance by the finding of Crawford and McCance (1960), that the chorioallantois of the pig actively transfers sodium ions from the foetal to the maternal surface. Such an active transport of Na^+ would establish a transplacental potential in favour of a passive movement of Na^+ and in fact all cations, across the placenta from mother to foetus.

More recently, the hypothesis that the total flux of Na^+ towards the foetus results from passage across the placenta, has been challenged. It has been suggested that in some species a path involving the amniotic fluid may make a significant contribution (Mellor, 1969; Mellor and Slater, 1971). However, in goats and sheep, this is unlikely as the

amnion seems to be relatively impermeable to ions. Even in other species, including rabbit, guinea-pig and man, the evidence of Na^+ transport via the amniotic fluid is not conclusive.

With respect to the transfer of water, Gellhorn and Flexner (1942), using tracer methods, have shown that water freely crosses the placenta. Rates of water movement, expressed as ml/hr/gm of placenta, have been given as 10 for man and 20 for guinea-pig (Hellman et al, 1948) for the direction from mother to foetus and a figure of 3.6 has been given for the reverse direction in the pig (Stanier 1965). These rapid rates confirm that the placenta offers little resistance to the transfer of water. Following a theoretical and experimental study of trans-placental diffusion using tritiated water; it has been stated by Meschia et al, 1967, that at least in the sheep, water diffusion is limited only by umbilical and uterine blood flows and is not a function of placental permeability.

Since the evidence suggests that there is very little resistance to the transplacental exchange of water, it is not surprising to find that this exchange can be altered by disturbing the osmotic equilibrium that exists between mother and foetus. This has been observed in a number of species. In the rabbit, an artificially induced increase in the osmolality of maternal plasma produced changes in the protein and electrolyte concentration and osmolality of foetal plasma that were consistent with a loss of water from foetus to mother (Dancis et al, 1957; Bruns et al, 1963). This response was presumably due to the establishment of an osmotic gradient across the rabbit placenta. The substance used in these experiments to increase the osmotic pressure of the maternal plasma, was mannitol. Other experiments in rats (Adolph and Hoy, 1963), sheep (Faber and Green, 1972) and monkeys (Bruns et al, 1964), using mannitol or other osmotically active substances have yielded similar results. It does appear, however, that in the rabbit

the placenta is more permeable to small solutes than is the case in the other species. Accordingly, about 50% of the increase in foetal plasma osmolality, following mannitol infusion into the doe, is due to the placental transfer of mannitol. A similar leakage of solute, across the placenta into the foetus probably occurs in all species; but the degree of leakage is much less and accordingly the osmotic gradient is more effective.

It is apparent, from the above reports, that it is possible to dehydrate foetuses by creating an osmotic gradient across the placenta. Bruns et al (1964) also dehydrated foetuses by producing a solute gradient between the amniotic fluid and foetal plasma. This was achieved by injecting a hypertonic dissacharide solution into the amniotic fluid of the monkey conceptus and resulted in a reduction of the total body water of the foetus.

Unfortunately, in none of these studies could the response of the foetal kidney to foetal dehydration be assessed, since foetal urine was not collected.

2.5 Hormonal influences on foetal kidney function

There is little doubt that the foetal kidney is under the influence of foetal hormones. It is known for instance, that anencephalic infants and infants without pituitaries have mal-developed kidneys, so it is logical to assume that the lack of circulating hormones from the pituitary have resulted in these abnormalities (Naeye et al, 1970; Naeye, 1970). Other evidence has been provided by Mellor and Slater (1974), who found that the intravenous infusion of ACTH was followed by changes in the ionic composition of allantoic fluid. Since allantoic fluid composition is largely influenced by foetal urine and since ACTH infusion causes adrenal hypertrophy (Liggins 1968; Nathanielsz et al, 1972) and an increase in the plasma levels of

corticosteroids (Liggins 1968; Liggins et al, 1973; Basset and Thorburn, 1969), it is probable that the corticosteroids alter the electrolyte concentration of foetal urine and hence, of allantoic fluid. However, the results obtained by Mellor and Slater (1974) may also indicate a corticosteroid influence on the ionic pumping mechanisms of the chorio-allantois (Mellor and Slater, 1972 b). To distinguish between these possibilities it would be necessary to directly assess the effect of ACTH treatment on the electrolyte concentration of foetal urine.

Secondly, since it appears that in dogs (Jackson et al, 1973) and rodents (Jost and Picon, 1970), the function of the foetal pituitary - adrenal axis is affected by that of the mother; the maternal endocrine system probably influences foetal kidney function. This possibility is strongly supported by the work of Alexander and Williams (1968) who found that when pregnancy is maintained in ovariectomised sheep, by administering progesterone, there is an excessive accumulation of allantoic fluid. If, however, a small amount of oestrogen is given in addition to the progesterone, this accumulation of fluid is prevented. The $[Na^+]$ of allantoic fluid also increased when progesterone was administered alone, but not when oestrogen was included. Thus, it appears that the ability of the foetal kidney to retain water and Na^+ is influenced by maternal levels of oestrogen and progesterone. Further, this effect is probably mediated by the foetal adrenals, since Eguchi (1962) has found that in pregnant ovariectomised rats, the foetal adrenals will atrophy unless oestrogen is administered in combination with progesterone.

It can be seen that the question of hormonal influence on the foetal kidney is potentially complex, with the probable involvement of both the maternal and foetal endocrine systems. The nature of these interactions and their effects on foetal kidney function are poorly understood and to discuss all of the possible influences would require

almost a complete review of the endocrinology of pregnancy. However, to provide a context for the possible interpretation of gestational trends in renal function, mention will be made of those hormones which show obvious changes in secretion rate and plasma concentration, during gestation. The role of foetal vasopressin and the renin-angiotensin system will also be considered. Much of this information relates to sheep, since the endocrinology of pregnancy has been studied intensively in that species.

In the pregnant ewe, the concentration of progesterone in the peripheral plasma reaches its highest level during the last 2 - 3 weeks of gestation and decreases before delivery (Bassett et al, 1969; Fylling, 1970). In contrast the level of unconjugated oestogens reaches a peak on the day of parturition (Challis, 1971; Challis et al, 1974). The major oestrogens in maternal plasma are oestrone, oestradiol-17β and oestradiol-17α in a ratio of approximately 2:1:1 (Thorburn et al, 1972).

In the foetus the hormonal patterns are quite different to those in the mother. Foetal oestrogens, formed in the placenta and present mainly in the form of sulphoconjugate oestrogens, reach a peak about 25 days before parturition (Findlay, 1970). Foetal cortisol levels show even more dramatic changes as gestation proceeds. These changes reflect the progressive maturation of the pituitary - adrenal system which has major significance with respect to the initiation of parturition and which may also effect renal function. During the last 7-10 days of gestation there is a gradual increase in the cortisol concentration of foetal plasma. Then, about 24 hours before parturition, there is a large and rapid rise in the cortisol level (Bassett and Thorburn, 1969; Comline et al, 1970). Analysis of the secretion and clearance rates of cortisol, suggests that the rise in plasma cortisol concentration is due to increased secretion of cortisol by the foetal adrenal (Liggins et al,

1973). This is confirmed by the fact that there is no simultaneous increase in the corticosteroid level of maternal plasma and the fact that the foetal levels exceed the maternal levels. Cortisol is not the only foetal corticosteroid to show a pre-parturient rise in concentration. Thomas et al (1976) have demonstrated that 11-deoxy cortisol increases in concentration during the last 8 days of pregnancy, although not as dramatically as cortisol. In contrast, the corticosterone concentration of foetal plasma decreased slightly in the last week of gestation.

The stimulus for the growth and increased secretory activity of the adrenal cortex is thought to be ACTH from the foetal pituitary. Although this is yet to be confirmed; hypophysectomy experiments support the proposal, as does the finding that ACTH is certainly present in the plasma of foetal sheep after the 130th day of gestation (Alexander et al, 1971). The lack of correlation between maternal and foetal ACTH levels indicates that there is little placental transfer of ACTH. With respect to the mechanism of ACTH stimulation, there is evidence that ACTH induces changes in the enzyme activity of the adrenal cortex and facilitates the increased biosynthesis of cortisol. Specifically, it is thought that ACTH stimulates the 11β hydroxylation of 11-deoxy-cortisol to cortisol. The simultaneous measurement of cortisol, corticosterone and 11-deoxy-cortisol in foetal plasma (Thomas et al, 1976) yielded concentration ratios which indicate that 17α and 11β-hydroxylases are largely inactive in the adrenals of foetal lambs before parturition. However, there does appear to be some increase in the activity of both enzymes during the 5 days prior to delivery. In contrast, Wintour et al, (1975) have shown that 17α and 11β hydroxylases are active in the adrenals of foetal sheep from as early as the 40th day of gestation. In the presence of ACTH, the adrenal of a 40 day-old foetus produced more cortisol per gm. of body weight than a term adrenal. Accordingly, an hypothesis other than increasing hydroxylase activity, has been proposed

to explain the pre-parturient rise in cortisol secretion. It is suggested that ACTH receptors within the foetal adrenals increase in number as gestation proceeds and, therefore, ACTH more effectively stimulates the adrenal cortex. Further, it is proposed that the increased cortisol level induces the operation of additional ACTH receptors resulting in further stimulation of the adrenals and greatly elevated cortisol levels.

A consideration of the activity of hormones from the foetal pituitary raises the question of the ability of the pituitary to produce vasopressin and of the influence of that hormone on foetal kidney function. Vasopressin has been isolated from the pituitaries of foetal sheep, guinea-pigs and seals (Vizsolyi and Perks, 1969) while Alexander et al (1971) have detected arginine vasopressin (AVP) in the plasma of sheep foetuses as early as the 107th day of gestation. Alexander and her associates also found that the basal levels of AVP (10-90μU/ml) were not correlated with foetal age but that there was a marked increase in AVP concentration in response to haemorrhage. Following haemorrhage, values of up to 1800μU/ml have been recorded in a 140 day-old sheep foetus.

Despite the fact that the presence of vasopressin in the foetus has now been established, there remains considerable debate on the responsiveness of the foetal kidney to this hormone. As the foetus produces dilute urine during intra-uterine life, it was thought that the foetal kidney is insensitive to the antidiuretic effect of vasopressin. In support of this idea, Alexander and Nixon (1961) reported that sheep foetuses do not respond to intra-muscular injections of vasopressin. Similarly, Ames (1953), found that human infants do not respond to vasopressin administered during the first three days of life. However, Vernier and Smith (1968) point out that failure to observe a decrease in urine flow rate or an increase in the osmolality of foetal urine, cannot be interpreted simply as unresponsiveness of the foetal kidney to

vasopressin. Vernier and Smith (1968) argue that other aspects of the renal concentrating mechanism must be considered, including the length of the loops of Henle and the existence of osmotic gradients within the kidney tissue. The involvement of the loops of Henle has previously been discussed, but the existence and influence of osmotic gradients within the kidney have not been considered.

It has been reliably established that in adult kidneys the counter-current mechanism proposed by Wirz (1961) is an integral part of the renal concentrating mechanism. The effectiveness of this mechanism depends upon the existence of osmotic gradients within the renal medulla. Therefore, information on the existence and magnitude of such gradients in the foetal kidney would be valuable in assessing whether a counter-current mechanism operates. One such study has been carried out by Stanier (1972), who found that a foetal lamb of 137 days gestational age had a steep intra-renal gradient for sodium. Unfortunately, these results are limited and no information about younger foetuses is available but it is likely that the gradient observed is the end-product of a gradual development during foetal life. However, none of what is currently known about intra-renal osmotic gradients or nephron anatomy provides much assistance in understanding the urine concentrating mechanism of the foetal kidney.

Much of the early work in which plasma levels of vasopressin were measured has been brought into question by Skowsky et al (1973), who examined the kinetics of AVP secretion in the foetus using radio-immunoassay measurements in a system where the high levels of vaso-pressinase were inactivated. They found, in contrast to the earlier findings, that mature monkey and sheep foetuses have a high rate of vaso-pressin production resulting in plasma levels significantly greater than in the adult. Skowsky et al (1973) offer no comment on the physiological significance of the high vasopressin levels in the foetus, but they

do reiterate Vernier and Smith's view, that the low osmolality of foetal urine is more probably the result of an inherent limitation in renal concentrating capacity than a lack of responsiveness to vasopressin.

Mellor and Slater (1973) have also disregarded the theory of foetal unresponsiveness to vasopressin. They have postulated that the foetus alters renal water retention by varying vasopressin secretion in response to changes of foetal blood volume and in older foetuses, to changes in plasma osmolality. The basis for this proposal was their observation that changes occurred in the osmolality of maternal plasma and foetal urine when ewes drank after feeding. They suggest that when the ewes drank, transplacental water exchange may have been altered, thereby altering foetal plasma volume and osmolality and stimulating changes in vasopressin secretion rate. Despite the logic of Mellor and Slater's argument, their evidence is indirect and other interpretations of their findings could be made. They provide no concrete evidence that vasopressin is involved in controlling water reabsorption in the foetal kidney and this remains a matter that requires further investigation.

Since the renin-angiotensin system (RAS) is an important mechanism in the maintenance of cardiovascular and electrolyte homeostasis in adults it is logical to consider its role during foetal life. Many of the physiological prerequisites for a functional RAS are present quite early in the foetal life of a sheep. Baroreceptor reflexes in foetal lambs are developed as early as 90 days gestation, since the acute bradycardia caused by the injection of adrenalin and nor-adrenalin is abolished by cutting the vagii (Dawes, et al 1956), and haemorrhage causes sustained bradycardia (Dawes and Mott, 1964). Also there is an increased involvement of the autonomic nervous system in the control of circulation in sheep foetuses near term (Born et al, 1956).

The major requirement for a functional RAS in foetal life, is of course, the ability of the foetal kidneys to produce renin. There is

little doubt that this is so. Granulated juxta-glomerular cells have been found in foetal pigs (Bing and Kazimierczak, 1963) foetal sheep (Smith et al 1974), human foetuses (Ljungqvist and Wagermark, 1966), newborn puppies (Granger et al, 1971) and perinatal rats (Eguchi et al, 1975). Also, Bing and Kazimierczak (1963) have demonstrated that kidney extracts from pig foetuses have a pressor activity probably due to the presence of renin. Similarly, Mott (1973), has reported a pressor response when extracts of foetal sheep kidney are injected into nephrectomised foetal lambs.

reduced maternal:foetal PRA ratio from in excess of 1, to as low as 0.03. In such circumstances, the maternal PRA was increased by as much as 335%, during furosemide treatment and no effect on foetal PRA was observed (Oakes, Catt and Chez, 1975). This is consistent with information obtained from an anephric human foetus at term (Symonds and Furler, 1973). In cord blood samples from this foetus there was no detectable plasma renin activity (PRA) and plasma renin concentration (PRC) was less than 10% of normal cord blood values. Not only does this indicate that the foetal kidney produces renin but it also implies that physiologically active renin does not cross the placenta.

Although it is well established that renal renin is present in foetal animals, the time in foetal life when renin secretion begins is unknown and the function of foetal renin has not been fully established. Mott (1973), has pointed out that the presence of renal renin, although necessary for the functioning of the RAS, does not necessarily imply that function. However, in the foetal lamb steps have been taken toward elucidating the function of the RAS.

With respect to the involvement of the RAS in cardiovascular activity, an arterial pressor response to angiotensin and renin has been established. Behrman and Kittinger (1968) infused high doses (0.5-2.5µg) of angiotensin I into the femoral vein of foetal monkeys and induced large

increases in mean arterial pressure (MAP). These workers reported similar but less dramatic effects using lower doses of angiotensin I (0.1µg/Kg). In the same study Behrman and Kittinger (1968) failed to induce any change of foetal blood pressure with an injection of 1.2µg of angiotensin I into the umbilical vein of a sheep foetus. This led to the suggestion that angiotensin injected by this route is largely inactivated during passage through the foetal liver.

Broughton - Pipkin et al (1974a), have found that foetal sheep in the last quarter of gestation, show an increased angiotensin II like activity in arterial blood following a 25% reduction of feto-placental blood volume. The levels reached under these circumstances were 0.2-1.3ng/ml as measured by bioassay. Comparable changes in angiotensin II like activity in the arterial blood of neonatal sheep, following similar treatment, had previously been observed (Broughton - Pipkin et al, 1971). Smaller reductions in blood volume (3%) have also been shown to increase PRA in foetal sheep (Broughton - Pipkin et al 1974). Thus it appears that in sheep, the mature foetus and neonate, possess converting enzyme and renin substrate in addition to renal renin. Also they are capable of responding to reduced extra-cellular fluid volume, by increasing PRA and increasing angiotensin II activation. Similarly Hyman et al (1975) have shown that the constriction of one renal artery in a foetal lamb induces marked arterial hypertension and Smith et al (1974) have shown that aortic constriction increases foetal PRA. Apparently the RAS is stimulated as a result of the reduced renal blood flow.

The ability of the RAS to function as a Na^+ conserving mechanism in the foetus has not been established. Trimper and Lumbers (1972) treated foetal sheep with furosemide which is known to be a potent natriuretic and to stimulate renin release in adult animals (Vander and Carlson, 1969). In 4 of the 5 foetuses treated, the PRC of arterial blood increased significantly over a period of 90 minutes. The youngest foetus to show this response was 110 days old. On the basis of these findings plus measurements of the relative increase in PRC induced by

similar doses of furosemide administered to non-pregnant ewes; Trimper and Lumbers (1972) concluded that the foetal kidney shows a greater response to furosemide than the adult kidney. This led them to further propose that the RAS has an important role in the maintenance of foetal circulatory homeostasis, possibly to compensate for the incomplete development of the nervous control of the circulation. Since these were acute studies, with the ewe under anaesthesia and the foetal head exteriorised, foetal stress was no doubt considerable. Under these circumstances renin secretion was probably increased due to stress as much as to the furosemide treatment.

Thus the basis for Trimper and Lumbers' proposals is tenuous. Furthermore, the natriuretic effect of furosemide in the foetus could not be confirmed in these experiments. There is no evidence that furosemide caused any increase in the urinary excretion of Na^+ nor is there any evidence that the $[Na^+]$ of foetal plasma was significantly changed. As a result, these experiments provide little information on the involvement of the RAS in the Na^+ conserving ability of the foetus.

In a recent study, Fleischman et al (1975) used 15 foetal sheep with chronic intra-vascular catheters to measure PRA in the foetus and ewe throughout gestation. They found that maternal PRA increased from base levels during the last third of gestation and remained elevated for 12 weeks after delivery. Foetal PRA levels were variable, but generally greater than maternal levels. This latter finding provides more substantial support for the proposals advanced by Trimper and Lumbers than does their own work. Fleischman et al (1975) also tested the response of foetuses to intra-venous injections of furosemide and found that the response was apparently dependent on the basal PRA. If the initial PRA was low, furosemide usually stimulated a significant increase. In contrast, foetuses with high initial PRA did not respond;

probably because renin secretion was near maximum prior to treatment. Although this work represents a more physiologically valid examination of the effect of natriuretic agents on the RAS of foetal sheep; again the natriuretic effect of furosemide in the foetus is unsubstantiated and no direct evidence regarding Na^+ homeostasis in the foetus is provided. Experiments of a similar nature were conducted as part of the present work, before the work of Fleischman et al was published. However, in the present work, not only was PRA measured in chronically catheterised foetuses, but the composition of simultaneously collected urine samples was analysed.

Despite the fact that the RAS appears to be functional in foetal animals, its role in Na^+ homeostasis is obscure. More specifically, it is not known whether there is any relationship between angiotensin II activation and aldosterone secretion. Nor, in fact, whether the foetal kidney responds to aldosterone in the same way as the adult kidney. It has been established that the adrenal cortex of foetal lambs secrete aldosterone, although the relationship between the changes that occur in the adrenal cortex of the sheep foetus near term and the secretion of aldosterone are confused. Wintour et al (1975) measured the peripheral blood levels of aldosterone in foetal lambs from 60 days of gestation until term. They found that the levels were significantly lower in 90 - 120 day-old foetuses than in younger or older ones and that ACTH was a potent stimulus to aldosterone secretion in even the youngest foetuses. Despite these findings, the responsiveness of the foetal kidney to endogenous aldosterone is unclear and consequently its importance in electrolyte homeostasis is unassessed.

The ability, if any, of progestins to influence foetal kidney function can only be speculated upon. Although there is no evidence that progesterone or any of its metabolites influence foetal kidney function, it is not inconceivable that such an influence exists. It could

be that progesterone influences foetal kidney function indirectly, by acting as a precursor for the production of an adrenal hormone with electrolyte conserving activity. Alternatively, progesterone or its metabolites could influence Na^+ metabolism in the foetus by acting as a mineralocorticoid antagonist. Visser et al (1964) have studied the effect of 17α-hydroxy-progesterone in a small number of newborn infants and have concluded that it acts as an aldosterone antagonist causing salt loss. A similar or modified effect could occur in foetal life.

Similarly, there is no evidence that oestrogens influence foetal kidney function. Nevertheless, since the RAS appears to be active in foetal life, and since oestrogens are known to raise the level of renin substrate in adults (Helmer and Griffith, 1952) it may be that unconjugated foetal oestrogens indirectly influence foetal kidney function via the RAS.

2.6 Thesis format

Since the methods used in most of the investigations reported in this thesis are basically similar, a single Materials and Methods section has been included in which the procedures used for establishing and maintaining the foetal preparation are described along with the sampling procedures and the laboratory methods. Some laboratory methods were used only in specific investigations but it was felt that all methods should be described in a common section of the report. During some experiments minor variations in sampling procedures were necessary and these variations are detailed in the Results sections as part of the description of the protocol of the individual experiments.

The Results sections fully document the data obtained in all areas of the present investigation. However because of the limited amount of work that has been carried out in this field, a need was felt to emphasise the relationship between the present information and information previously reported. In an endeavour to provide such a context the results section includes not only the results obtained but also limited comment on the comparison of that data with other comparable results.

Finally in the Discussion section an attempt is made to integrate the findings reported in this thesis. The experimental data is discussed and interpreted and the information obtained is used in an attempt to understand the function of the foetal nephron and to explain the observed gestational variations in foetal kidney output. The reported homeostatic capacity of the foetal kidney is also considered in the light of information gleaned from the foetal experiments.

3. *MATERIALS AND METHODS*

3.1 Animals

3.1.1 Supply

The sheep used in this research were Merino crossbred ewes which ranged in age from 5-7 years. They were supplied by the Mortlock Experimental Station (Mintaro, South Australia), and had been mated to rams fitted with a harness and crayon (sire-sine) so that the date of service could be recorded. If a ewe was serviced more than once, she was assumed to have come back into oestrus and the day of gestation was calculated from the date of the last service.

3.1.2 Housing

At about the 90th day of gestation the sheep were brough to Adelaide and housed in the Queen Elizabeth Hospital Animal House. They were kept in a constant temperature (25^{o}C) environment, and subjected to a 12 hour light-dark cycle. For 2 or 3 days prior to surgery, the sheep were kept in metabolism cages to prepare them for similar confinement after surgery. Animals being used in investigations were fed 900g of lucerne chaff per day and the feeding time was unrestricted. The ewes were also allowed free access to water and the average intake was 4.5L/day (n=13). Individual measurements of water intake were not related to stage of pregnancy.

3.2 Pre-Operative procedures

3.2.1 Pregnancy confirmation

In animals selected for surgery, pregnancy was confirmed by palpating the abdomen. In doubtful cases, an ultrasonic Dopper was used to detect foetal heart beat, or the plasma progesterone level of the ewe was assayed.

3.2.2 Pre-Anaesthesia

The pregnant ewe was starved for 24 hours prior to surgery to minimise gaseous distension of the rumen and sedated with a 0.2mg/Kg intra-muscular dose of Xylazine (Rompun, Bayer). Xylazine produces

depression of the central nervous system ranging from mild sedation to deep basal narcosis. It was a particularly useful drug, not only for pre-medication, but also as a supplement to the analgesia induced with an epidural injection of lignocaine hydrochloride (Xylocaine). There is no contra-indication in sheep to the combination of Xylazine and lignocaine hydrochloride.

3.2.3 Lumbar Epidural Analgesia

An epidural nerve block provided excellent analgesia for abdominal surgery. A lumbosacral injection was made at a site immediately behind the spinous process of the last lumbar vertebra. The area around this site was shorn, disinfected and infiltrated with 2ml of 2% lignocaine. A sterile needle (25G 1½"), was inserted through the lumbo-sacral space to a depth of about 2-3cm. When the needle had penetrated the ligamentum flavum, a loss of resistance was felt and 10ml of 1.5% lignocaine with adrenalin (adrenalin 1:100,000) was slowly infused. In earlier work, up to 15ml of 2% lignocaine was used, but this occasionally caused irritation of the meninges, as noted at post-mortem, and increased the risk of foetal death (Morishima et al, 1972). The lower dose avoided these problems, yet provided satisfactory analgesia for about two hours.

Fig. 1 shows that the maternal dose of lignocaine and xylazine may effect the composition of foetal urine. This is of little consequence since all experiments and normal collections were not commenced until several days after surgery.

3.3 Surgical Procedures

Once the infusion of lignocaine was finished, the sheep was placed on its back in a wooden cradle to aid the dispersal of the lignocaine solution and to ensure bilateral analgesia. In this position preparation of the abdomen could proceed.

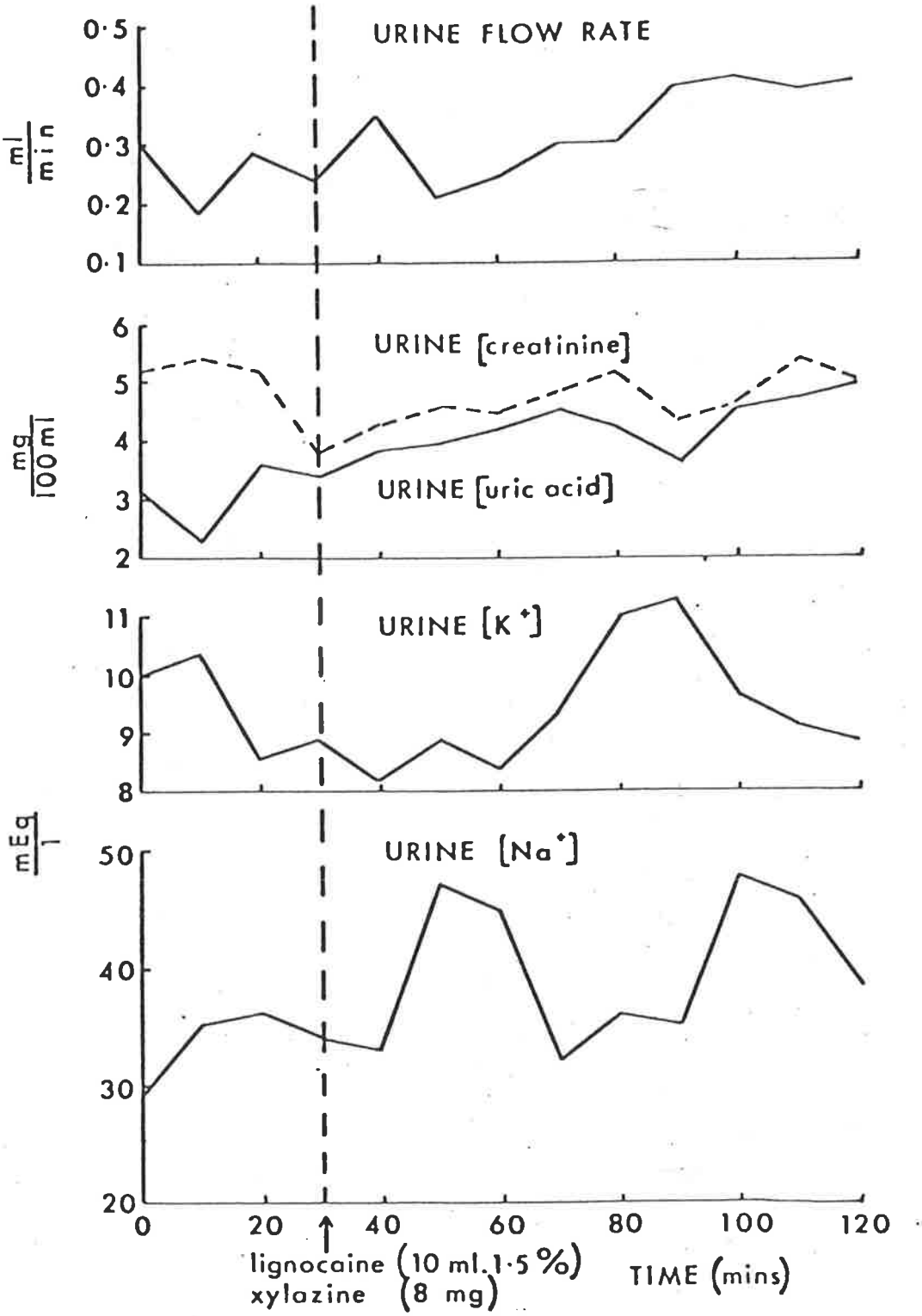


FIGURE 1. Changes in the flow rate and composition of foetal urine following the administration of lignocaine and xylocaine to the ewe. (See text page 44)

3.3.1 Preparation of the Surgical Field

The entire abdomen of the sheep was closely shorn and the following steps were employed in the preparation of the surgical field.

(i) The shorn abdomen was washed with Sapoderm surgical soap and a depilatory cream applied. The cream was removed after about 5 minutes and the abdomen washed again.

(ii) The skin was rinsed with a solution of chlorohexidine and cetrimide in alcohol and after a few minutes, dried, using sterile abdominal sponges. This step was repeated twice.

(iii) The surgical field was sprayed with antibiotic powder (Polybactrin, Parke Davis).

Finally, the sheep was transferred to the surgery and draped as quickly as possible to prevent re-contamination of the surgical site. A plastic drape with an adhesive backing (Steridrape, Ethnor) was placed over the incision site and covered with linen drapes.

Proper preparation of the surgical field was vital, as swabs taken from the skin of incoming sheep revealed a mixed population of aerobic spore bearing bacilli, coliform bacteria, pseudomonas aeruginosa and, invariably, streptococcus faecalis. Foetal organs and fluids, sampled during the post-mortem examination of unsuccessful preparations were often contaminated with these bacteria. However, by adhering to a strict format of skin preparation and sterile technique, the incidence of foetal contamination was reduced. The development of a good sterile technique, plus the gradual refinement of the surgical procedure, resulted in 75% of all post-operative animals surviving beyond 3 weeks.

3.3.2 Exposing the foetus

The maternal abdomen was opened with a mid-ventral incision. The underlying muscles were parted by blunt dissection and the peritoneal membrane cut. At this point, a wound protector (Parke-Davis) was

into the incision. The wound protector has a flexible plastic ring which was pushed into the ewes' peritoneal cavity through the incision. The plastic drapes attached to the ring were then spread to allow access to the gravid uterus and to form a barrier between the edges of the abdominal incision and the uterus. This further reduced the danger of contaminating the foetus with pathogenic bacteria from the surrounding skin. To reinforce this innovation, all materials, including gloves, swabs and instruments which had been in contact with the maternal skin, were discarded.

Once exposed, the gravid uterus was positioned at the abdominal opening and palpated to locate a foetal hind limb. The foetus was then re-orientated to bring the hind limb into apposition with the area of the uterus selected for incision. Care was taken when opening the uterus to avoid areas of placental attachment and large inter-placental blood vessels.

Next, the chorioallantois and amnion were punctured and Allis clamps positioned to hold the cut edges of both the uterine wall and the membranes. The edges of the incision could then be raised to minimise the loss of foetal fluid.

By grasping its hind limbs, the foetus was withdrawn from the uterus to a point midway between the peritoneum and the umbilicus and was held in this position by clamping its skin to the uterine incision. Exposing the foetus in this way minimised amniotic fluid loss and limited disturbance to the umbilical blood flow (see Plate 1).

3.3.3 Cannulating the foetus

One hind leg was then selected for cannulation, and the remaining exposed areas of the foetus wrapped in an abdominal sponge which had been soaked in warm isotonic saline. By keeping the foetus warm in this way, the drop in deep body temperature could be limited to less than $1^{o}C$.

A pulse was located on the inner surface of the hind leg to determine the correct site for exposing the femoral artery and femoral vein. The skin overlying this site was cut and the muscle junction immediately below was parted by blunt dissection to isolate the femoral artery and vein. These vessels were cleared of surrounding tissue and cannulated (see Plate 2).

The polyvinyl (PV) cannulae used were 2.0mm and 1.4mm outside diameter (OD) for the artery and vein respectively and were pre-sterilised with ethylene oxide gas. Linen sutures (size 2/0) were used to tie the cannulae in place and the foetal skin was closed with 2/0 gut.

To cannulate the bladder, the foetus was held with its ventral surface upward and its back slightly arched. In this position the bladder was raised into contact with the ventral surface of the foetal abdomen.

The bladder was then exposed through a 2cm incision made near the mid-line in the posterior third of the abdomen and a single purse-string suture was laid through the bladder wall using 3/0 silk on an atraumatic needle. Care was taken to avoid the small blood vessels in the bladder wall and the umbilical arteries which are apposed to the lateral surfaces of the bladder. A small incision was made within the suture ring and a cannulae pushed through this incision into the bladder. The cannula was secured by closing and tying the purse-string suture.

The cannula used in the bladder was specially designed. It consisted of PV tubing (3.0mm OD) and had a 3cm tip separated from the rest of the tube by a small silastic cuff. The tip was rounded to avoid damage to the interior of the bladder and had a number of holes along its length to facilitate urine drainage (see Plate 3). When cannulating the bladder, the silastic cuff was pushed through the incision and

included in the purse-string suture. This prevented the catheter pulling out and helped seal the bladder incision. To close the abdominal incision in the foetus, the muscles and skin were sutured independently using 2/0 gut. (See Plates 3 and 4).

While the foetus was still exposed, a fourth PV cannula (3.0mm OD) was stitched to the skin of the unoperated hind limb. This cannula was included to enable the urine draining from the bladder to be re-circulated into the amniotic cavity.

3.3.4 Replacing the Foetus

Once the four cannulae were in place, the clamps holding the foetus were removed and it was eased back into the uterus. Particular care was taken to avoid tearing the foetal membranes or the uterine wall. About 40cm. of each cannula was fed into the uterus to allow for foetal movement. Then the edges of the uterine incision and the foetal membranes were gathered together using Allis clamps and closed with a continuous line of chromic 2/0 sutures. This was oversewn with interrupted sutures of chromic 0 to ensure an effective seal. Both the muscle layer of the maternal abdomen and the peritoneal membrane were closed in a single suture line which was oversewn with interrupted sutures for additional support (chromic 0). The skin was closed with continuous sutures of 2/0 gut. (See Plate 5).

All cannulae were brought out through the laparotomy incision and sewn to the skin on the ewe's flank. Precautions were taken during exteriorization of the catheters to ensure an unobstructed flow.

3.3.5 Cannulating the ewe

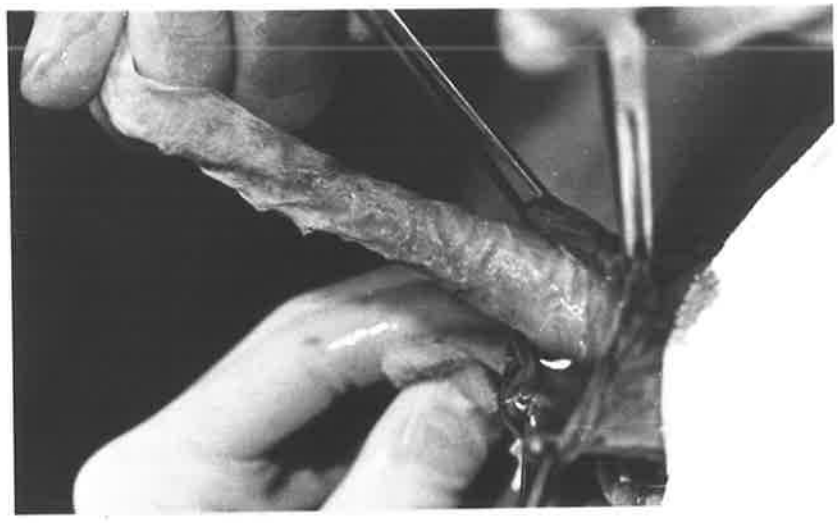
For continuous sampling of maternal blood, or for infusion into the ewe, one or both of the external jugular veins was cannulated using a PV cannula (1.4mm OD), passed into the vein through a 16-gauge needle. If

PLATE 1.

A foetus partially withdrawn through a uterine incision and secured in position with Allis clamps.

The intact amnion exposed through incisions in the uterus and chorio-allantois.

The tail of the foetal lamb withdrawn through an incision in the amniotic sac. The cut edges of the uterus and foetal membranes are secured in Allis clamps.



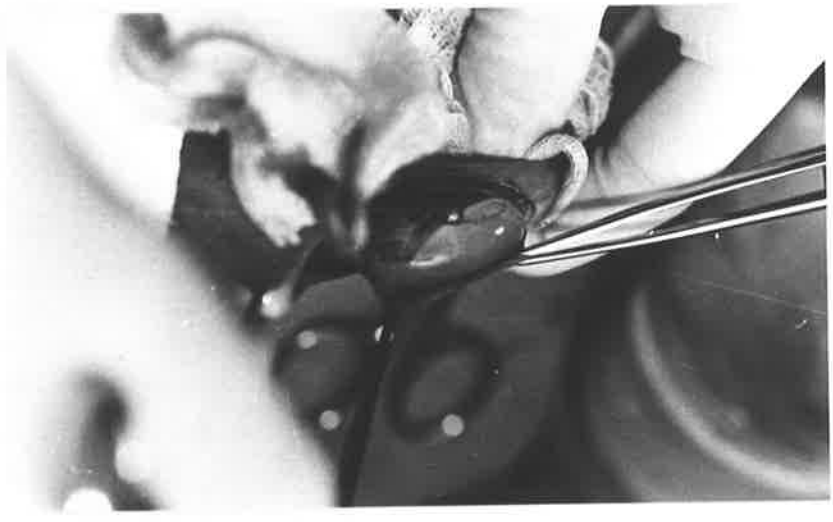
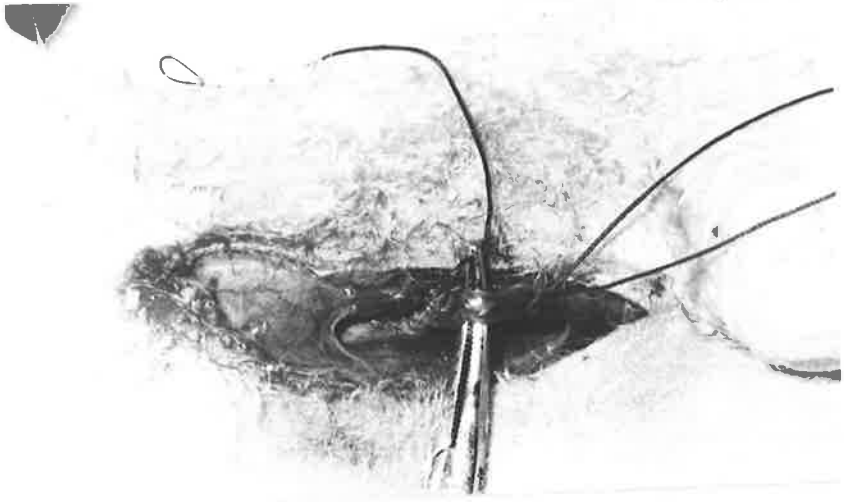


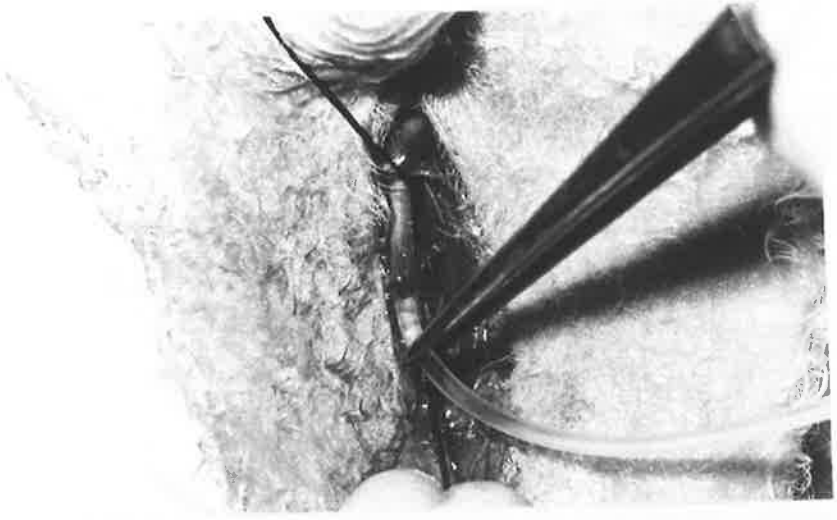
PLATE 2.

An incision in the hind-limb of the foetus exposing the femoral artery. Cotton sutures are being placed around the artery in preparation for cannulation.

A cannula being introduced into the femoral artery of the foetal lamb.

The femoral artery after cannulation and the femoral vein exposed in preparation for placement of the venous cannula.





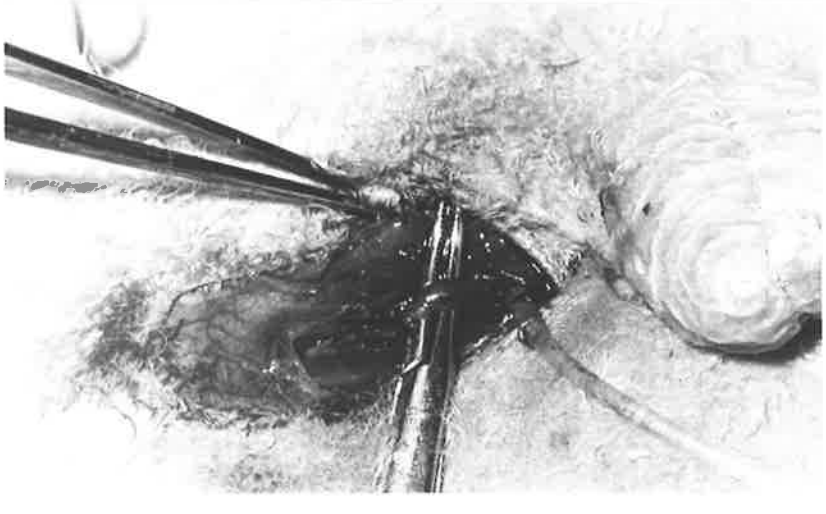
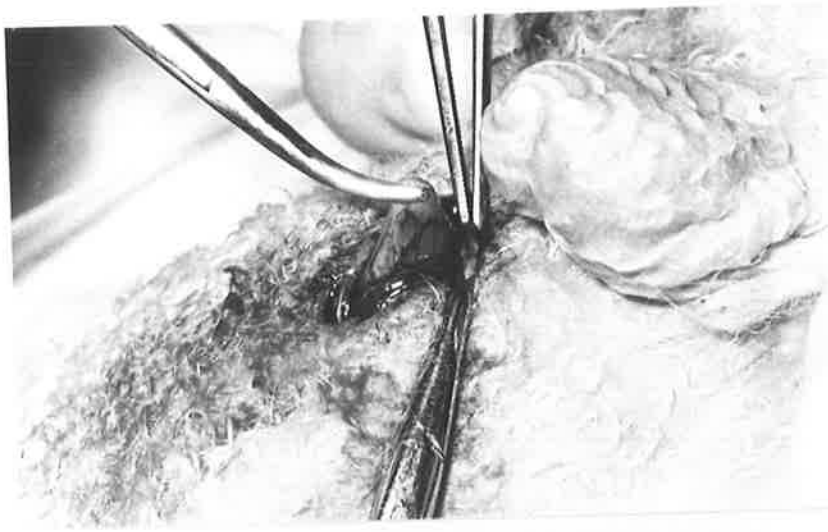


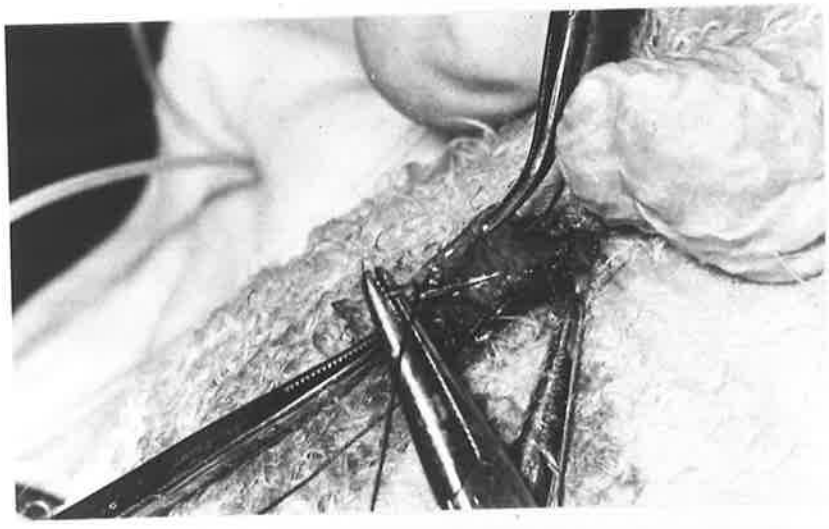
PLATE 3.

A section of the bladder wall of the foetus exposed through an incision in the foetal abdomen.

A purse-string suture being placed in the wall of the foetal bladder.

The tip of the bladder cannula. Note the rounded end, lateral drainage holes and silastic cuff. The entire tip is placed within the foetal bladder.





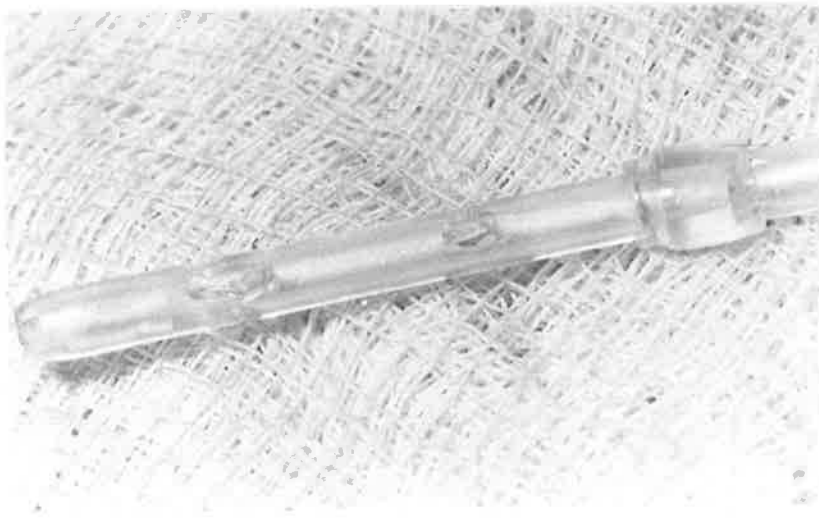
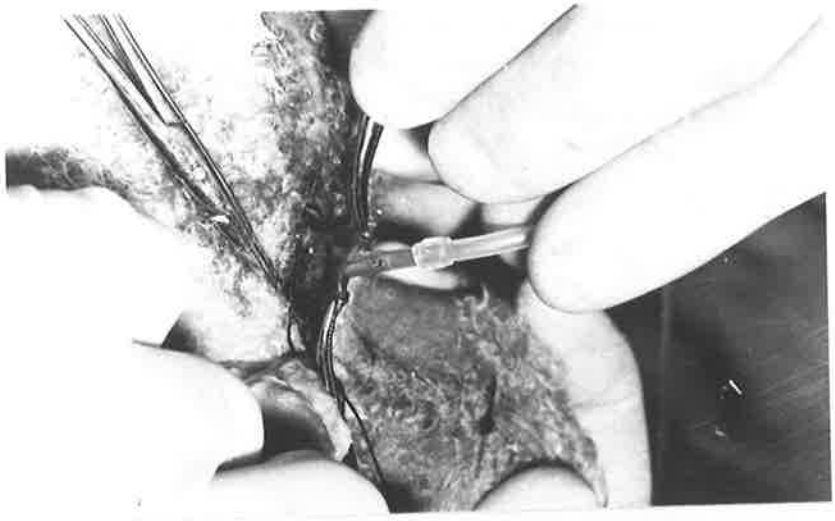


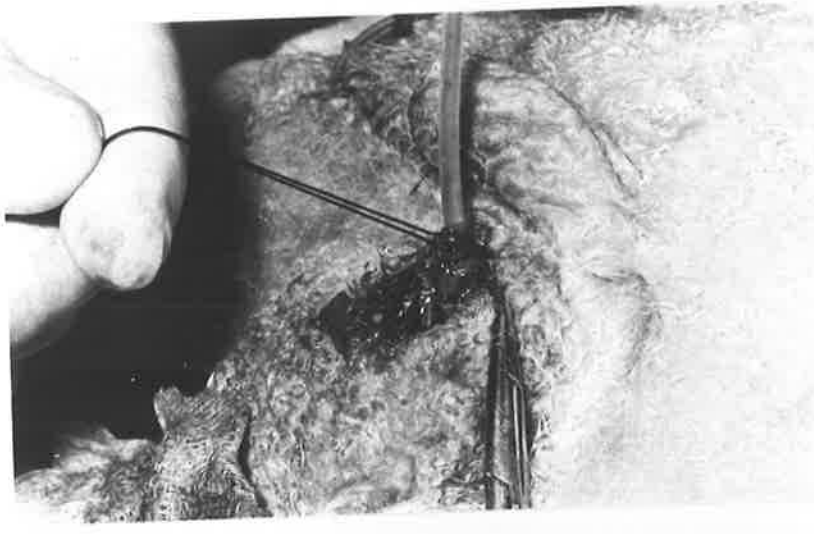
PLATE 4.

The bladder cannula being introduced into the foetal bladder through an incision in the wall. The incision is within the purse-string suture.

The bladder cannula secured in position by the purse-string suture.

An incision in the foetal hind-limb prior to suturing.





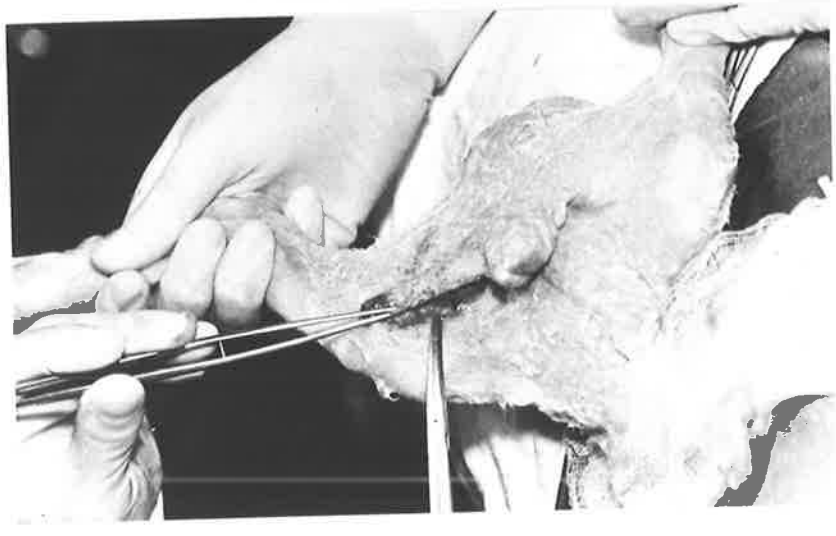
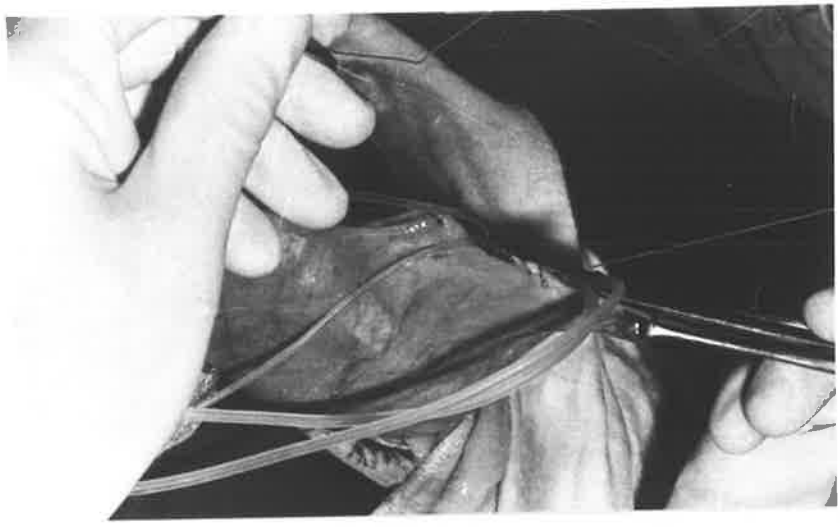


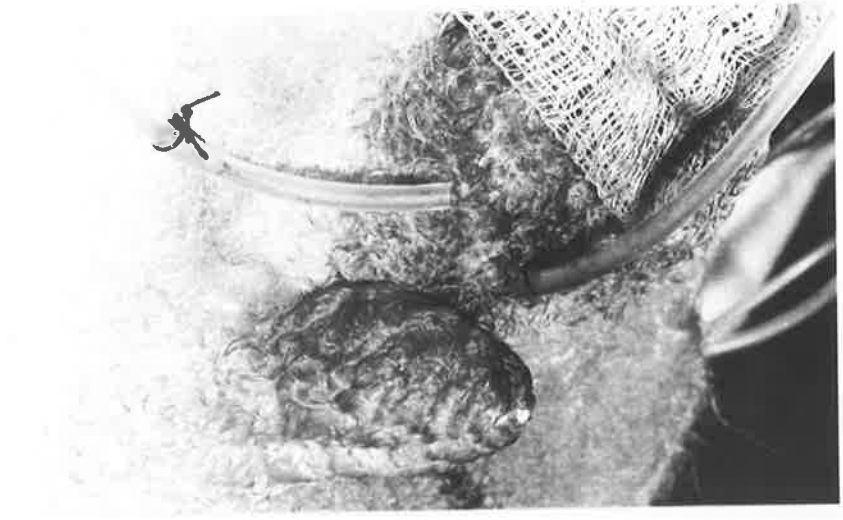
PLATE 5.

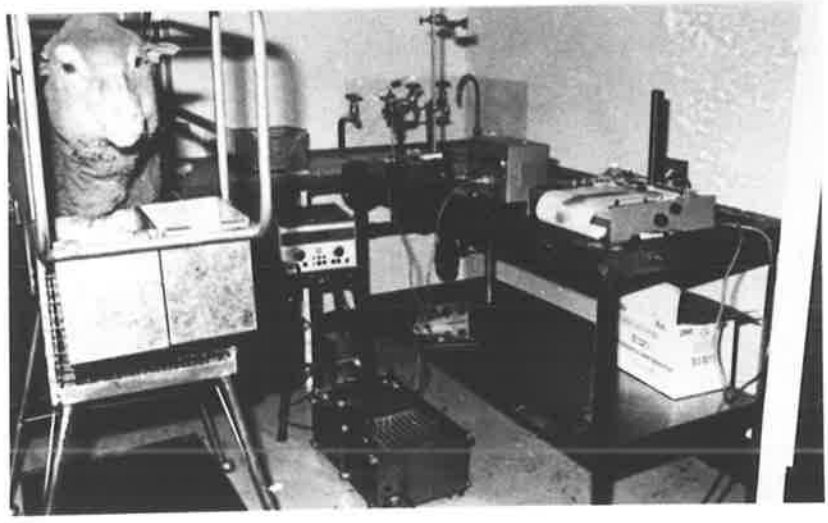
The uterus being sutured. Note the cannula passing through the uterine incision.

The foetus prior to replacement in the uterus. The bladder catheter is shown after closure of the abdominal incision and the cannula for recirculation of foetal urine is shown sutured to the unoperated hind-limb.

A ewe confined in a metabolism cage prior to an experiment. The equipment used in the experiments, including a fraction collector, chart recorder and electronic counter are shown.







urine was to be sampled, the maternal bladder was cannulated using a Foley FG14 catheter (Folatex).

3.4 Post-Operative Management

All post-operative animals were kept in metabolism cages. Despite the inability of sheep to exercise normally in metabolism cages, this confinement was the only satisfactory means of preventing the sheep from dislodging the indwelling catheters. Sheep which were confined for long periods were occasionally removed from their cages and allowed to exercise. During exercise, the indwelling catheters were put into a canvas bag strapped around the ewe's abdomen.

All post-operative sheep were fed 1 kgm. of lucerne chaff per day and allowed free access to water. Some animals did not regain their appetite immediately after surgery, and were given B complex vitamins (Hoechst) by intra-muscular injection until they resumed eating.

3.4.1 Urine Recirculation

Approximately 50cm of each indwelling catheter was left outside of the ewe. The cannula into the amniotic sac was connected to the cannula from the foetal bladder via a stainless steel stop-cock. This enabled the urine draining from the bladder to pass into the amniotic fluid. When a urine sample was required, the stop-cock was adjusted to interrupt this recirculation and allow the urine to drain through a side arm into a collecting tube. When not in use, openings in the stop-cock were sealed.

A similar technique for the recirculation of foetal urine into the amniotic sac was described by Buddingh et al (1969). Buddingh and his co-workers claimed that recirculation of urine was essential for the survival of the foetus during the last trimester of pregnancy (Buddingh et al, 1969, 1971). However, Gresham et al (1972), had two

foetuses in which the urinary output was drained for 7 and 14 days, and apparently normal lambs were delivered by caesarian section on the 148th day of gestation. They did note a virtual absence of amniotic and allantoic fluid in these preparations.

In the present study, the catheter into the amniotic sac was occasionally dislodged, preventing the recirculation of urine. In these cases, urine flow rates and composition were comparable with preparations in which urine was recirculated. On one occasion, urine was drained from a foetus for 22 days, and a lamb weighing 4.6kg was delivered vaginally on the 150th day of gestation. All cannulae were cut during the delivery of this foetus and samples were obtained from the lamb until 10 days after birth. During the 22 days of urine drainage, the foetus lost a total of 12.7 litres of urine. Table 2 shows estimates of the daily and total losses of Na^+, K^+, creatinine and uric acid during the drainage period.

The loss of large quantities of substances, which under normal circumstances are recirculated back to the mother and foetus via the amniotic and allantoic sacs, may have subtle effects which impair the reliability of such preparations as experimental models. Therefore, recirculation of urine is desirable, but the assertion of Buddingh et al (1969 and 1971), that recirculation is essential for foetal survival is refuted.

3.4.2 Capping Intravascular Cannulae

Cannulae from both the foetus and the ewe were fitted with blunted hypodermic needles and sealed with metal plugs. The fittings were pre-sterilized and the cannula ends with their attached fittings were kept immersed in sterilizing solution.

3.4.3 Antibiotic Treatment

Immediately after all operations, samples of foetal blood,

TABLE 2: SOLUTE LOSSES DURING CONTINUOUS DRAINAGE OF FOETAL URINE

GESTATIONAL AGE (DAYS)	DAILY URINE VOLUME (mls)	DAILY Na^+ EXCRETION (mEq)	DAILY K^+ EXCRETION (mEq)	DAILY URIC ACID EXCRETION (mg)	DAILY CREATININE EXCRETION (mg)
128	331	7.6	2.6	4.0	41.0
129	820	11.1	7.4	7.4	58.2
130	696	7.3	3.5	3.8	17.4
131	768	13.1	10.0	10.6	50.7
132	931	15.4	5.6	6.5	16.8
133	364	7.1	4.0	3.6	23.7
134	244	2.4	2.0	1.7	17.8
135	320	4.9	4.2	2.6	13.5
136	800	14.4	8.8	6.4	36.0
137	750	12.4	6.8	4.5	31.5
138	550	7.9	5.5	4.4	23.7
139	815	14.7	4.9	5.7	35.9
140	840	7.1	8.4	6.7	36.1
141	580	10.2	2.9	5.8	53.4
142	1040	19.8	5.2	7.3	46.8
143	810	10.1	4.1	4.9	33.2
144	650	8.1	3.9	3.9	17.6
145	105	1.6	0.7	1.8	14.8
146	225	4.5	2.0	3.6	20.7
147	520	4.4	3.1	5.2	24.4
148	168	4.3	3.9	6.4	36.3
149	192	4.9	7.8	20.7	115.2
TOTALS	12719	193.3	107.3	126.6	764.7

(See text page 50)

urine and amniotic fluid were collected for culture and bacteriological examination. Also, immediately after surgery, the foetus was given 20 mg/Kgm. of Cephaloridine (Glaxo) and 5 mg/Kgm. of Gentamicin (Schering) via the venous catheter. (The weight of the foetus was estimated from an age - weight nomogram; see Fig. 2). The ewe was given an intramuscular injection of 200 mg. of Caphaloridine and 50 mg. of Gentamicin.

If the cultures of the blood and foetal fluids, collected immediately after surgery, showed contamination, antibiotic treatment was repeated on the 3rd day after surgery and further samples were taken for culture. This entire procedure was repeated every 3 days until no pathogenic organisms could be detected. Although results obtained from foetuses being treated with antibiotics showed no disruption of urine composition that could be attributed to the antibiotics, (see Fig. 3), no foetus was used in experiments while an infection persisted and antibiotics were being used.

3.4.4 Maintaining Cannula Patency

Because of the continuous flow of urine through the bladder catheter, it rarely became blocked. However, when partial or complete blockage did occur, it was usually because the drainage holes were covered by foetal tissue. This could be corrected by flushing a small volume of saline into the bladder and the saline introduced was cleared in a few minutes, depending on the rate of urine production. A similar technique was used to clear blockage of the amniotic cannula.

Intra-vascular catheters proved to be more difficult to keep patent. All vascular cannulae were flushed daily with saline containing 50 U/ml of heparin. The concentration of heparin was kept to a minimum to offset any effects of heparin on sodium metabolism (Bailey and Ford, 1969). The lumen volume of each catheter was known, so the volume of saline flushed into the foetus could be regulated. This volume never

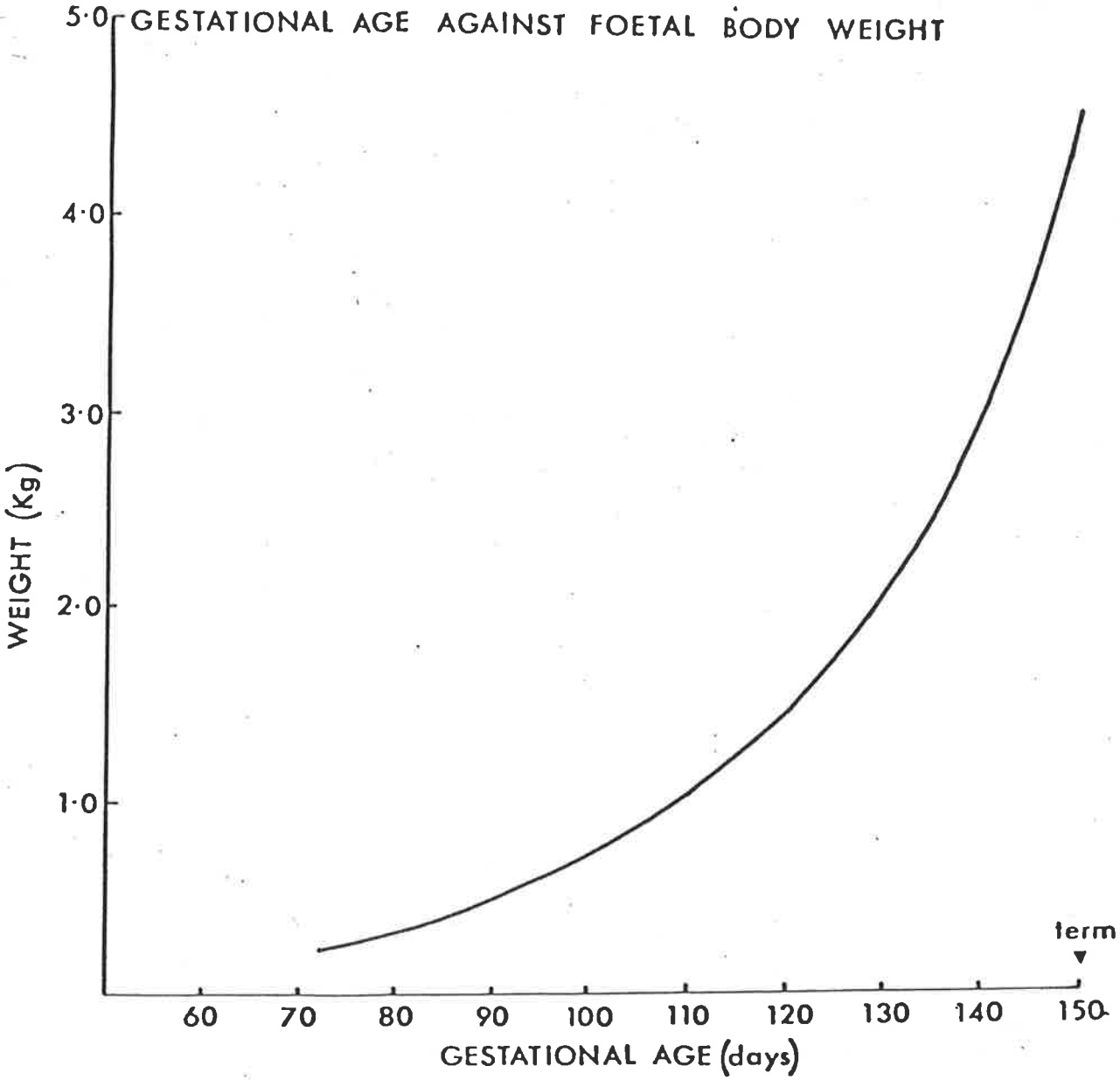


FIGURE 2. The relationship between gestational age and foetal weight in Merino crossbred sheep. (See text page 51)

[The relationship has been established by the author and other workers in this laboratory (Seamark R.F., and Kennaway D.) using foetuses of known gestational age]

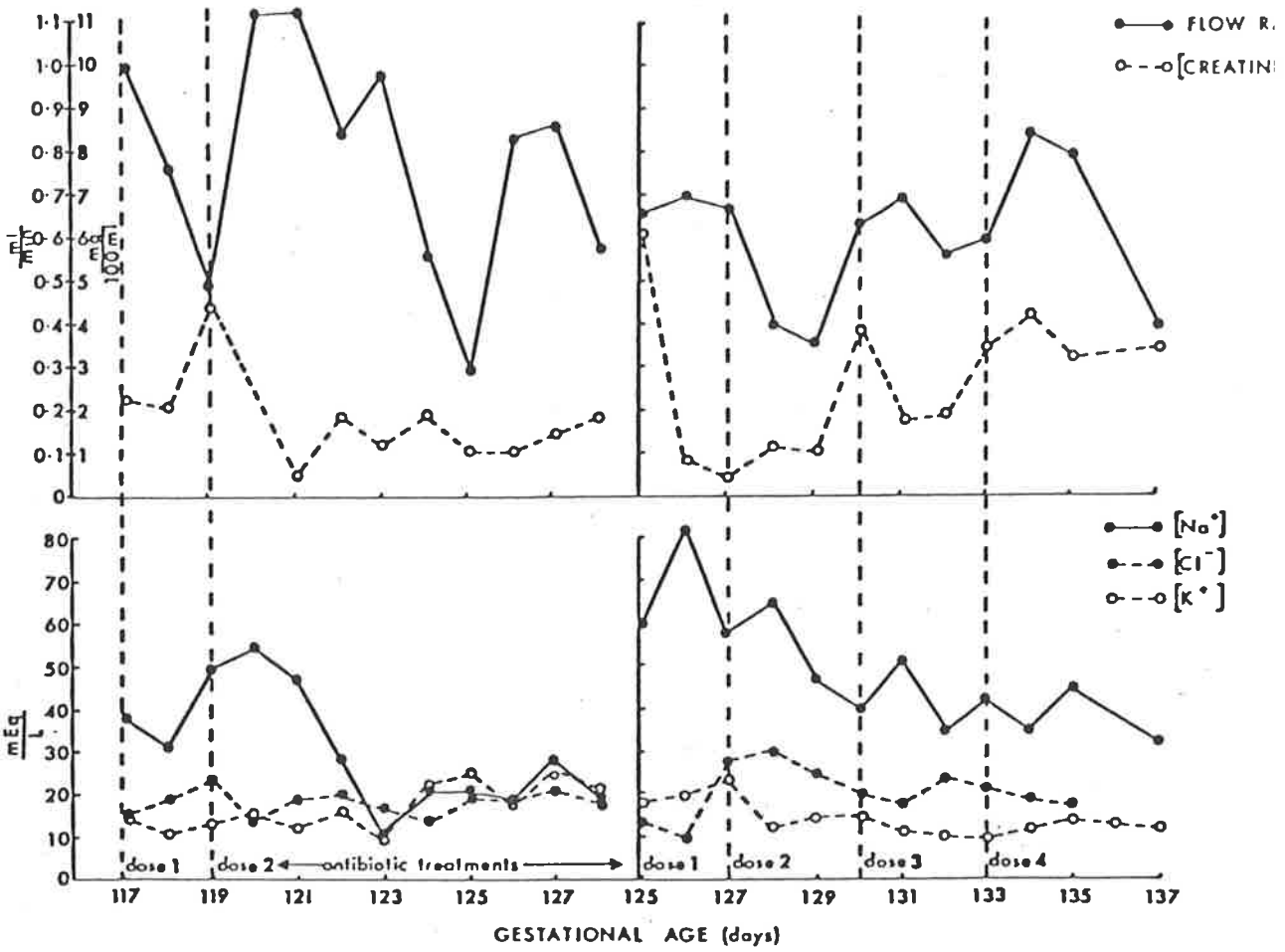


FIGURE 3. The effect of antibiotic treatments (cephaloride 20mg/Kg and gentamicin 5mg/Kg), on the flow rate and composition of foetal urine. (See text page 51)

exceeded 1ml.

If blood clots formed in a catheter, a 2000 U/ml solution of fibrinolysin (thrombolysin - Merck, Sharpe and Dohme), was forced into the catheter to lyse the clot. If the clot dissolved, the tube was refilled with heparinised saline.

3.4.5 Checking Cannula Placement

At times it was found that fluid could be infused into a venous cannula, yet withdrawal of blood was impossible. Fibrinolysin did not correct this problem. Post-mortem examinations revealed that it was caused by the formation of a flap of tissue over the tip of the catheter. Usually, this did not seriously impair the usefulness of the preparation because blood could be obtained from the arterial cannula and the venous tube could still be used for infusion. However, it was necessary to verify that the venous cannula was in fact still in the femoral vein. To do this, 2 ml. of 10% methylene blue was injected into the venous cannula and flushed in with 0.5ml. of saline. If the dye subsequently appeared in the foetal urine, proper placement of the cannula was confirmed. In all experiments, the placement of the cannula used for infusion was checked either by this method or simply by drawing blood into the tube.

3.5 Sample Collection

In untreated foetuses used to establish normal data, blood and urine samples were collected daily between 9.00 am and 10.30 a.m.

3.5.1 Urine Collection

When single samples of foetal urine were collected, the stop-cock on the bladder cannula was adjusted to allow the urine to flow directly into a measuring cylinder. The collection period was timed with a stopwatch and the volume of urine recorded. Usually about 8ml of foetal urine was collected over a period of 15-30 minutes, depending on flow rate. An aliquot of each urine sample was stored at -10^{o}C.

For continuous sampling of urine, the bladder catheter was

connected to a linear fraction collector (Paton Industries, S.A.). The fraction collector was modified to allow the simultaneous collection of maternal and foetal urine and the time base control permitted the collection of urine over precisely measured intervals. Also, the photo-electric drip counter in the fraction collector was connected to a chart recorder (Servoscribe) to plot variation in the rate of urine flow within a given collection period.

After a serial collection, the volume of urine in each tube was measured and an aliquot frozen. In experiments where urine storage would be delayed, 3-4 drops of a preservative solution (10% thymol in butanol) was added to all collection tubes before sampling.

3.5.2 Comments on Foetal Urine Collection

In studies of foetal kidney function, the aim is to obtain urine samples that represent, as nearly as possible, the direct output of the kidney and to offset possible modification of the composition of urine after it is formed.

Foetal bladder volumes were measured at post-mortem by filling the bladders with saline and measuring the volume aspirated. The volume of a fully distended bladder, varied between 14 and 21ml. in foetuses aged 120 days or over. This volume, plus the volume of the urine catheter (1.9ml.), was assumed to represent the dead space in the urine collection system. To estimate the time interval between urine production and collection, urine was collected from two foetuses at 1 min. intervals, following an intra-venous injection of inulin ^{14}C. Radioactivity was detected in the 5th and 7th samples respectively. (See fig. 4).
In two further experiments, tritiated water was injected directly into the exposed bladder of two foetuses during surgery. The femoral artery cannula had previously been implanted to enable blood samples to be taken at 1 min. intervals after the injection of tritiated water.

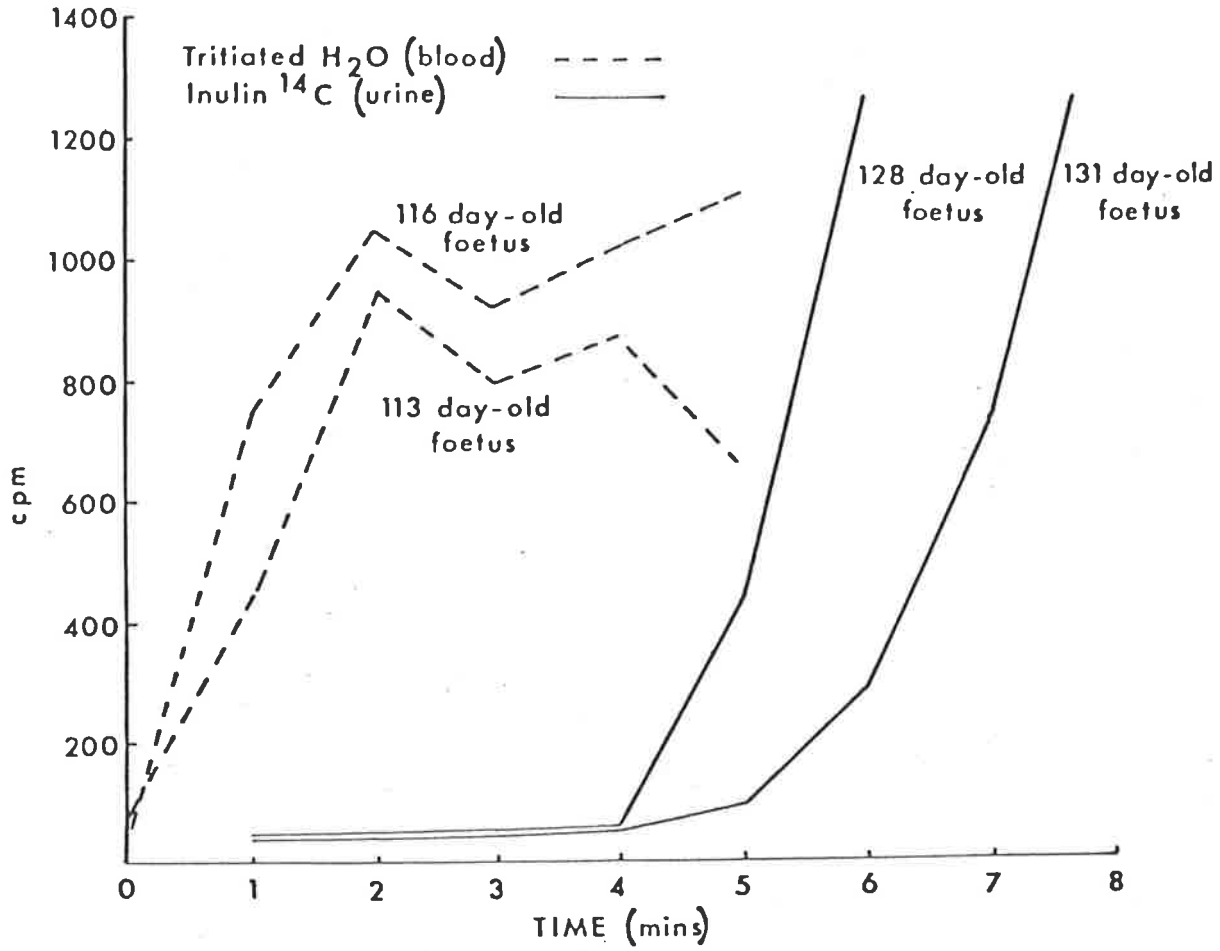


FIGURE 4. The rate of appearance of radioactivity in the urine of foetuses following intra-venous injection of inulin ^{14}C; plus the rate of appearance of radioactivity in the blood of foetuses following the injection of tritiated water into the foetal bladders. (See text page 53)

Radioactivity was present in the first sample in both foetuses, indicating a rapid efflux of water from the foetal bladder. (See Fig. 4). However, this was only a one-way measurement; the work of France et al (1974) indicates that there is a continuous influx and efflux of water and electrolytes which result in only minor nett changes. Nevertheless, the fact that such changes are occurring at a rate faster than the time necessary for urine to pass through the urinary tract, suggests that modifications of urine composition will occur before the urine is sampled. The extent of these modifications cannot be accurately defined until further studies of transport across the bladder wall have been conducted.

In view of these difficulties, a more suitable technique would seem to be to cannulate the ureters of the foetus. In the early stages of this study, chronic ureter cannulations were attempted, but were rarely successful. The trauma caused to the foetus, by exposing and handling the kidneys while locating the ureters, was a major problem. In those ureter samples collected and analysed, the concentration of the various urine solutes was comparable with the concentration of solutes in samples of bladder urine collected from foetuses of similar ages.

Apart from the technical difficulties of the operation, the desirability of ureter cannulations is questionable. Tanagho (1972), found that partial obstruction of the ureters of foetal lambs caused rapid and massive hydronephrotic atrophy of the renal parenchyma. Such gross pathological changes were usually found in preparations attempted in this study. Similarly, Bernstine (1970) reported that when the ureters of foetal sheep were cannulated, changes occurred in the foetal kidney which were similar to those accompanying polycystic disease of the kidney. Therefore, despite the possible problems of collecting urine via a bladder catheter, it remains the most practical and physiologically reliable means of sampling foetal urine.

When the foetal bladder is cannulated, there is also a problem of urine leakage, either voluntary, or involuntary through the urethra or urachus. In older foetuses, this would be predominantly through the urethra. To prevent this leakage, some workers ligate the penile urethra in male foetuses and avoid using female foetuses (Bernstine 1970). However, Rankin et al (1972), claim that if the free end of the bladder catheter is some distance below the level of the foetus, this will create a slight negative pressure in the foetal bladder and prevent urine leakage. In the dog foetus, it has been shown that discharge of urine via the urethra usually occurs when the pressure within the bladder exceeds 15mm Hg. (Bernstine 1970). A similar pressure is probably required in the sheep foetus where spontaneous micturition has been demonstrated (Robillard et al, 1973). Thus, if a negative pressure is applied to the foetal bladder, continuous drainage will be promoted and it is unlikely that the pressure within the bladder will reach the point where urine drainage through the urethra will be significant. In the present work, the free end of the bladder catheter, at the point of collection, was 90cm below the level of the foetus. Therefore, urine leakage would be minimal.

3.5.3 Blood Collection

To obtain a blood sample, the heparinised saline in the vascular cannula was aspirated into a syringe; then using a clean syringe, the required amount of blood was withdrawn (usually 2 ml). The blood was transferred immediately to a heparinised tube and mixed gently. Finally, the blood was flushed out of the cannula. Throughout these steps, care was taken to avoid contaminating the cannula fittings. When a number of blood samples were to be taken in a short time, the packed cell volume (PCV) of each sample was measured. If, during an experiment, the PCV changed significantly, the rate of sampling was reduced.

Blood required for progestin analysis was placed directly into a glass extraction tube containing an equal volume of redistilled cyclohexane. The extraction tube was weighed before and after the blood was added to measure the exact weight of blood. Finally, the tube was capped and shaken vigorously to commence extraction.

Where plasma samples were required, the blood was centrifuged (3000rpm, 10 min.), and the plasma decanted. All samples were stored at -10^{o}C until analysed.

3.6 Infusion and Injection Techniques

As mentioned above, no injection or infusion was made into a vascular cannula unless the placement of the cannula was confirmed. Drugs injected via a cannula were flushed in, with a small volume of saline. Normally, injections and infusions into the foetus were made via the catheter into the femoral vein and blood withdrawn via the catheter into the femoral artery.

Single blood samples were obtained from the ewes by jugular venepuncture, while for serial sampling or for infusion, one or both of the external jugular veins was cannulated.

Infusions were carried out with a variety of syringe pumps, including constant speed clockwork pumps and variable speed electric pumps. For long term infusions, a drip system or constant speed roller pump, drawing from a reservoir, was used.

3.7 Blood Pressure Measurement

Foetal blood pressure was recorded by connecting the arterial catheter via a pressure transducer (Statham P23AA), to a Servoscribe chart recorder. A 3-way stopcock on the arterial cannula allowed blood samples to be taken with little disruption to the blood pressure record.

3.8 Laboratory Methods

3.8.1 Non-Hormone Analyses

(i) The pH of maternal and foetal urine was measured using an ATHOM pH meter model 22R. The instrument was calibrated using standard pH solutions.

(ii) The sodium and potassium concentration of plasma and urine was measured using an IL flame photometer (Model 143) with an automatic dilutor and Lithium standard.

(iii) The Osmolality of urine samples was measured on a Fiske osmometer model G.

(iv) The urea concentration of urine was measured using the method of Natelson et al (1951).

(v) A Technicon autoanalyser (series II) was used for the determination of uric acid and creatinine concentration in plasma and urine. Creatinine was determined using Technicon programme AA 11-11.

Uric acid was determinedusing Technicon programme AA 11-13a.

(vi) To measure packed cell volume (PCV), whole blood was drawn into a heparinised capillary tube, and the tube sealed. It was then centrifuged for 5 minutes at 12000G and the results read using a Hawksley micro-haematocrit reader.

(vii) Glomerular Filtration Rate (G.F.R.) was measured using a technique similar to that of Rankin et al (1972). Inulin - carboxyl - ^{14}C (New England Nuclear) was injected into the femoral vein of the foetus (10μc in 2 ml saline). Blood samples (1ml) were drawn from the femoral artery of the foetus at intervals of 200, 300, 400, 500 and 600 minutes after the inulin injection. Foetal urine was drained via the bladder catheter throughout the experiment and collected in 30 min. fractions.

The plasma and urine samples were analysed by mixing 0.2ml of each sample with 1ml of tissue solublizer (Biosolv. BBS-3 Beckman Instruments) in a scintillation vial. The vials containing plasma were shaken for 24 hours and after solublizing, 10ml of toluene-triton scintillation fluid was added to each vial. The radioactivity was measured in an ISOCAP 300 liquid scintillation counter (Nuclear Chicago) and counting efficiency determined using the channels ratio method. The data was analysed as described by Rankin et al (1972) and inulin ^{14}C clearance calculated as an estimate of GFR.

3.8.2 Hormone Analyses

(i) Progesterone, 17α Hydroxy-Progesterone (17αHP) and 20α Hydroxy-Pregn -4-En-3-One (20αHP) were measured using an assay developed by Seamark and Lutwak-Mann (1972). In this assay, the progestins were extracted from whole blood with cyclohexane and separated using alumina columns. The columns were eluted with cyclohexane containing ethanol at various concentrations. The elution procedure was as follows: 5ml 0.5% (v/v) ethanol (discard), 3 ml 0.6% ethanol (progesterone fraction), 3ml 1.25% ethanol (20αHP fraction), 2.0ml 2.0% ethanol (17αHP fraction). The progesterone and 17αHP fractions were then assayed using a competitive protein binding assay developed by Obst and Seamark (1970). This assay was based on earlier work by Murphy (1967) and Bassett and Hinks (1969). The binding protein used was cortico-steroid binding globulin (CBG) obtained from dog or hen plasma. The residues of the steroid fractions were incubated with the CBG solution (45^{o}C for 30 mins) and then an aliquot of each sample was transferred to a small Sephadex column (0.5gm of G25 fine). The protein bound steroid was eluted with phosphate buffer into scintillation vials

containing dioxane scintillator and the activity measured. The steroid concentrations were calculated by reference to standard curves.

The 20αHP fraction was reacted with a specific enzyme, 20α hydroxy steroid-oxido reductase prepared from foetal sheep blood (Seamark, Herriot and Nancarrow, unpublished) (Seamark, McIntosh and Moor 1973). The reaction mixture contained 1ml bicarbonate buffer (0.1M, ph 9), 0.27μmol NADP, 5.7μmol nicotinamide and 0.02ml enzyme and was incubated at 37°C for 20 minutes. The reaction products were extracted with cyclohexane and the progesterone concentration measured using the above method. All samples were pre-equilibrated with a small amount of tritiated 17αHP, 20αHP and progesterone to be assayed as internal recovery standards. Therefore the level of each progestin could be calculated from the assay result and the percent recovery. For further details, see appendix.

(ii) Corticoid concentration in plasma was determined by the method of Bassett et al (1969).

(iii) Plasma Renin Activity (PRA). The method used to estimate PRA measures the amount of angiotensin-I, produced by the action of renin on its substrate. The rate of angiotensin-I formation in plasma incubated in vitro is proportional to the time of incubation. A known volume of each plasma sample was dialysed for 16 hours against pH 7.5 buffer at 8°C. The buffer used was dibasic-monobasic phosphate buffer containing 0.005m EDTA (ethylene diamine tetra acetate) and made 0.16M with NaCl. After dialysis angiotensinase inhibitors were added to the plasma (0.01ml dimercaprol/ml and 0.3m EDTA/ml) and the original volume restored, if necessary, by the addition of pH 7.5 buffer. Next the plasma samples were incubated at 37°C and aliquots taken for angiotensin-I assay at 30, 60 and 90 mins. When these results were determined, the amount of

angiotensin generated per hour in each sample could be calculated. This value indicated the intial velocity of reaction of renin with renin substrate and was an estimate of the activity of renin on renin substrate in the original samples. The angiotensin-I assay used was a radio-immunoassay developed by Lumbers, Seamark and Pickles (1971 unpublished), and was similar to a method described by Haber et al (1969). The antiserum used was raised in goats by injecting angiotensin-I coupled to poly-L-lysine (Haber et al, 1965) and the tracer was produced by labelling angiotensin-I with I^{125} (Hunter and Greenwood, 1962). Bound and free hormones were separated using Dextran (T-40) coated charcoal (Norit A), (Herbert et al, 1965) and sample activity was counted in a Packard Tri-carb liquid scintillation counter with a gamma spectrometer attachment.

3.8.3 Histology

For morphological study of foetal kidneys; the kidneys were removed from the foetuses as quickly as possible after the ewes were slaughtered and a slice of tissue was taken down the long axis of each kidney. This slice included tissue from the cortex and medulla. The kidney slice was placed into a glass dish containing cold fixative solution and smaller pieces of tissue cut from the regions selected for study. These were transferred to a drop of fixative and trimmed into about 1mm cubes. Preparation of the specimens then proceeded as follows:-

(a) Fixed for 2 hours with 2% gluteraldehyde in Sorensen's phosphate buffer (0.2 M pH 7.2) at $4^{o}C$..

(b) Washed overnight in Sorensen's phosphate buffer.

(c) Post-fixed for 2 hours in 1% osmium tetroxide in Sorensen's buffer.

(d) Washed in distilled water for ½ an hour.

(e) Dehydrated in graded acetones; 30%, 50%, 70%, 90% (2 times), 100% (2 times).

(f) Infiltrated for 2 hours in 100% Epon 812 (under light vacuum).

(g) Embedded in fresh epon for 30 hours at $60^{o}C$.

Thick sections (1-2μ) were cut for light microscope examination and studied either unstained using phase contrast or stained with basic fuchsin and crystal violet.

Ultra thin sections (500-700Å) were cut for electron microscope examination. These sections were stained with a saturated solution of uranyl acetate for 20 mins. and lead citrate solution for 5 mins. They were examined with an AEI - EM801 electron microscope.

4. *RESULTS FROM UNTREATED FOETUSES*

4.1 *Presentation of Results*

The first section of this study involved daily collection of samples of urine, blood and amniotic fluid from sheep foetuses with indwelling catheters. The aim was to establish the concentration of specific components of these fluids and to determine their relationship to foetal age and foetal kidney development.

Daily samples were obtained from 26 foetuses which, at the time of the first collection, were between 112 and 139 days of gestational age. Sampling continued for an average of 18 days and 14 of the foetuses were delivered normally. The average age at delivery was 147 days. The samples obtained from the foetuses were analysed as described in section 3.

The data from some individual foetuses will be presented, but for the determination of normal ranges and gestational trends, composite results have been derived. The mean ($\bar{x}$) and standard error (SE) has been calculated for each parameter, on each day of gestation observed, using the results from all foetuses. Foetal age is given as either, "days after mating" or "days prior to parturition". In the latter cases, the results used are from the 14 foetuses which were delivered normally. Three foetuses in which the plasma cortisol levels remained low, immediately prior to delivery, were regarded as abnormal and were excluded from all results.

The individual values for the concentration of urine constituents have been combined with the flow rate measured at the time of collection, to determine excretion rates. Also the creatinine concentration in simultaneously collected plasma and urine samples have been combined with flow rate to calculate endogenous creatinine clearance. Average excretion rates and clearance values have been calculated for each

day of gestation on which samples were collected. Finally, all absolute and derived values relating to urine output and composition have been corrected for changes in foetal kidney weight. Standard kidney weights, based on measurements made in foetuses of known gestational age, were used for these corrections. (See table 3).

4.2 Foetal Sodium

4.2.1 Plasma Sodium

A feature of these data and most of the subsequent data is the small number of samples collected between days 115 and 120 and days 145 and 150 compared with the number of samples collected within these extremes. Because of this, the results have been grouped to produce averages for each 5 day period after day 115. Nevertheless, considerable information can be obtained from the ungrouped data which are presented in the appendix tables. (Appendix table 1).

Figure 5 illustrates the difficulties caused by the limited number of results at the extremes of the foetal age span. The plasma [Na^+] varied considerably between days 115 and 120 and between days 145 and 150, but within these limits the variation was reduced and there was a gradual increase in [Na^+]. Over the total range sampled, there was a significant correlation between foetal age and plasma [Na^+] ($r = 0.223$, $0.01 > P > 0.001$, $n = 156$) (Pearsons coefficient), but examination of the grouped data suggests that this correlation was not retained after day 139. Between days 115 and 120 the average plasma [Na^+] was 134.6 mEq/L ($n = 9$) and rose to 146.5 mEq/L ($n = 27$) between days 130 and 135 and then fell to 145.3 mEq/L ($n = 8$) between days 144 and 148. Mellor (1970) reported that the mean plasma [Na^+] for sheep foetuses aged between 76 and 140 days was 152 mEq/L ($n = 14$) which is higher than the value obtained in the present work. There are no other reports of gestational trends in foetal plasma [Na^+] similar to those reported here.

4.2.2 Urine Sodium

As might be expected, the variation between the daily averages for urinary [Na^+] was greater than for plasma [Na^+]. Such variability is

TABLE 3: TOTAL KIDNEY WEIGHT OF FOETUSES COMPARED WITH GESTATIONAL AGE

(These total kidney weights were used as standard values when parameters relating to kidney function in foetuses of different ages were standardised on ths basis of unit kidney weight)

DAY OF GESTATION	TOTAL KIDNEY WEIGHT (gm)
115	15.30
116	15.70
117	16.20
118	16.70
119	17.10
120	17.50
121	17.90
122	18.30
123	18.80
124	19.20
125	19.60
126	19.90
127	20.20
128	20.60
129	20.90
130	21.20
131	21.50
132	21.80
133	22.10
134	22.40
135	22.60
136	22.80
137	23.00
138	23.20
139	23.40
140	23.50
141	23.55
142	23.60
143	23.65
144	23.70
145	23.70
146	23.70
147	23.70
148	23.70
149	23.70
150	23.70

(See text page 63)

apparent in all comparable studies of foetal kidney function. However, despite short term fluctuations in the composition of urine from individual foetuses and variation in urinary composition between foetuses of the same age, a gestational trend was apparent. Urinary [Na^+] was negatively correlated with foetal age ($r = -0.258$, $P < .001$, $n = 183$). (See fig. 5). In the period between days 115 and 120 the average concentration was 42.6 mEq/L ($n = 8$) while between days 130 and 135 and days 145 and 150, the averages were 24.0 mEq/L ($n = 35$) and 20.4 mEq/L ($n = 17$) respectively. When the values for urinary [Na^+] were corrected for changes in total kidney weight, the correlation with foetal age was more significant ($r = -0.389$, $P < .001$, $n = 183$). (Appendix table 2).

When urinary [Na^+] was plotted against "days prior to parturition", the distribution of observations was changed. The total number of observations is reduced from 156 to 99 of which 75 relate to the period between day -16 and term. (See fig. 5). Again a decline in urinary [Na^+] was evident, but only until day -6 after which there was a rapid increase in [Na^+] from 11.5 mEq/L at day -6 to 53.3 mEq/L at day -1. This rise in the last 6 days of gestation was highly significant ($r = .46$, $P < .001$, $n = 29$). (Appendix table 3).

In other studies, Alexander et al (1958a) obtained a mean of 71 mEq/L for the urinary [Na^+] of foetuses aged between 104 and 117 days and a mean of 20 mEq/L for foetuses aged between 130 and 142 days. Bernstine reported an average of 60.7 ± 40.6 mEq/L ($n = 7$) for foetuses aged between 108 and 125 days and an average of 60.7 ± 31.5 mEq/L ($n = 7$) for foetuses aged between 126 and 142 days. In the present study, the average urinary [Na^+] of young foetuses (115 - 125 days) was approximately 40 mEq/L lower than in either of these reports. For older foetuses (130 - 142 days) the present average (27.88mEq/L $n = 88$) was comparable with Alexander et al's result but

about 35 mEq/L lower than Bernstine's figure. These discrepancies are probably due to the limited number of observations in the earlier studies.

More recently, Mellor and Slater (1972b) used chronically catheterised foetuses to determine the average concentration of urinary solutes. The results they obtained are generally similar to those reported here. However, Mellor and Slater found that the mean $[Na^+]$ in the urine of foetuses aged between 141 and 150 days (28.6 mEq/L) was only marginally greater than the average for the preceding 10 days (28.1 mEq/L). This is not consistent with the pre-parturient rise in urinary $[Na^+]$ observed in the present work. There were 36 individual observations made on foetuses aged between 141 and 150 days in the present study and 28 in Mellor and Slater's work, so there is no statistical reason for the discrepancy. However, since Mellor and Slater were unable to plot their data in relation to time of parturition, the pre-parturient rise may have been obscured.

In general, there was an overall decline in urinary $[Na^+]$ as gestation proceeded, although the trend was reversed over the last 6 days of pregnancy. This pre-parturient reversal of the overall gestational trend has not been reported elsewhere.

4.2.3 Amniotic Fluid Sodium

The $[Na^+]$ of amniotic fluid was extremely variable, both within and between foetuses. However there was a small negative correlation with foetal age ($r = -0.211$, $0.05 > P > 0.01$, $n = 94$), (See fig. 5) which is consistent with the findings of Malan et al (1937), and Mellor and Slater (1971 and 1972b). (Appendix table 5).

4.3 Foetal Potassium

4.3.1 Plasma Potassium

Plasma $[K^+]$ showed no significant relationship to foetal age

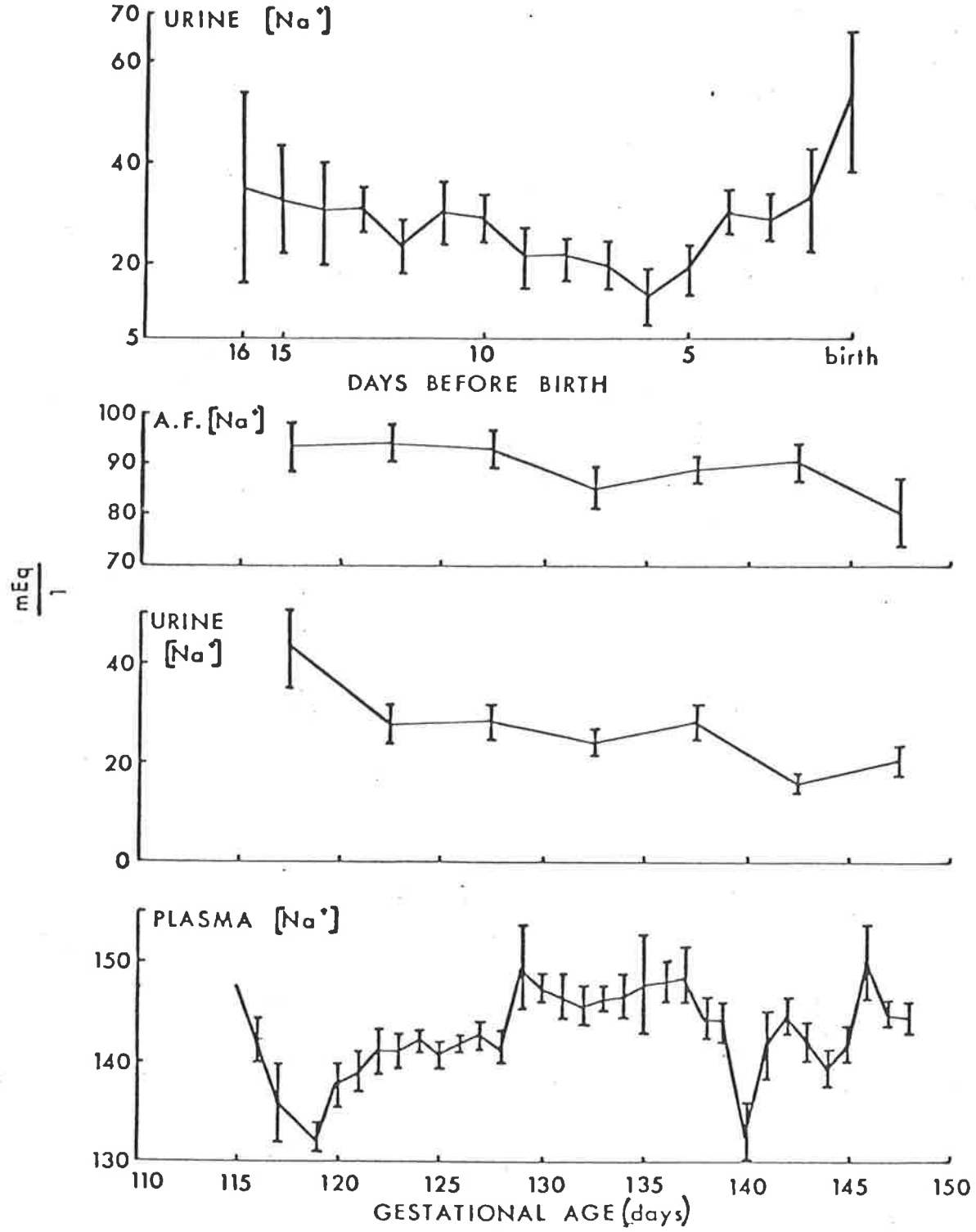


FIGURE 5. The concentration of Na^+ in foetal urine, foetal plasma and amniotic fluid during the last 35 days of gestation. (See appendix tables 1-5 and text pages 63-65)

(In Figures 5-16 either the mean and standard error for each day of gestation is shown or the mean and standard error for each successive 5-day period.)

(r = .102 NS n = 157) (See Fig. 6). However, between the 115th and 135th days of gestation there was a gradual increase in the daily average for plasma $[K^+]$. The mean $[K^+]$ for the period between days 115 and 120 was 4.30 mEq/L (n = 10) compared with 4.76 mEq/L on day 135, thereafter the daily averages fell to a minimum of 4.1 mEq/L on day 140. After day 140 the plasma $[K^+]$ increased, but in this period the number of observations was low and the standard errors of the means high. Accordingly, this trend was less significant than that of the preceding 20 days. (Appendix table 1).

Mellor, (1970) reported that the average plasma $[K^+]$ for foetuses aged between 76 and 140 days was 5.7 ± 1.6 mEq/L (n = 15) but there are no other reports of gestational trends in plasma $[K^+]$ similar to those reported here.

4.3.2 Urine Potassium

Urinary $[K^+]$ was significantly correlated with foetal age ($r = 0.315$, $P < 0.001$, $n = 190$) (see fig. 6) and the correlation was retained when the data was corrected for foetal kidney weight, although the degree of correlation was reduced. The average urinary $[K^+]$ for the period between days 115 and 120 was 7.5 mEq/L (n = 8) and for the period from day 120 to day 132 was 13.71 mEq/L (n = 35). After day 132 there was a decrease in urinary $[K^+]$ and a minimum daily average of 9.0 mEq/L was recorded on day 137. Throughout this period the sample sizes were sufficient to suggest that the observed trend was significant. Following this brief reversal the daily averages for urinary $[K^+]$ increased until day 143 but declined again from day 143 until term. (Appendix table 2).

In summary, apart from the fact that urinary $[K^+]$ showed a small positive correlation with foetal age, only the fall in concentration during the 4 days from day 134 to day 137 could be significant. It is interesting

that this decrease in urinary [K^+] resembles that which occurred in plasma [K^+] although the latter trails the former by about 5 days. The physiological significance, if any, of these changes is not apparent.

When K^+ excretion rates were calculated they were found to be highly variable. Despite this, there was an overall correlation between potassium excretion rate and foetal age ($r = 0.245$, $0.01 > P > 0.001$, $n = 183$). this correlation was lost when the data was corrected for changes in foetal kidney weight which indicates that although changes in urinary [K^+] are relatively independent of kidney weight, changes in K^+ excretion rate are not. (Appendix table 4).

Alexander and Nixon (1961) reported urinary [K^+] in mid-gestation (days 104 to 117) to be 4 mEq/L ($n = 2$) and in late gestation (days 130 to 142) to be 8 mEq/L ($n = 2$). In comparison, Bernstine (1970) reported an average urinary [K^+] for mid-gestation (days 108 to 125) of 17.7 mEq/L ($n = 7$) and for late gestation (days 126 to 142) of 14.6 mEq/L ($n = 7$). Corresponding values in the present study were: mid-gestation (days 115 to 125) 10.6 mEq/L ($n = 30$), and late gestation (days 130 to 142) 17.44 mEq/L ($n = 139$). For the period from day 142 to day 150 the average was 21.5 mEq/L ($n = 34$).

The positive correlation between foetal age and urinary [K^+] reported here substantiates the findings of Mellor and Slater (1972b). However, the values for urinary [K^+] quoted by Mellor and Slater are lower than those of the present work and lower than those reported by Bernstine (1970).

4.3.3 Amniotic fluid potassium

The daily averages for the [K^+] of amniotic fluid varied widely, as did the individual values contributing to those averages. Accordingly, there was no correlation between amniotic fluid [K^+] and foetal age ($r = 0.130$, NS, $n = 96$). However, when the results were grouped as in figure 6, some increase in [K^+] was seen, which is consistent with earlier

reports by Mellor and Slater (1971 and 1972b). The absolute values for amniotic fluid [K^+] reported here, are comparable at all stages of gestation, with those reported by Mellor and Slater (1971) (Appendix table 5).

4.4 Foetal Uric Acid

4.4.1 Plasma uric acid

The average concentration of uric acid in foetal plasma decreased from 1.07 mg/100ml (n = 11) for the period between days 115 and 120 to 0.80 mg/100ml (n = 9) between days 145 and 150 (Appendix table 6). This downward trend was not without fluctuation and, in fact, there was no significant correlation between plasma uric acid concentration and foetal age ($r = 0.142$, NS, $n = 151$). However, when the results from those foetuses that delivered normally were considered alone, a rapid rise in plasma uric acid concentration, particularly in the 15 days prior to parturition was revealed. (See fig. 7). Over the final 25 days of pregnancy, the correlation between plasma uric acid concentration and foetal age was highly significant ($r = 0.401$, $P < .001$, $n = 82$). In fact, the increase during this period appeared to be exponential.

4.4.2 Urine uric acid

Urinary uric acid concentration showed significant correlation with foetal age ($r = 0.199$, $0.01 > P > 0.001$, $n = 194$). This correlation was particularly evident in the period between days 115 and 135. When urinary uric acid concentration was considered in relation to time before parturition, an even more significant correlation emerged ($r = 0.361$, $P < 0.001$, $n = 109$). (See fig. 7). This correlation was the result of an increase in uric acid concentration from an average of 1.03 mg/ml (n = 7) in the period between 25 and 20 days before birth,

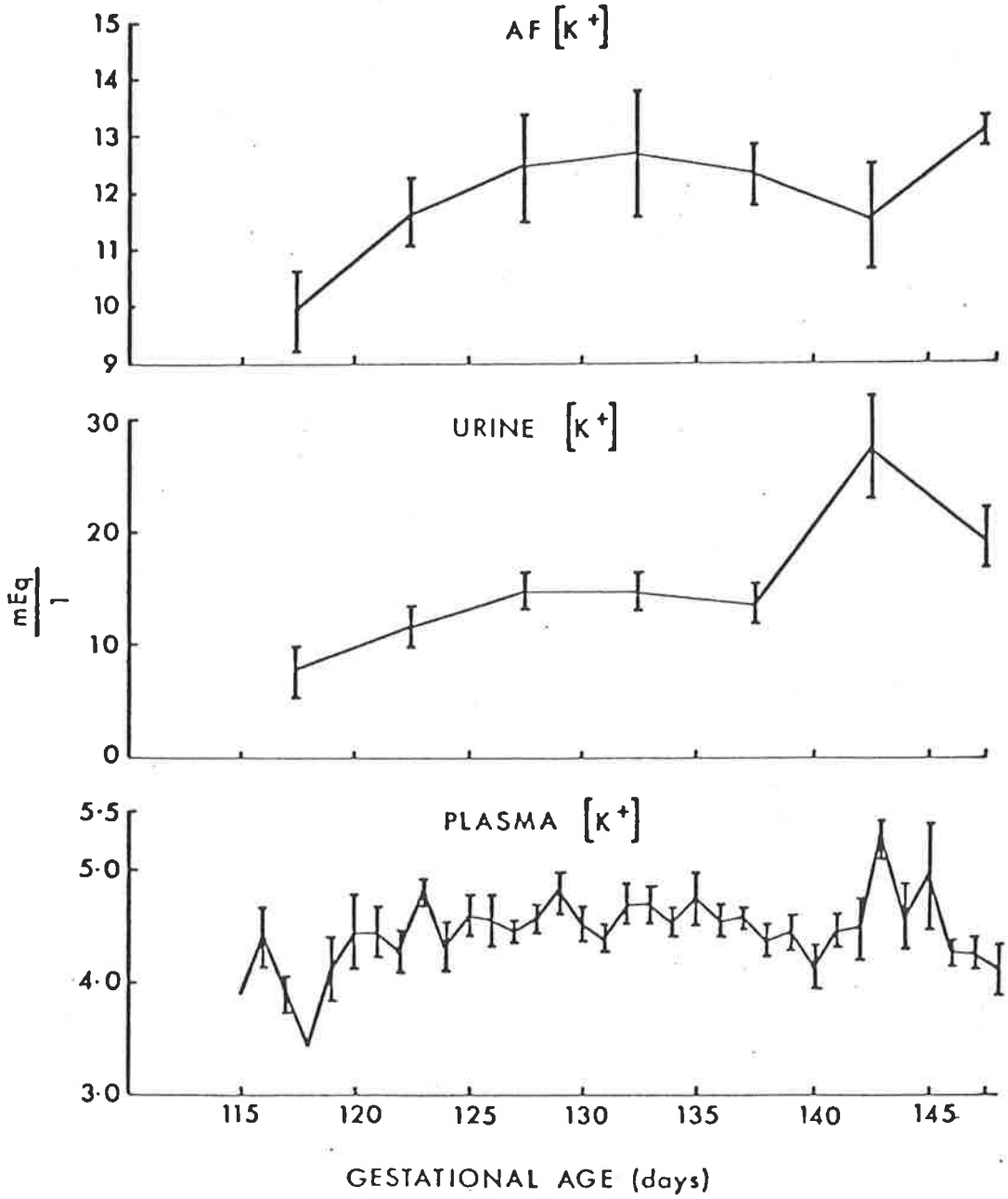


FIGURE 6. The concentration of K^+ in foetal urine, foetal plasma and amniotic fluid during the last 35 days of gestation. (See appendix tables 1-5 and text pages 65-68)

to 3.48 mg/100ml (n = 30) in the last 5 days before parturition. (Appendix table 7). The pre-parturient increase in the urinary concentration of uric acid was only slightly modified when the uric acid measurements were corrected for changes in foetal kidney weight. This was not surprising since at the time of the most dramatic changes in uric acid concentration, foetal kidney weight increases only slightly. However, the corrected data does imply that the real increase in urinary uric acid concentration, began within 15 days of birth. Therefore, the urinary changes closely parallel the observed plasma changes.

The excretion rate of uric acid showed a small significant correlation with foetal age ($r = 0.179$, $0.01 > P > 0.001$, $n = 194$). (Appendix table 10). However, this correlation was lost when the values were corrected for changes in kidney weight ($r = 0.100$, NS, $n = 194$) and it appears that about 40% of the original relationship was due to kidney growth. When the data was related to parturition, a significant correlation was retained ($r = 0.301$, $0.01 > P > 0.001$, $n = 82$) and the correlation remained significant ($r = 0.240$, $0.05 > P > 0.01$, $n = 82$) after kidney weight correction (appendix table 8). These correlations resulted primarily from increases in the uric acid level of foetal urine during the last 15 days of foetal life.

4.4.3 Amniotic fluid uric acid

The concentration of uric acid in amniotic fluid increased during pregnancy ($r = 0.503$, $P < 0.001$, $n = 98$) (see fig. 7). However, the rate of increase was not regular. Before day 140 the increase was relatively slow, but thereafter there was a more rapid rise. This biphasic pattern of uric acid increase in amniotic fluid closely reflected the changes in the urinary concentration of uric acid (appendix table 9).

No other reports of uric acid concentration in sheep amniotic

fluid are available, although Harrison (1972) found that in humans the uric acid concentration of liquor increased as pregnancy advanced.

4.5 Foetal Creatinine

4.5.1 Plasma creatinine

The concentration of creatinine in the plasma of foetal sheep decreased as gestation proceeded. The youngest foetuses showed concentrations of around 4.5 mg/100ml (average for days 115 to 120 = 4.44 mg/100ml, n = 14), while in near term foetuses the values were about 1.0 mg/ml (average for days 146 to 150 = 1.11 mg/100ml, n = 6) (appendix table 6). Accordingly, there was a significant relationship between plasma creatinine and foetal age ($r = -0.270$, $0.01 > P > 0.001$, $n = 153$) (see fig. 8). However, when the results from those foetuses that lambed were considered alone, there was no significant correlation. It can be seen in figure 8 that the most rapid fall in plasma creatinine concentration occurred between days 115 and 125 after which the decline was slower and more variable. Since a large proportion of the observations from the foetuses that lambed fall into the last 25 days of gestation, the lack of correlation may simply reflect the slow variable change in creatinine concentration that occurred during that period. The only other study of plasma creatinine concentration in foetal sheep is that of Alexander et al (1958a). In contrast to the present study, they found that plasma creatinine concentration increased from an average of 2.1 mg/100ml (n = 2) on day 117 to 4.2 mg/100ml (n = 4) on day 142. However, apart from the fact that Alexander et al used acute preparations, their limited number of observations precludes any reliable prediction of gestational trends.

4.5.2 Urine creatinine

In contrast to foetal plasma, the concentration of creatinine in foetal urine was positively correlated with foetal age ($r = 0.171$,

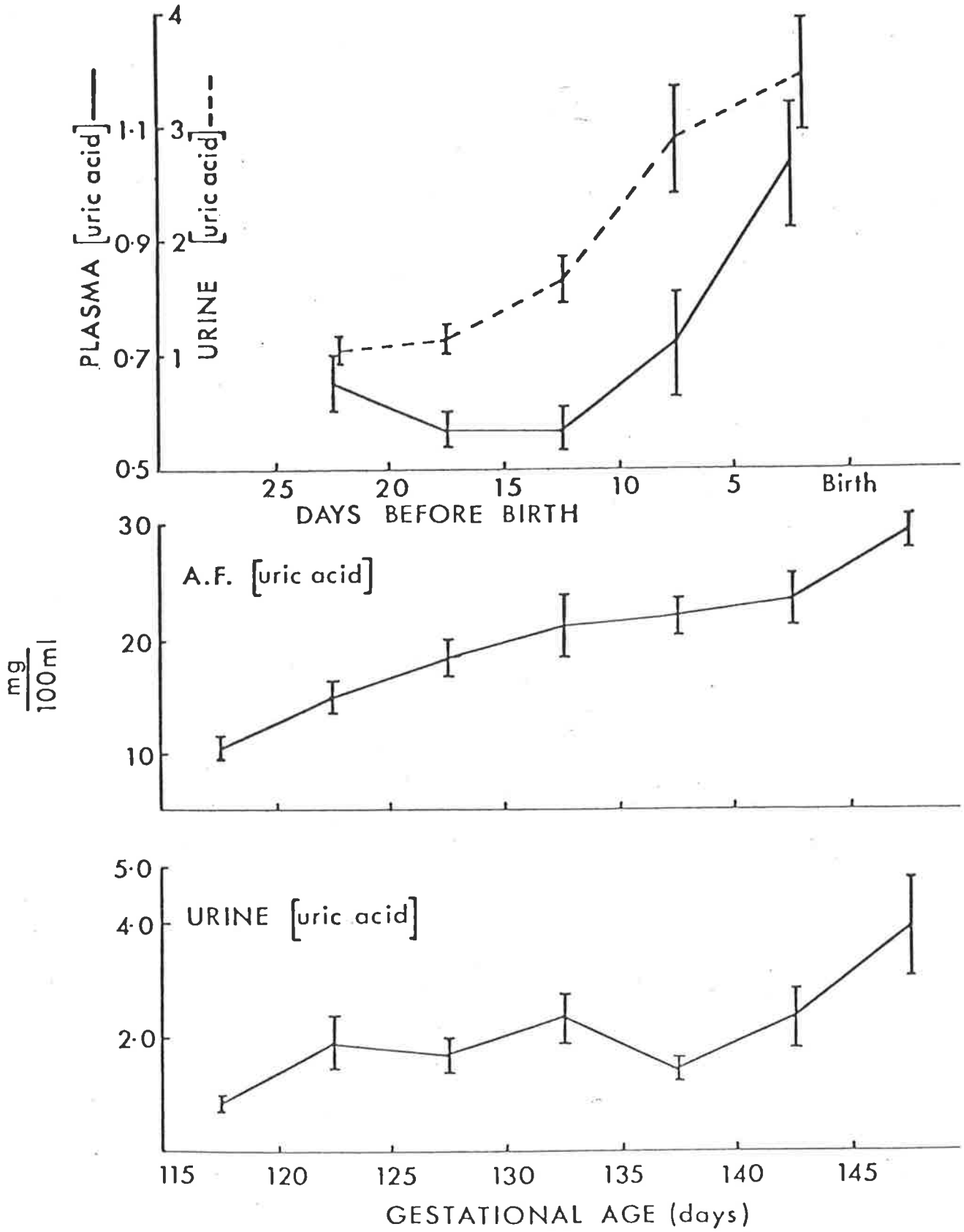


FIGURE 7. The concentration of uric acid in foetal urine, foetal plasma and amniotic fluid during the last 35 days of gestation. (See appendix tables 6-10 and text pages 68-70)

$0.05 > P > 0.01$, $n = 196$) (see fig. 8). There was threefold increase in concentration from an average of 5.60mg/100ml for the 5 days from day 115 to 120 to an average of 16.17 mg/100ml for the period between days 144 and 150 (appendix table 7). However, this correlation was lost when the results were corrected for kidney weight ($r = 0.063$, NS, $n = 196$), indicating a close relationship between increasing kidney size and the changes in the concentration of urinary creatinine. When the results from those foetuses which delivered normally were analysed separately, a high correlation between urinary creatinine concentration and foetal age was revealed ($r = 0.320$, $0.01 > P > 0.001$, $n = 109$) and this relationship was largely unaffected when the data was corrected for changes in foetal kidney weight ($r = 0.305$, $0.01 > P > 0.001$, $n = 109$) (appendix table 8). It can be seen in figure 9 that the relationship between urinary creatinine concentration, and time before birth was exponential rather than linear and it was only during the final 15 days of gestation that significant increases in creatinine concentration began.

In summary, between the 115th and 135th days of gestation, there was a small increase in the creatinine concentration of foetal urine which was closely related to increasing kidney weight. Over the remaining 15 days of gestation, the creatinine concentration of foetal urine increased approximately threefold and the increase was independent of changes in foetal-kidney weight.

In other studies of urinary creatinine concentration, trends similar to those reported here have been observed, although the sample sizes were usually limited. For example Alexander et al (1958a), quoted a single value of 6.4 mg/100ml for a 117 day-old foetus and other single values of 24.4 and 28.8 mg/100ml for 2 foetuses aged 137 and 142 days respectively. Bernstine (1970) reported an average value of 12.3 mg/100ml ($n = 7$) for the period between days 108 and 125 and an average of 19.5 mg/100ml ($n = 7$) for the period between days 126 and 142. Neither

study rivals the present work in which 196 individual measurements were made.

Creatinine excretion rates, plotted against foetal age, are shown in figure 9. It can be seen that there was a progressive increase in creatinine excretion rate as gestation proceeded ($r = 0.274$, $0.01 > P > 0,001$, $n = 109$) (appendix table 10). When the values for creatinine excretion were corrected for foetal weight, the correlation with foetal age was reduced ($r = 0.173$, N.S., $n = 109$) to a degree which indicates about a 35% involvement of kidney-weight change in the original correlation. In view of the observed changes in creatinine concentration, it is likely that this 35% involvement was primarily in the period before day 135.

The pattern of change, for creatinine excretion rate, was relatively uniform throughout the period of gestation examined. This is in contrast to the changes in creatinine concentration.

4.5.3 Amniotic fluid creatinine

The creatinine concentration of amniotic fluid was positively correlated with gestational age ($r = 0.277$, $0.05 > P > 0.01$, $n = 79$) (see fig. 8), although variation within and between daily samples was high. The average value for creatinine concentration in the interval between day 115 and 120 was 39.3 mg/100ml, this increased to an average of 79.93 mg/100ml in the period between days 130 and 135, after which the average for each 5 day period decreased, with the exception of a small rise between days 145 and 150 (appendix table 9).

It is interesting to note, that until day 140 the rise in the concentration of creatinine in amniotic fluid (approx. 2 times) was of a similar magnitude to the increase in creatinine excretion rate over the same period. After day 140 the relationship ended and amniotic fluid creatinine decreased while the creatinine level in foetal urine

and creatinine excretion rate increased.

In all cases where paired samples of urine and plasma were obtained and creatinine concentrations measured, endogenous creatinine clearance was calculated. It was found that endogenous creatinine clearance was correlated with foetal age ($r = 0.469$, $P < 0.001$, $n = 149$) (see fig. 9). This was not surprising since it had already been established that the creatinine concentration of foetal plasma decreased with increasing foetal maturity, and that conversely, creatinine excretion rate increased with increasing foetal age. Over the period of gestation observed, creatinine clearance increased about sevenfold.

When the creatinine clearance values were corrected for foetal kidney weight, there was some reduction of the correlation with foetal age, although a highly significant relationship remained ($r = 0.406$, $P < 0.001$, $n = 149$). this reduction was a reflection of the involvement of kidney size in creatinine clearance. As with creatinine excretion rate, the influence of kidney weight on endogenous creatinine clearance was most significant before the 135th day of pregnancy. The rate of increase of creatinine clearance per gm of kidney, in the period between days 115 and 135, was not as great as for the uncorrected data. Nevertheless, there was a tenfold increase in creatinine clearance per gm of kidney, and most of this increase occurred between days 135 and 150. This reflected the large increase in urinary creatinine concentration that occurred in that period.

Alexander et al (1958b) reported a value for creatinine clearance of 0.88 at day 117 of gestation which is comparable with the present results. However, Alexander et al also quoted a value of 1.4 for a 142 day-old foetus and that is lower than the range of values reported here.

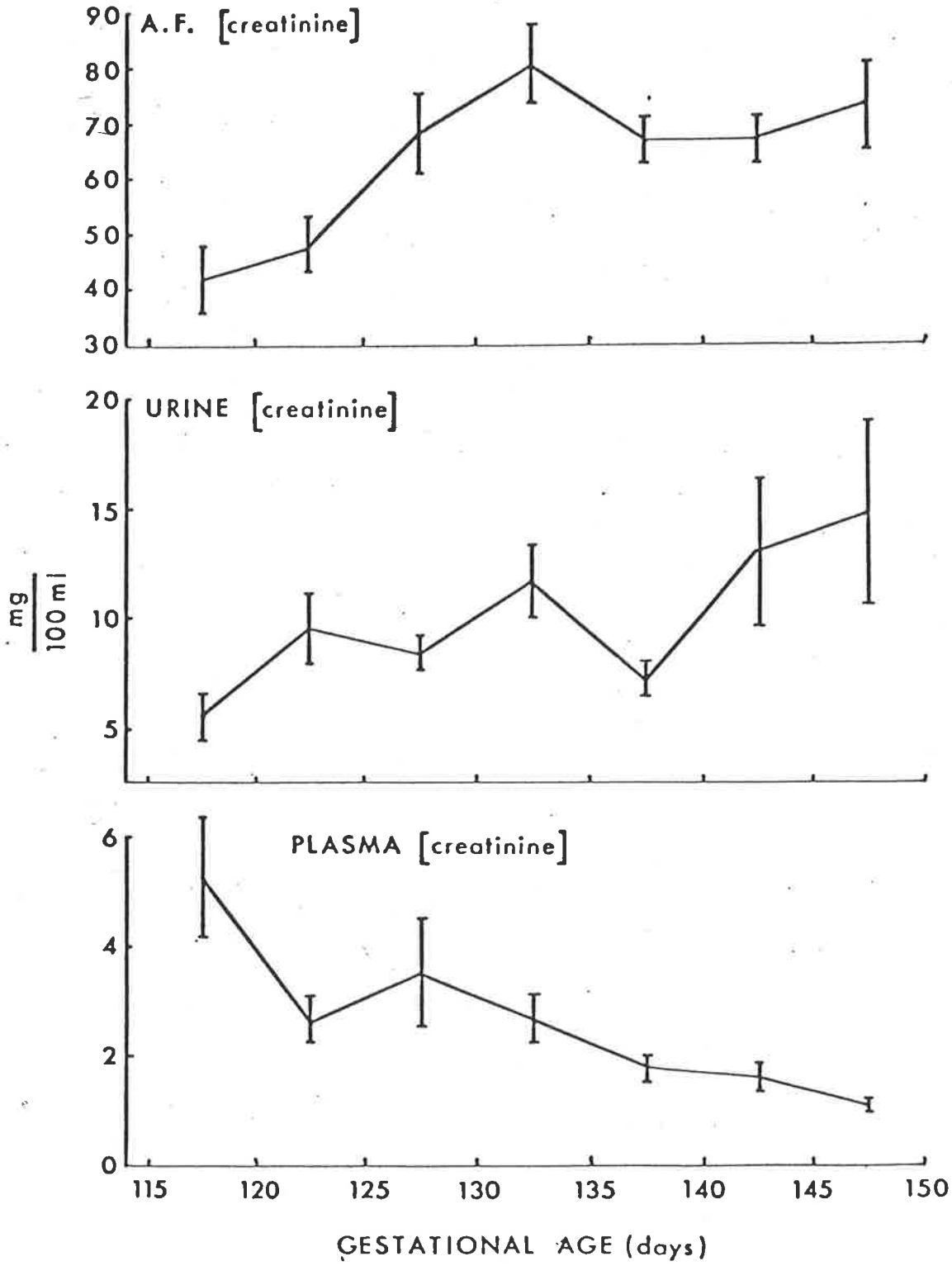


FIGURE 8. The concentration of creatinine in foetal urine, foetal plasma and amniotic fluid during the last 35 days of gestation. (See appendix tables 6-10 and text pages 70-73)

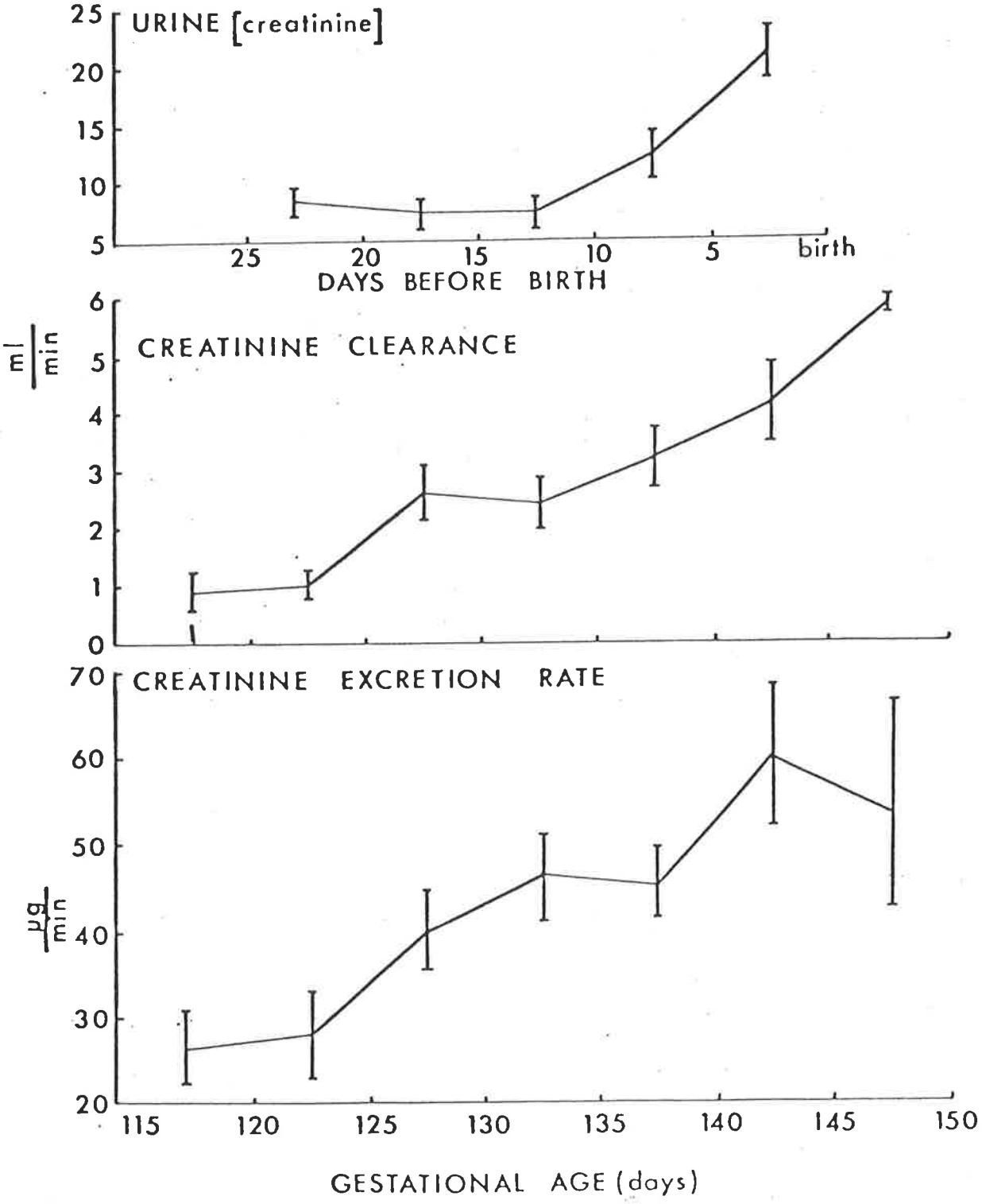


FIGURE 9. The concentration of creatinine in foetal urine during the 25 days prior to parturition; plus the excretion rate and clearance of endogenous creatinine throughout the last 35 days of gestation.
(See appendix tables 6-10 and text pages 70-73)

4.6 Foetal Urea

4.6.1 Urine urea

Urinary urea concentration increased significantly with increasing gestational age ($r = 0.311$, $0.01 > P > 0.001$, $n = 140$). The average urea concentration for the period between days 115 and 120 was 156.6 mg/100ml, compared with an average of 293.7 mg/100ml between days 145 and 150 (appendix table 7). However, an examination of the average values for each 5 day interval indicates that the relationship between urea concentration and foetal age was not linear (see fig. 10). The values increased most rapidly from day 130 until term (with the exception of the period between days 135 and 140). An exponential relationship was also seen when the results from foetuses which lambed were considered alone (see fig. 10). Here again, a significant correlation existed but was reduced, presumably because of the decreased number of observations ($r = 0.281$, $0.01 > P > 0.001$, $n = 78$). In the period from 22 days before birth to 10 days before birth, the average concentration of urea in foetal urine was 118.2 mg/100ml ($n = 28$), yet over the 5 days immediately before delivery the value was 264.1 mg/100ml ($n = 22$) (appendix table 8).

When the urinary urea values were corrected for total kidney weight the correlation with foetal age was reduced ($r = 0.201$, $0.05 > P > 0.01$, $n = 140$) indicating that over the entire range, between days 115 and 150, increasing kidney size contributed to the rise in urea concentration. However, when the weight correction was applied only to the data from those foetuses which were delivered normally, a highly significant relationship between urinary urea and foetal age was revealed ($r = 0.292$, $0.01 > P > 0.001$, $n = 78$). This is not surprising since in this case there were a large number of observations near parturition and it is in this period that substantial increases in urinary urea concentration occurred, while

changes in foetal kidney weight are minimal. therefore, factors other than kidney size were responsible for the rapid rise in urinary urea concentration during the last 15 days of gestation. It was possibly related to the increasing ability of the foetal liver to produce urea. (Kennan and Cohen 1959; Colombo and Richterich 1968).

The relationship between urea excretion rate and foetal age was similar to that for urinary urea concentration, although variation was increased due to the inclusion of flow rate variability (appendix table 10). Urea excretion rate was correlated with foetal age ($r = 0.353$, $P < 0.001$, $n = 120$) and as might be expected, there was a particularly rapid increase in the rate of urea excretion between day 135 and term. When the values for urea excretion rate were corrected for kidney weight, the correlation was reduced ($r = 0.280$, $0.01 > P > 0.001$, $n = 120$) indicating that increasing kidney size contributes to the rise in the excretion of urea.

The average concentrations of urinary urea obtained in this study are similar to those recorded by Alexander (1958a, 1961) for foetuses of comparable ages. They are, however, higher than those reported by Mellor and Slater (1974). With respect to gestational trends, Mellor and Slater's results showed only a slight increase in the urea concentration of foetal urine during the 10 days from day 139 to day 148. Even in this period, the average value for urea concentration was lower than for the same period in both Alexander's work and the present study.

4.7 Foetal Urine pH

The daily averages for urine pH are shown in figure 11. There was no correlation between individual pH values and foetal age ($r = -0.04$, NS, $n = 184$) (appendix table 11). There was however, a decrease in urine pH on days 149 and 150, but the small number of observations obtained for those days, limits the reliability of that

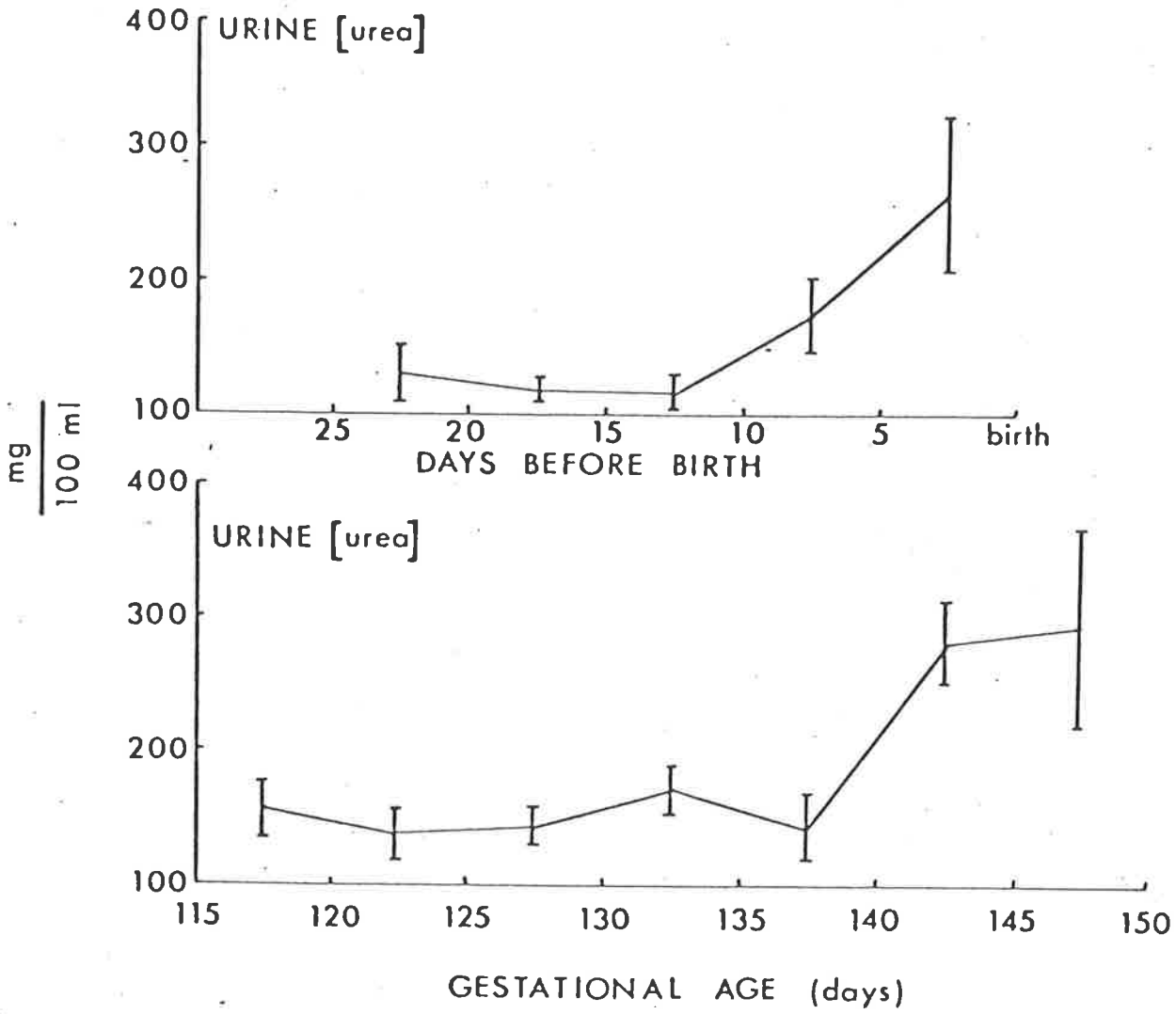


FIGURE 10. The concentration of urea in foetal urine during the last 35 days of gestation.
(See appendix tables 7, 8 and 10 and text pages 74 and 75)

finding. When the results from those foetuses which delivered normally were considered alone, the pre-parturient decline in foetal urine pH was more apparent. Figure 11 shows that from 25 days before parturition until delivery, there was a steady decline in the average pH of foetal urine ($r = -0.340$, $0.01 > P > 0.001$, $n = 105$), however during the 5 days immediately before birth, the decline in pH was small.

A surprising difference between these two sets of data is that the average values obtained from foetuses which lambed are comparatively high in the period between 25 and 15 days before birth. Also, despite the marked pre-parturient fall in urine pH, the lowest value recorded was 6.90 and the overall average for urine pH during the last 5 days of pregnancy was 7.49. Thus, even with the obvious decrease in uring pH during the last 25 days of gestation, the urine pH in those foetuses which delivered normally, was never as low as the average values for day 149 and 150 in the general data. Nevertheless, the decline over the last 25 days of gestation was a significant feature of the urine pH changes and when the kidney weight variation was removed from these observations, the pH changes were even more closely correlated to foetal age ($r = -0.511$, $P < 0.001$, $n = 105$). This suggests that the pH changes reflect physiological developments that are independent of kidney growth.

Mellor and Slater (1972a), measured pre-parturient changes of foetal urine pH in 10 foetuses. In all cases the pH fell sharply near term, beginning between days 130 and 145. Mellor and Slater found that before the decline, the pH values were quite stable, varying between 7.0 and 7.4, but then, over a 5 to 9 day period, the pH fell as low as 5.4.

There is general agreement between Mellor and Slater's results and those of the present study, although the average pH values before the pre-parturient decline, were higher in the present work. Mellor and Slater also found that the decline in foetal urine pH could commence as early as 13 days before birth which agrees with the findings of the

present study.

4.8 Foetal Urine Flow

As mentioned, urine flow rate showed a great deal of variation, not only from day to day during gestation, but also within any one day. The standard errors of the daily averages were usually large and there was no significant correlation between flow rate and foetal age ($r = -0.039$, NS, $n = 202$). However, when the flow rates were corrected for changes in foetal kidney weight, a small negative correlation emerged which was not quite significant at the 5% level ($r = -0.121$, NS, $n = 202$) (see fig. 11). The mean flow rate per gm of kidney, for the period between days 115 and 120 was 0.03 ml/min compared with 0.019 and 0.017 ml/min. between days 130 and 135 and days 145 and 150 respectively (appendix table 12). These results agree in part, with the findings of Alexander et al (1958b) who observed that foetal urine flow increased to a maximum of 0.64 ml/min. on the 117th day of gestation and then declined to a minimum of 0.14 ml/min. near term. Alexander et al (1958b) found that when the urine flow rates were corrected for foetal body weight, this negative relationship was more apparent.

Finally, although the flow rate averages were influenced by the inherent variability of urine flow, thereby making comparisons difficult, it is interesting that the average, for uncorrected flow rate between days 125 and 145 in the present study, was as high as 0.58 ml/min. This is considerably higher than the average of 0.28 ml/min. reported by Alexander et al (1958b) for the period between days 123 and 142 and the average of 0.24 ml/min. for a similar period reported by Bernstine (1970).

4.9 Foetal Glomerular Filtration Rate (GFR)

GFR was measured in foetuses at various stages of gestation using the inulin ^{14}C method described in Section 3. The GFR values obtained

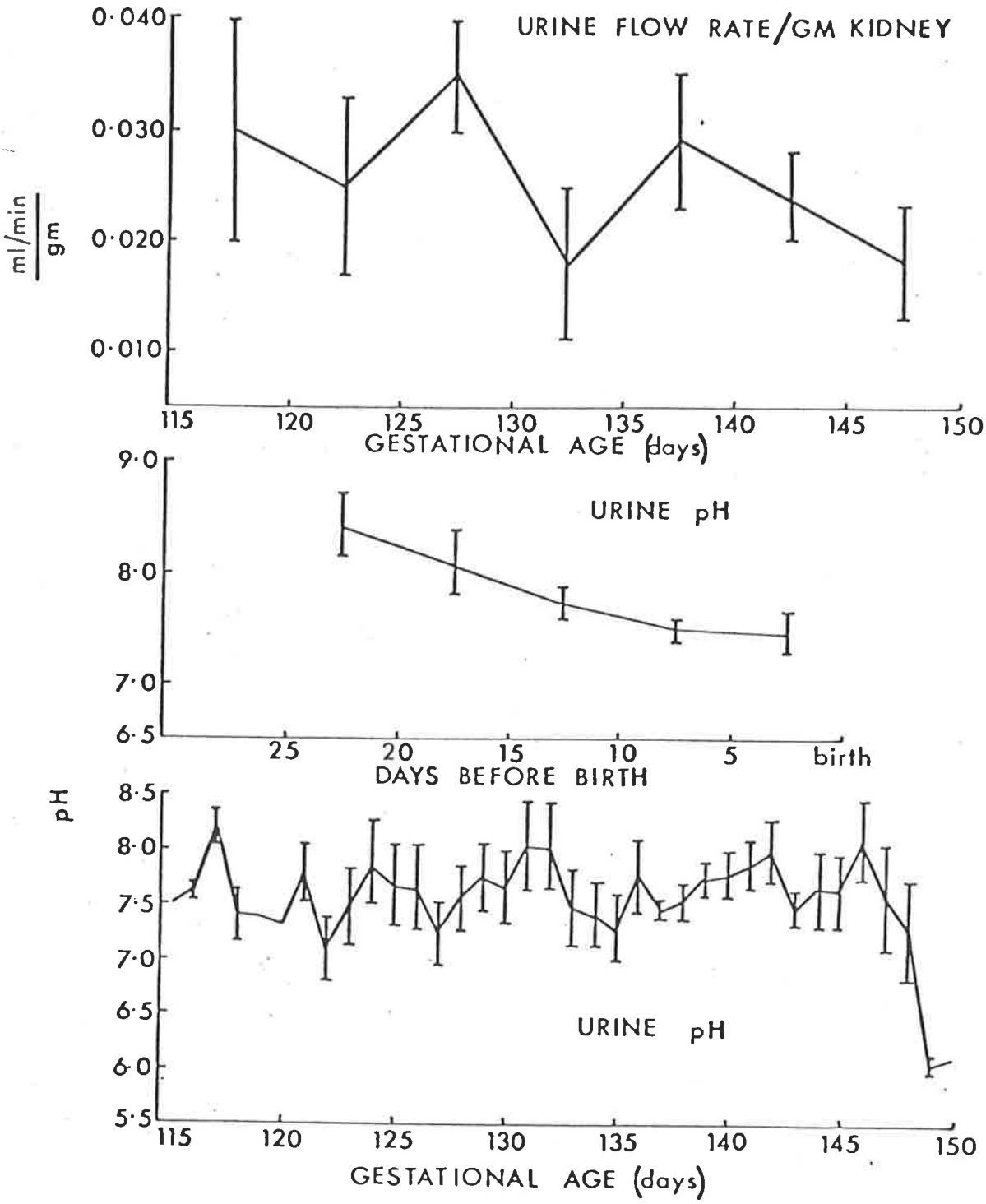


FIGURE 11. The flow rate and pH of foetal urine during the last 35 days of gestation.
(See appendix tables 11 and 12 and text pages 75-77)

using untreated foetuses were significantly correlated with foetal age ($r = 0.842$, $P < 0.001$, $n = 18$) (see fig. 12) and even when the individual values were corrected for total kidney weight, the correlation remained significant ($r = 0.791$, $P < 0.001$, $n = 18$) (appendix table 12). In addition to these composite results, table 4 shows GFR measurements in a single lamb (274) from the 128th day of gestation until 2 days after birth.

All samples of plasma and urine obtained for GFR measurement were divided into 2 aliquots. One aliquot was prepared for the measurement of inulin ^{14}C activity while the other aliquot was subjected to the normal analyses to determine the concentration of creatinine, uric acid, Na^+ and K^+. Accordingly, the clearance values for these solutes could be determined in additon to inulin ^{14}C clearance. Table 5 shows the clearance values for these solutes compared with the simultaneously determined inulin clearances.

Since endogenous creatinine clearance is often used as an estimate of GFR, it is important to note the very close correlation between inulin ^{14}C clearance and endogenous creatinine clearance. When all simultaneously determined values for inulin ^{14}C clearance and endogenous creatinine clearance are considered, including those from experimental situations, the close relationship between the two estimates of GFR is confirmed ($r = 0.920$, $P < 0.001$, $n = 32$)

Gresham et al (1972) carried out studies of GFR using foetuses aged between 122 and 134 days and simultaneously determined the clearance rates of fructose, urea, creatinine, sodium and chloride. The results obtained from Gresham et al concerning those solutes which were also studied in the present work, are shown in table 6. In the original report these data were corrected for foetal body weight, but to permit comparison with the results obtained in the present study, these adjustments have been reversed.

TABLE 4: GFR DURING GESTATION AND THE EARLY POST-NATAL PERIOD (LAMB 274)

GESTATIONAL AGE (days)	GFR (Inulin ^{14}C clearance) (ml/min) mean ± SE	
128	3.86 ± 0.28	(n=2)
130	2.84 ± 0.41	(n=5)
132	4.25	(n=1)
139	4.60	(n=1)
140	5.07 ± 0.17	(n=2)
142	7.42 ± 0.30	(n=3)
144	6.76 ± 0.20	(n=2)
146	6.21 ± 0.21	(n=2)
2nd day post-partum	10.15 ± 2.54	(n=4)

(See text page 78)

TABLE 5: SUMMARY OF FOETAL RENAL CLEARANCES

GESTATIONAL AGE (days)	CLEARANCE				
	INULIN	^{14}C	CR	UA	Na^+	K^+
	(ml/min)				
128	3.47		3.09	0.77	0.0599	0.75
128	4.25		3.32	0.77	0.0645	0.83
130	1.09		0.73	0.10	0.1072	0.37
130	2.76		2.16	0.60	0.1567	0.95
130	3.69		3.03	0.34	0.0421	0.53
130	3.49		3.59	0.24	0.0327	0.49
130	3.16		2.58	0.18	0.0241	0.43
139	4.60		4.23	0.53	0.0689	1.01
140	5.30		4.98	0.25	0.1688	2.15
140	4.83		3.85	0.38	0.1117	2.03
142	7.54		4.07	0.39	0.2323	2.11
142	7.98		4.94	0.15	0.2397	2.32
142	6.75		5.50	0.46	0.2222	1.61
144	6.90		6.48	0.34	0.1924	1.99
144	6.61		6.07	0.21	0.1393	1.40
146	6.05		5.84	0.55	0.3799	3.26
146	6.36		5.84	0.32	0.3243	2.48

(See text page 78)

TABLE 6: SUMMARY OF FOETAL RENAL CLEARANCES (Gresham et al 1972)

GESTATIONAL AGE (Days)	CLEARANCE		
	INULIN	^{14}C	Cr	Na^+
	(ml/min)		
122	1.41	-	0.0284
125	1.77	1.92	0.0425
126	1.65	-	0.0240
128	2.00	2.09	0.0964
128	1.85	2.85	0.1014
129	1.63	3.17	0.3420
131	2.34	3.46	-
131	3.08	3.76	-
132	2.52	4.04	-
132	1.91	3.59	-
134	2.54	-	-

(See text page 78)

Apart from the work of Gresham et al, other clearance studies have been carried out on exteriorised foetal lambs (Alexander et al 1958b; Alexander and Nixon 1962; Smith et al 1966; and Smith and Schwarz, 1970). The results of these experiments are not comprehensive and are highly variable.

4.10 Foetal Blood Pressure

The mean arterial pressure (MAP) of foetal lambs was variable but showed a general increase as gestation proceeded (see fig. 12) as did GFR (determined using inulin ^{14}C). Individual GFR values were closely correlated with the corresponding flow rates ($r = 0.776$, $P < 0.001$, $n = 3$]) (appendix table 12).

4.11 Tubular Reabsorption

In the preceding section it was established that simultaneously determined values for inulin ^{14}C clearance and endogenous creatinine clearance are highly correlated. Each endogenous creatinine clearance value was usually about 80% of the corresponding value for inulin ^{14}C clearance ($80.47 \pm 2.25\%$ $n = 32$). This relationship was very consistent. Since inulin ^{14}C clearance is a reliable index of GFR, it follows that endogenous creatinine clearance, despite the 20% variation, can be used to monitor GFR changes. Having established the usefulness of endogenous creatinine clearance as an estimate of GFR, the creatinine clearance value, calculated from the creatinine concentrations in each paired urine-plasma sample was combined with the $[Na^+]$ and $[K^+]$ of the plasma to calculate the rate of filtration of these electrolytes. It was assumed that Na^+ and K^+, are freely filtered at the foetal glomerulus. These filtration rates were then compared with the corresponding electrolyte excretion rates, and the percentage reabsorption of each electrolyte

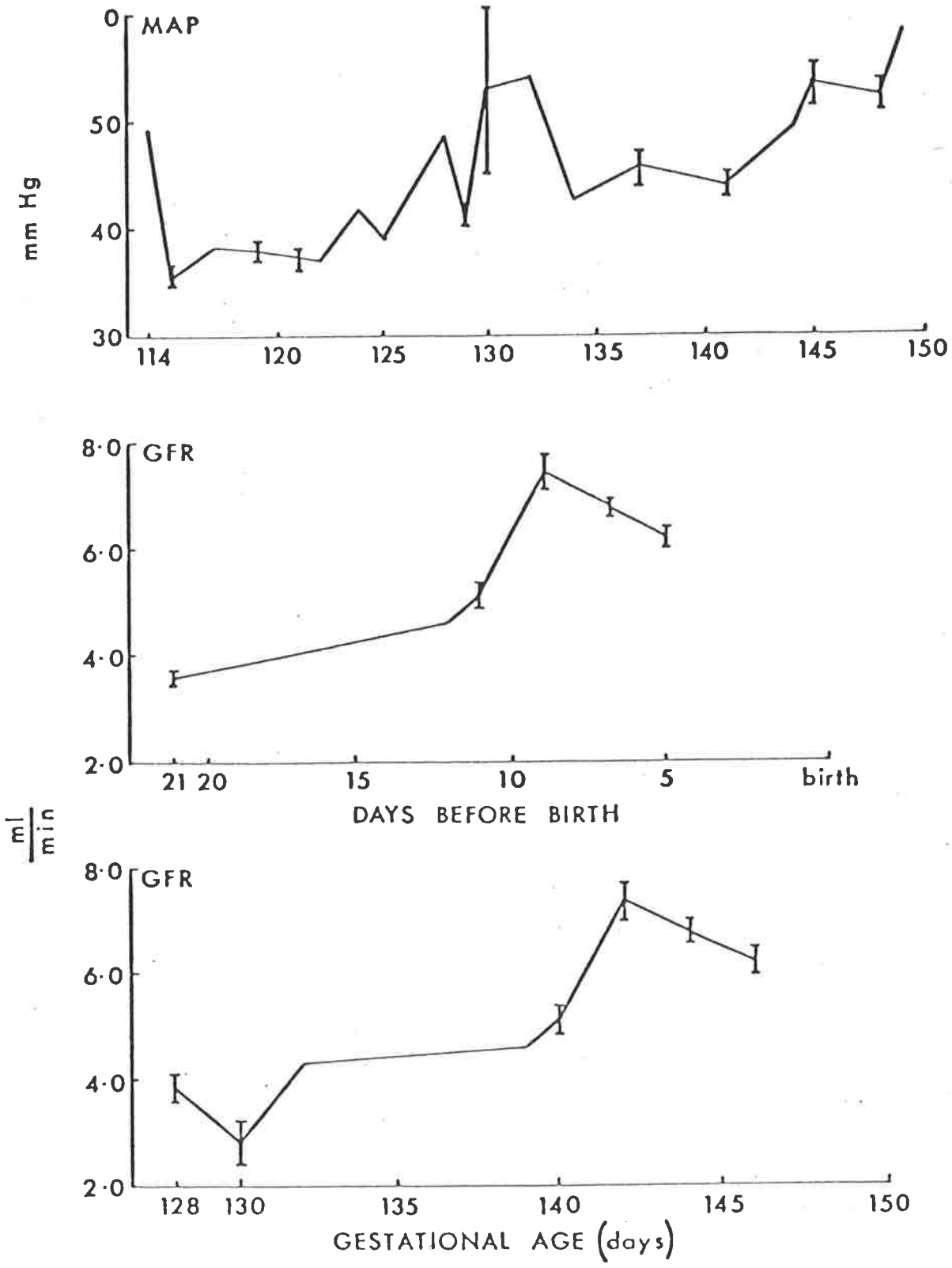


FIGURE 12. Foetal mean arterial pressure (MAP) and glomerular filtration rate (GFR) during the last 35 days of gestation.
(See appendix tables 12 and 13 and text pages 77-79)

was determined. The percentage reabsorption of water was calculated for each pair of samples simply by comparing the GFR (as estimated by creatinine clearance) with the urine flow rate measured, during collection of the urine sample.

4.11.1 Reabsorption of water

The percentage reabsorption of water was positively correlated with foetal age ($r = 0.201$, $0.05 > P > 0.01$, $n = 144$). It increased from an average of 75.7% ($n = 10$) between days 115 and 125 to an average of 86.3% ($n = 10$) between days 145 and 150 (see fig. 13) (appendix table 14). This is consistent with the general observation, both in this and other studies, that foetal urine flow decreases with increasing foetal age.

4.11.2 Reabsorption of sodium

When the individual values for sodium reabsorption were compared with gestational age, no significant correlation emerged ($r = 0.002$ NS $n = 126$). However, when the values were considered in relation to the time of parturition a negative correlation was found ($r = -0.283$, $0.05 > P > 0.01$, $n = 66$) (see fig. 13). Between the 25th and 15th days before birth, the average value for percentage sodium reabsorption was 98.3% ($n = 9$) compared with an average of 89.9% ($n = 12$) in the 4 days immediately before birth (appendix table 14). Thus there was some evidence that the tubular reabsorption of sodium decreased as term approached. This appears to contradict the earlier finding that urinary $[Na^+]$ decreased as gestation proceeded. However, when it is considered that Na^+ excretion rate, and in particular Na^+ excretion rate per gm of kidney was positively correlated with foetal age ($r = 0.190$, $0.01 > P > 0.001$, $n = 180$), then the contradiction is not so apparent.

4.11.3 Reabsorption of potassium

The reabsorption of potassium showed an overall decrease

whether considered in relation to gestational age, or to time of parturition. When considered in relation to gestational age, the correlation coefficient was -0.188 ($0.05 > P > 0.01$, n = 113) (see fig. 13). The average value for percentage K^+ reabsorption between days 115 and 125 was 56.2% (n = 9) compared with an average of 45.4% (n = 22) between days 135 and 140. However, in the last 5 day period (days 144 to 149) there was an increase in K^+ reabsorption, to an average of 50.6% (n = 14) (appendix table 14). When potassium reabsorption was considered in relation to the time of parturition, the negative correlation was retained ($r = -0.249$, $0.05 > P > 0.01$, n = 61), but there was less evidence of an increase in percentage reabsorption in the period immediately before birth, possibly because of the reduced number of observations.

4.12 Microscopy

The kidney of the foetal lamb develops successively through 3 stages; pronephros, mesonephros and metanephros (Tanagho 1972 and Lewis 1958). In the present work, all studies have been carried out after the 100th day of gestation, by which time the metanephros has long been established. Accordingly the data obtained relates solely to metanephric function, but there is little information available on the maturational changes that occur during the metanephric phase.

As reported, some of the urinary parameters examined during the present work showed patterns of change that were correlated with foetal age and often these correlations were retained even when the results were corrected for changes in foetal kidney weight. Therefore it was decided to examine kidney morphology to determine what structural changes are evident that may be related to the functional changes described above.

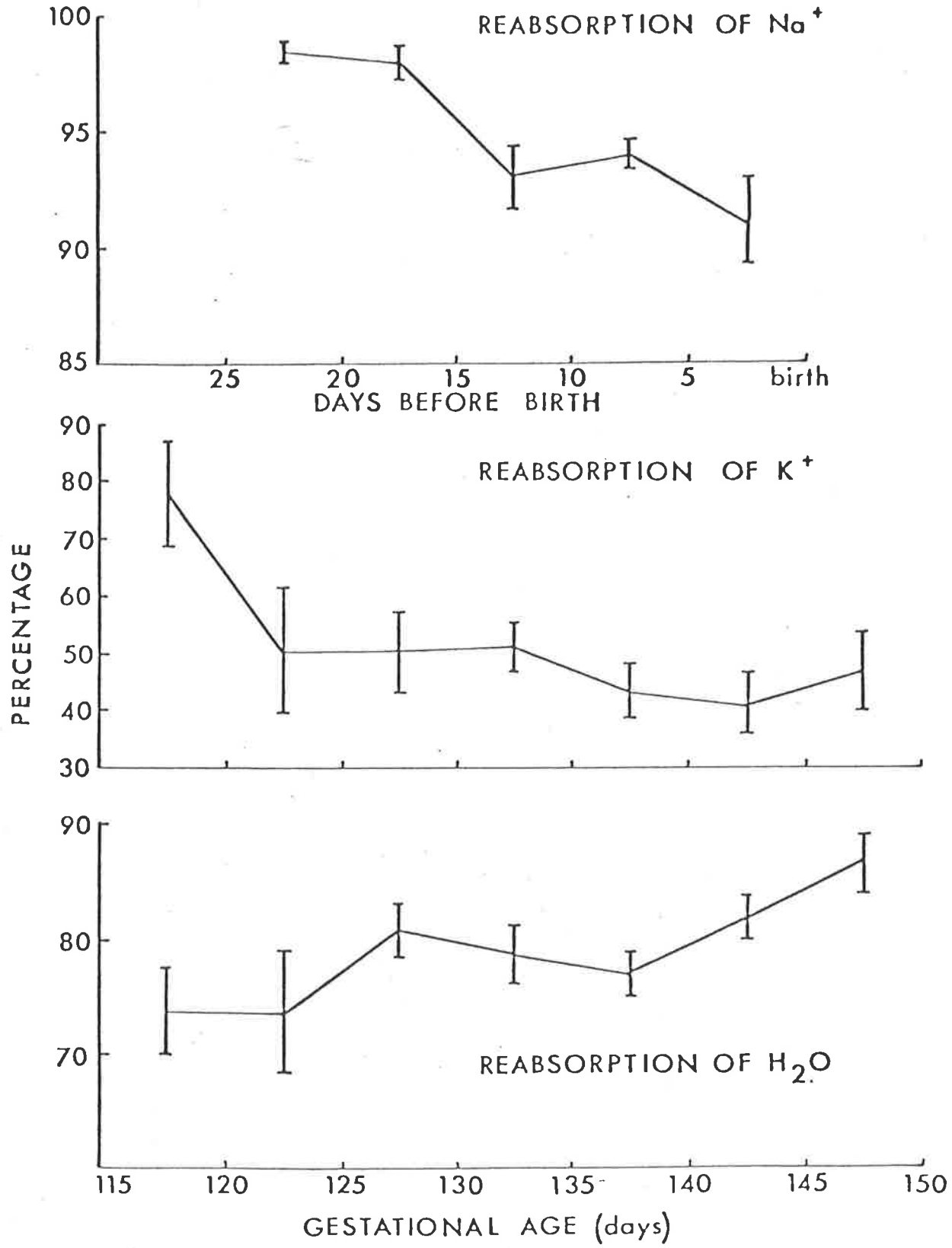


FIGURE 13. The percentage reabsorption of Na^+, K^+ and water by the foetal kidneys during the last 35 days of gestation.
(See appendix table 14 and text pages 80 and 81)

4.12.1 Light microscopy

The right kidney was taken from 6 foetuses aged between 119 and 145 days and sections from each kidney were prepared for light microscopy as described in section 3. Photo-micrographs of these sections were produced at precisely determined magnifications and the micrographs used for qualitative and quantitative analyses of kidney morphology.

(a) Micrographs of low magnification were used to count the number of glomerulii in 4 separate fields in each of the 6 kidneys. The average number of glomerulii per mm^2 of cortex was calculated from the 4 measurements (See plates 6 and 7).

(b) Micrographs of low magnification were also used to measure the depth of cortex in each kidney. To minimise errors caused by variation in the plane of section, measurements of cortical depth were made only where medullary rays had been sectioned longitudinally. It is known that the tubules which constitute the medullary rays follow a relatively straight course, from the cortex to the medulla. Therefore, the distance between the renal capsule and the cortico-medullary junction, was measured adjacent to these longtitudinally sectioned medullary rays. Several measurements were made for each kidney and the average calculated. (See plate 6).

The depth of the medulla was also measured. These measurements were obtained by extending the line of measurement used for cortical depth through to the base of the renal pyramids and measuring along this line, from the base of the pyramids to the cortico-medullary junction.

(c) Micrographs of high magnification were used to measure the size of glomerulii. Since the glomerulii were invariably oval in section, the long and short diameter of each glomerulus was measured and an average calculated. The parietal layer of Bowman's capsule was taken as the outer perimeter of the glomerulii for the measurement of diameters (see plate 8).

Forty glomerulii were measured in micrographs from each of the 6 kidneys and an average glomerular diameter calculated for each kidney. A large number of glomerulii were used for each average, to allow for differences in diameter resulting from variation in the plane of section.

The results of this work are shown in table 7 which also lists the age and weight of the foetuses used and gives the weight of the kidneys taken for examination. It is apparent, from the table, that despite regular increases in body weight and kidney weight, there was no comparable increase in the number of glomerulii per mm^2 of cortex. This is contrary to findings in human foetuses. Vernier and Birch-Andersen (1962) reported that the number of nephrons and therefore the number of glomerulii seen in foetal kidney sections, increased from about 35×10^4 at 20 weeks of pregnancy to 82×10^4 at 40 weeks. In an earlier study, MacDonald and Emery (1959) found that new glomerulii were formed up until the 36th week of gestation and Potter and Thierstein (1943) suggested that the production of new glomerulii was closely related to the increase in foetal body weight.

The difference between these earlier findings and those of the present study may be due in part to the greater immaturity of the human foetus at birth, compared with the foetal lamb. Unfortunately, no other studies of glomerular development in foetal lambs have been carried out.

With the exception of the 119 day-old foetus, there was a general increase in the depth of the cortex with increasing foetal age and the cortex formed an increasing proportion of the total depth of the kidney. Presumably therefore, the volume of cortical tissue occupied an increasing proportion of the total kidney volume. Accordingly, it could be argued that even though the number of glomerulii per unit of cortical tissue did not appear to increase with foetal age, the increasing size of the cortex would result in a greater number of glomerulii. There was

TABLE 7: QUANTITATIVE ASSESSMENTS OF FOETAL RENAL MORPHOLOGY

GESTATIONAL AGE (Days)	FOETAL WEIGHT (kg)	KIDNEY WEIGHT (gm)	GLOMERULAR DIAMETER (μ) $\bar{X} \pm SE$	NO. GLOMERULII/ mm^2 OF CORTEX $\bar{X} \pm SE$
119	1.5	7.6	69.8 ± 1.1 (n=40)	27.6 ± 1.8 (n=4)
123	1.6	8.1	65.9 ± 1.5 (n=40)	22.4 ± 0.8 (n=4)
132	2.6	8.4	66.9 ± 1.4 (n=40)	22.1 ± 1.3 (n=4)
138	2.9	9.1	65.8 ± 1.0 (n=40)	23.1 ± 1.2 (n=4)
141	3.1	9.9	63.2 ± 1.6 (n=40)	21.1 ± 1.1 (n=4)
145	3.3	10.2	67.9 ± 1.5 (n=40)	20.9 ± 0.5 (n=4)

(See text page 83)

TABLE 7: QUANTATIVE ASSESSMENTS OF FOETAL RENAL MORPHOLOGY (Cont.)

GESTATIONAL AGE (Days)	DEPTH OF CORTEX (mm) $\bar{X}$ ± SE		DEPTH OF MEDULLA (mm) $\bar{X}$ ± SE		CORTICAL DEPTH AS % TOTAL DEPTH $\bar{X}$ ± SE	
119	2.53 ± 0.04	(n=3)	4.55 ± 0.30	(n=3)	35.9 ± 1.1	(n=3)
123	2.60 ± 0.18	(n=3)	8.42 ± 0.04	(n=3)	23.6 ± 1.3	(n=3)
132	2.82 ± 0.23	(n=3)	6.73 ± 0.27	(n=3)	29.4 ± 1.5	(n=3)
138	2.65 ± 0.21	(n=3)	6.14 ± 0.16	(n=3)	31.3 ± 1.1	(n=3)
141	2.48 ± 0.11	(n=3)	5.12 ± 0.15	(n=3)	32.7 ± 1.5	(n=3)
145	3.43 ± 0.22	(n=3)	6.63 ± 0.09	(n=3)	34.0 ± 1.2	(n=3)

in fact a decline in the number of glomerulii per mm^2 as the cortex expanded suggesting that the cortical expansion was due to the growth and extension of nephron tubules rather than to the development of new nephrons. This is true in part, but if the youngest foetus is ignored, as it yielded results which are not consistent with those of the other foetuses, it can be seen by comparing the 123 day-old foetus with the 145 day-old foetus that there was a 7% decrease in the number of glomerulii/mm^2 of cortex. In contrast, there was a 35% increase in the proportion of cortical tissue in the kidney sections. Presumably therefore, in the intact kidneys of the older foetus there would have been a greater number of cortical glomerulii. The remaining kidneys would have had intermediary increases in the number of glomerulii.

Apart from the number of glomerulii seen in kidney sections, table 7 shows that there was no regular increase in the diameter of foetal glomerulii during the last 1/3 of gestation. The average glomerular diameter in the 6 foetuses studied, varied between 65 and 70μ. This represents about 50% of the adult diameter. In humans, the glomerulii at birth are approximately 100μ in diameter which is nearly ½ of the adult glomerular diameter.

Apart from these quantitative analyses of foetal kidney morphology, other qualitative features were noted. For example, there appeared to be little, if any, variation in the stage of development of the glomerulii of the 6 foetuses. Vernier and Birch-Andersen (1962) identified 3 stages of glomerular development in human foetal kidneys. They found that all 3 stages co-exist throughout gestation but that the proportion of the more mature glomerulii increases as gestation proceeds. In the present work, no glomerulii corresponding to the first stage of maturity, as described by Vernier and Birch-Andersen, were seen. The glomerulii in all foetuses showed a number of capillaries containing erythrocytes and therefore correspond to stage II and probably stage III glomerulii, as defined by

Vernier and Birch-Andersen.

The tissue between the cortical glomerulii appeared to contain the usual array of tubules. Proximal and distal tubules could be distinguished. (See plate 8). However, in the medulla some variation in tissue morphology was seen (see plates 9 and 10). The number of tubules in the medulla of the oldest foetus (145 days) was greater than in the youngest (119 days) and the other foetuses appeared to have intermediate quantities. Fetterman et al (1965) reported that in humans, 20% of the loops of Henle were still within the cortex at birth and that they gradually extended toward the renal pelvis. A similar process probably occurs during foetal life in sheep, producing the observed changes in the morphology of the renal medulla.

4.12.2 Electron microscopy

Electron micrographs were prepared, as described in section 3, and used to examine the fine structure of foetal glomerulii and renal tubules. A total of 120, in focus, electron micrographs of various magnifications were examined.

Vernier and Birch-Andersen (1962) carried out an electron microscope study of glomerulii in human foetuses. They noted a morphological sequence in the formation of the basement membranes plus the progressive attentuation of capillary endothelium and the appearance of greater numbers of endothelial fenestrae. They also noted increased development of epithelial cell foot processes in the more mature foetuses.

In view of these findings, the present study paid particular attention to similar features of glomerular ultra-structure but no comparable changes were observed. Even in the youngest foetuses examined, the glomerular fine structure resembled that of the mature glomerulii described by Vernier and Birch-Andersen (see plate 10). Well developed basement membranes were present. Also, epithelial cell foot processes were present around much of the circumference of the glomerular capillaries and long

sections of thin endothelium, containing numerous fenestrae were evident. In some cases these fenestrae were crossed by a diaphragm and other diaphragms were seen extending between adjacent foot processes. These diaphragms were not always present and it was impossible to assess whether their absence was normal or an artefact of preparation. (See plates 11 and 12).

The glomerular features mentioned above were present in all of the foetal kidneys examined, but there was no evidence of any sequential change in these or any other features of glomerular ultra-structure. No attempt was made to quantify the relative number of fenestrae or foot processes in the various foetuses, but estimates were made of the thickness of the basement membranes, diameter of the endothelial fenestrae and the size of the filtration slits.

The basement membranes were estimated to be between 400Å and 800Å thick and where a well defined lamina densa was apparent, it was usually about 500Å wide. The endothelial fenestrae were between 150Å and 350Å in diameter while the filtration slits were 250 to 500Å wide.

The fine structure of the kidney tubules was also examined. All of the structural features found in adult renal tubules were present in the foetuses and there was no apparent variation between foetuses. The apical surfaces of the proximal tubule cells showed transverse or longitudinal sections of the micro-villi which form the brush border (see plate 13). In some cases apical vacuoles and tubules were present immediately beneath the cell membrane. These structures have been described in adult human kidneys by Rhodin (1958, 1963) and by Tisher et al (1966), and are thought to be involved in micropinocytosis. The most striking feature of the proximal tubule cells in all foetuses was the large number of organelles, including round or oval mitochondria and vacuoles, plus numerous cytoplasmic granules. Unlike the apical membranes, the basal membranes were smooth and continuous and there was no indication, in any

of the foetuses, of the invaginations of the basal membranes which produce the laminated appearance in the cytoplasm of adult proximal tubules (Rhodin 1963, Tisher et al 1966).

Sections of distal tubules are shown in plate 14. It can be seen that the lumen of each tubule was open and there were no microvilli on the apical surfaces of the cells. The number of mitochondria in distal tubule cells was less than in proximal tubule cells and there were few other organelles; although occasional cytosomes and vacuoles were evident. Some distal tubule cells showed in folding of the basal membrane, a feature which has been reported in the distal tubules of mice (Clark 1957). However, none of these features of distal tubule fine structure were peculiar to a particular foetus. As with the proximal tubules there was no evidence of ultra-structural changes which might reflect maturational developments.

4.13 Hormonal Influence on Foetal Kidney Function

To study the influence of hormones on foetal kidney function, three approaches were used. Firstly, studies were carried out on the concentration of various hormones in foetal blood during the period of gestation already studied with respect to urine output and composition. The hormones considered were those of importance during gestation and for which reliable assay techniques were available. Cortisol was studied as a means of assessing the functional maturity of the foetal adrenals while PRA was estimated to examine the possible involvement of the renin-angiotensin system in perinatal kidney function. Progestins were measured because of their importance during gestation and because both progesterone and 17∝-hydroxy-progesterone (17∝ HP) have been reported to be aldosterone antagonists(Landau and Lugibihl 1958; Visser et al 1964). Another progestin, 20∝-hydroxypregn-4-en-3-one (20∝HP), was measured because it has been demonstrated in foetal sheep that progesterone

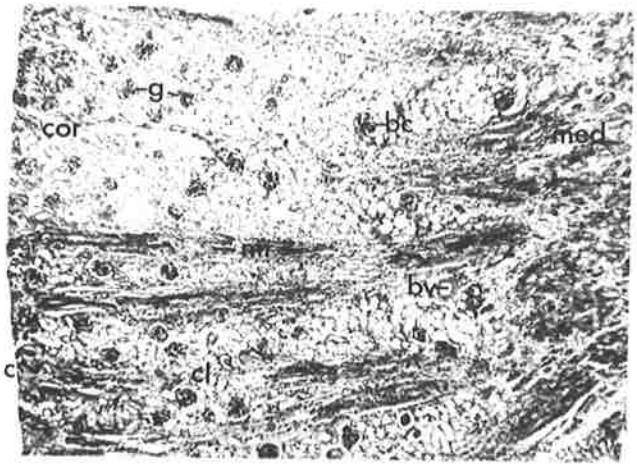
PLATE 6.

A section of renal cortex (123 day-old foetus: x25).

A section of renal cortex (141 day-old foetus: x25).

An area of renal cortex midway between the capsule and the cortico-medullary junction (138 day-old foetus: x120).

Key: bc = Bowmans Capsule, bv = blood vessel
c = capsule, cl = cortical labyrinth
cor = cortex, cd = collecting duct
g = glomerulus, med = medulla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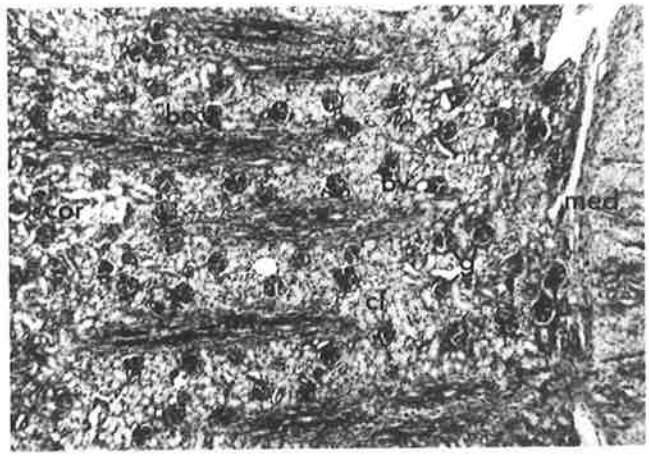
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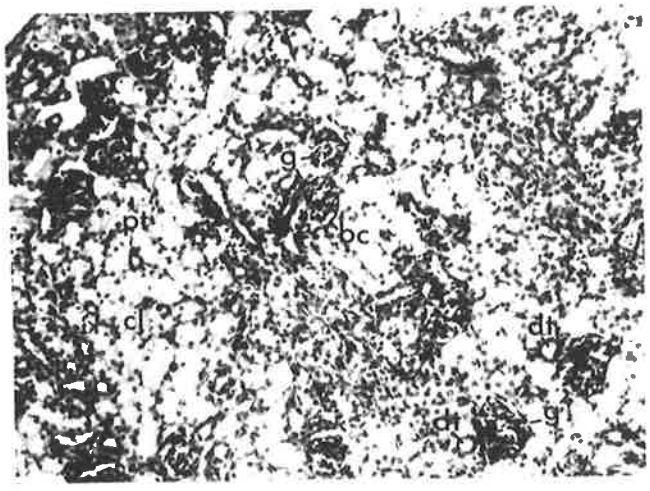
PLATE 7.

An area of renal cortex immediately below the capsule (119 day-old foetus: x120).

An area of renal cortex midway between the capsule and the cortico-medullary junction (141 day-old foetus: x120).

Cortical glomerulii and associated tubules (141 day-old foetus: x250).

Key: bc = Bowmans Capsule, c = capsule
cl = Cortical labyrinth, dt = distal tubule
g = glomerulus, pl = parietal layer
pt = proximal tubule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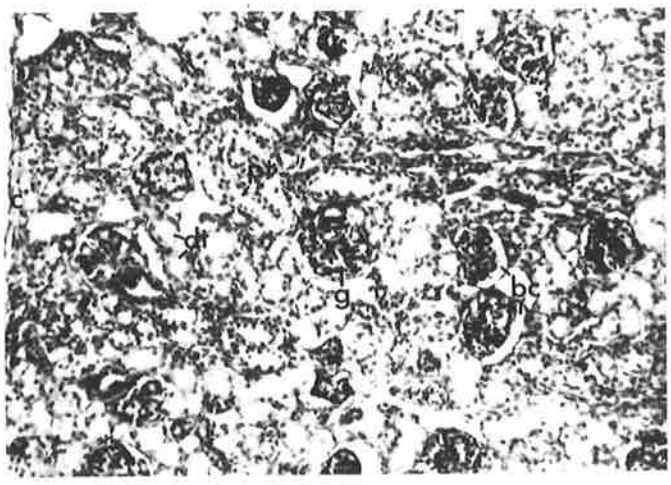
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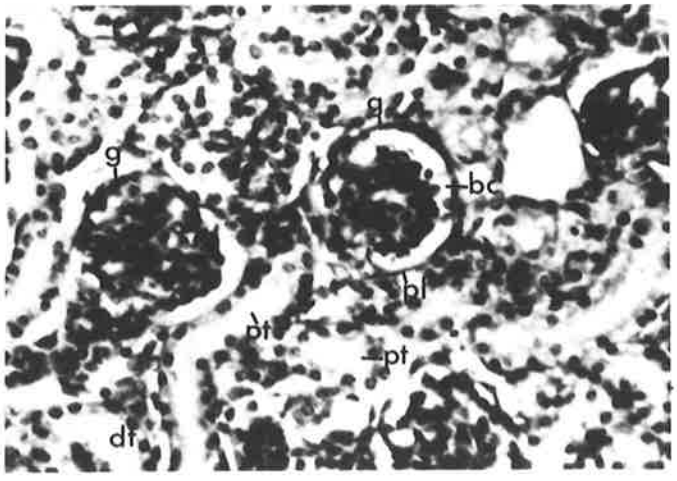
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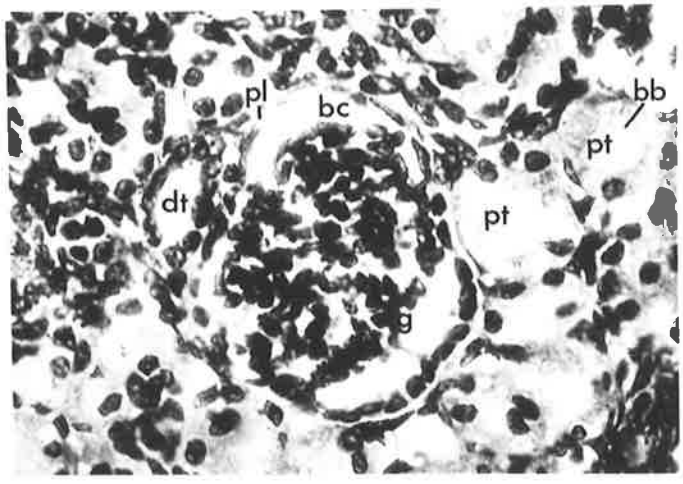
PLATE 8.

A cortical glomerulus and associated tubules, situated midway between the capsule and the cortico-medullary junction (119 day-old foetus: x600).

A cortical glomerulus and associated tubules, situated immediately beneath the renal capsule (132 day-old foetus: x600).

A cortical glomerulus and associated tubules, situated midway between the capsule and the cortico-medullary junction (141 day-old foetus: x600).

Key: bb = brush border, bc = Bowmans Capsule
dt = distal tubule, g = glomerulus
pl = parietal layer, pt = proximal tubule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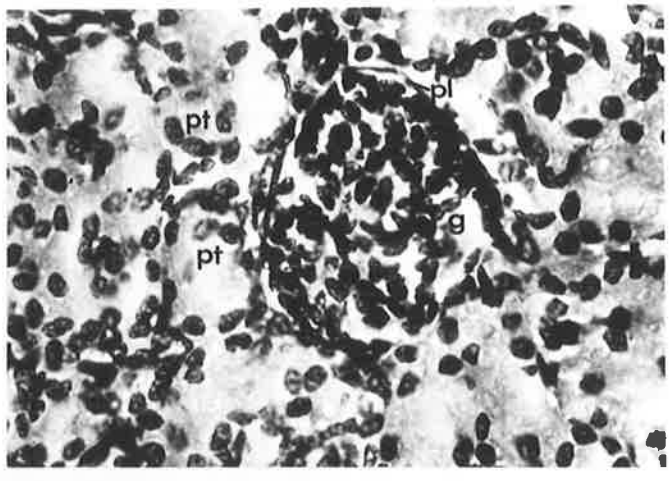
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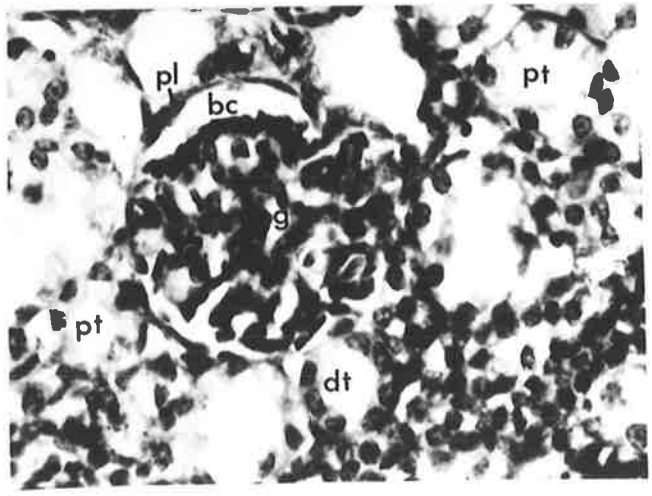
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PLATE 9.

An area of renal medulla, immediately beneath the cortico-medullary junction (123 day-old foetus: x120).

An area of renal medulla immediately beneath the cortico-medullary junction (141 day-old foetus: x180).

Medullary tubules located midway between the cortico-medullary junction and the renal sinus (145 day-old foetus: x600).

Key: cd = collecting duct, lh = loop of Henle
tklh = thick loop of Henle
tnlh = thin loop of Henle


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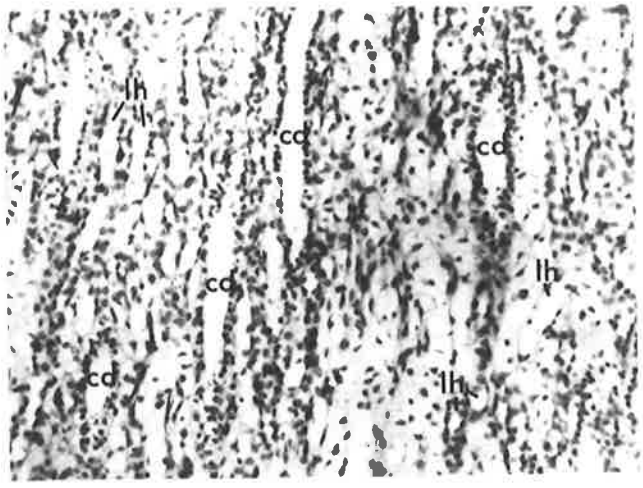
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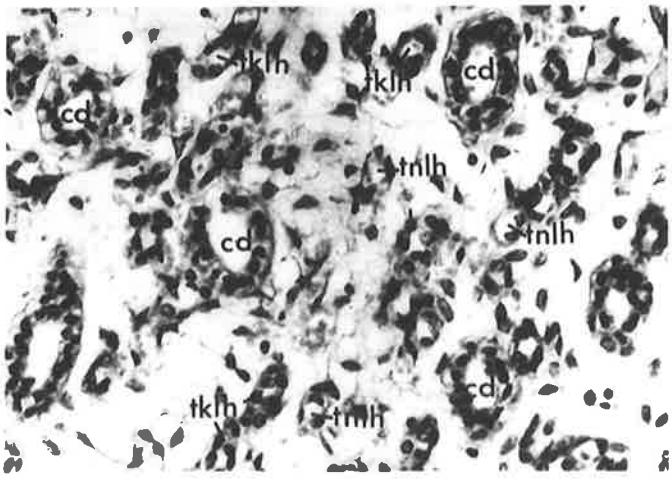
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cd
cd
tnlh
cd
tnlh
cd
tklh
tnlh

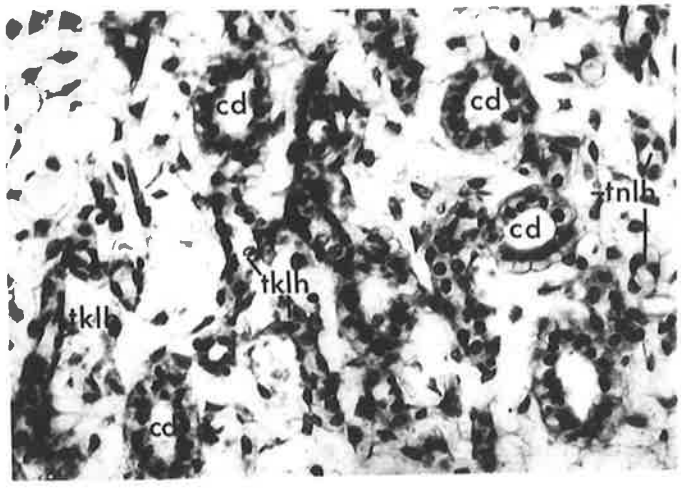
PLATE 10.

Medullary tubules located immediately beneath the cortico-medullary junction (132 day-old foetus: x600).

A section of a cortical glomerulus showing its general ultrastructure (123 day-old foetus: x1660).

A small area of a cortical glomerulus plus a section of an apposed proximal tubule (141 day-old foetus: x4200).

Key: ca = capillary, cd = collecting duct, bm = basement membrane
ep = epithelial cell, fp = foot process
pl = parietal layer, pt = proximal tubule
rc = red blood cell, tklh = thick loop of Henle
tnlh = thin loop of Henle


cd
cd
tnlh
cd
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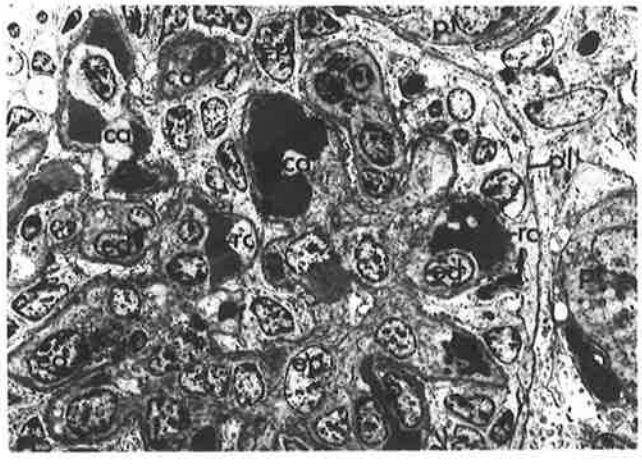
tkll
cd


pf
ca
ca
pl
rc
rc

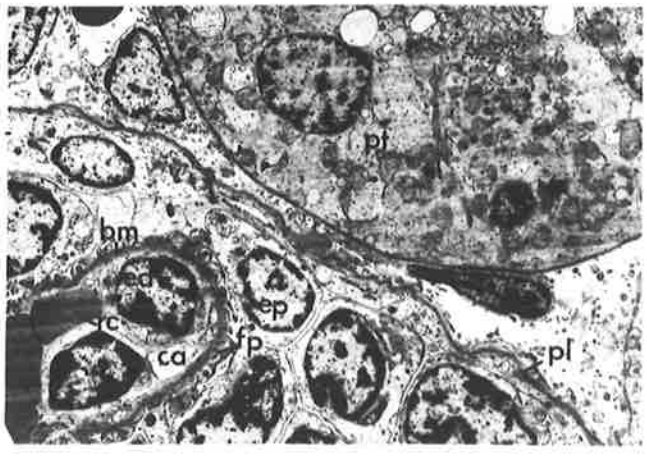

pf
bm
ep
rc
ca
fp
pf

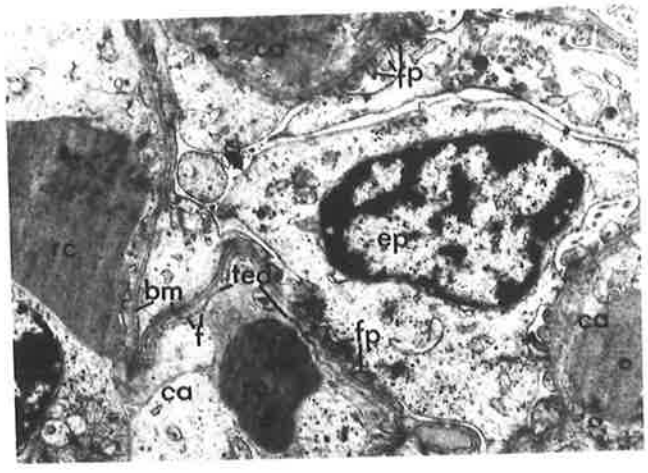
PLATE 11.

An epithelial cell with its foot processes apposed to the thin endothelium of glomerular capillaries (119 day-old foetus: x6600).

A section of epithelial cell cytoplasm with the associated foot processes apposed to the thin endothelium of a glomerular capillary (132 day-old foetus: x16,600).

An epithelial cell with its foot processes apposed to the thin endothelium of a glomerular capillary (138 day-old foetus: x6600).

Key: bm = basement membrane, ca = capillary
ed = endothelial cell, ep = epithelial cell, f = fenestra
fp = foot process, m = membrane
rc = red blood cell, ted = thin endothelium


fp
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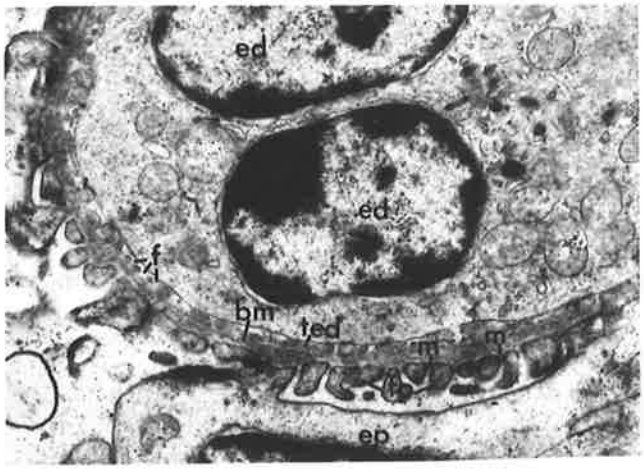
ed
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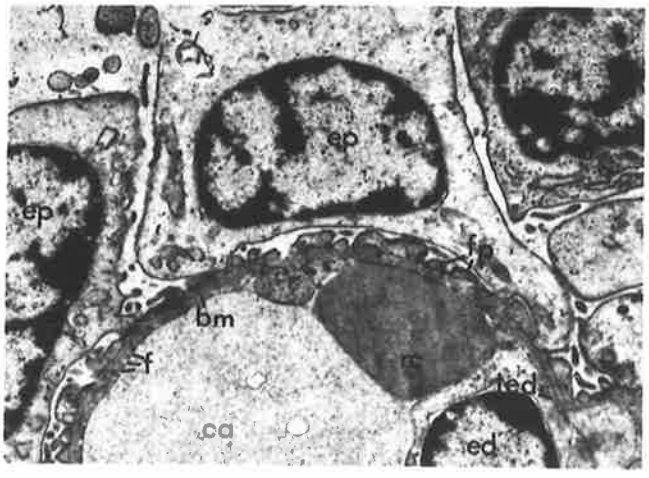
ep
ep
fp
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f
rc
ted
ca
ed

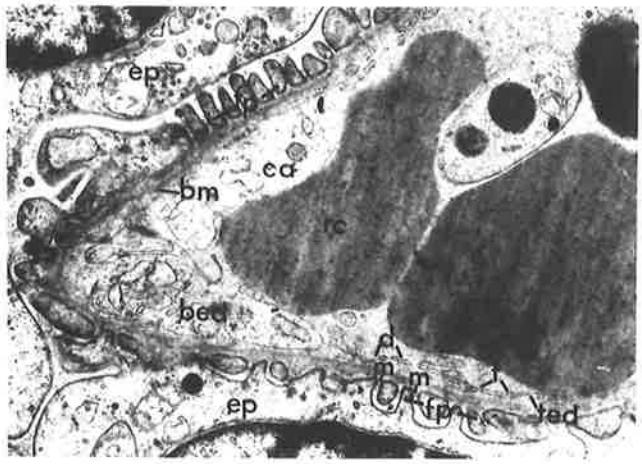
PLATE 12.

Sections of the cytoplasm of two epithelial cells with the foot processes apposed to a glomerular capillary (141 day-old foetus: x16,600).

An epithelial cell and an endothelial cell in apposition (145 day-old foetus: x16,600).

A section of epithelial cell cytoplasm formed into foot processes which are apposed to the thin endothelium of a glomerular capillary (145 day-old foetus: x18,300).

Key: bed = broad endothelium, bm = basement membrane
ca = capillary, d = diaphragm, ed = endothelial cell
ep = epithelial cell, f = fenestra, fp = foot process
m = membrane, rc = red blood cell, ted = thin endothelium
v = vacuole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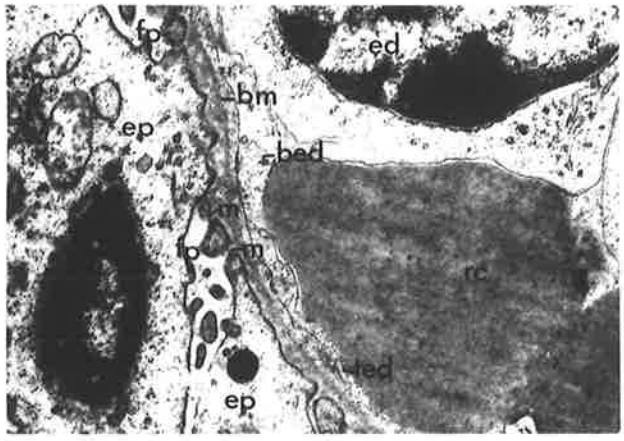
fp
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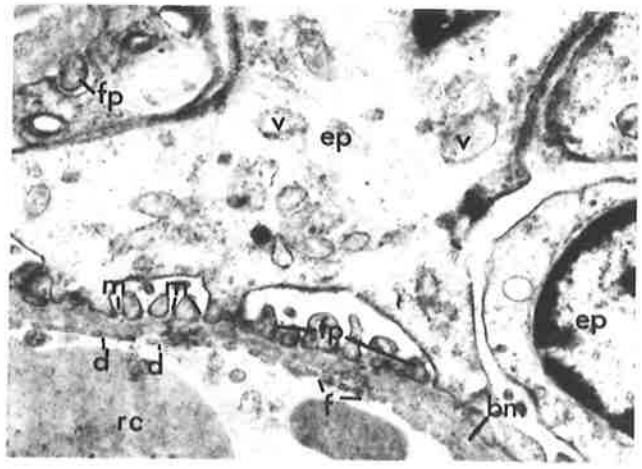
fp
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d
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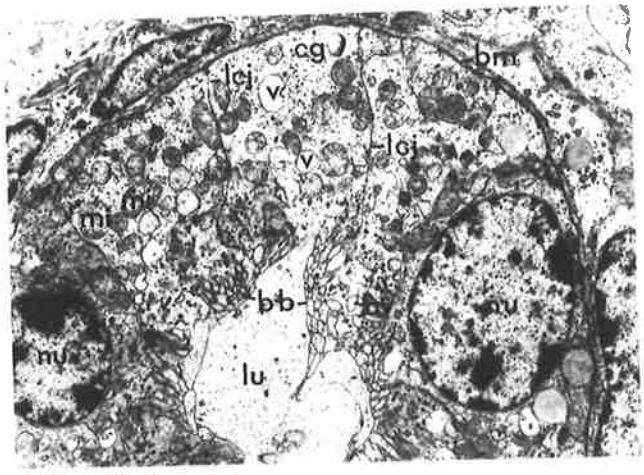
PLATE 13.

A cross section of a proximal tubule (123 day-old foetus: x4000).

A cross section of a proximal tubule (132 day-old foetus: x4200).

A cross section of a proximal tubule (141 day-old foetus: x10,000).

Key: bm = basement membrane, bb = brush border, cg = cytoplasmic granules
lcj = lateral cell junction, lu = lumen
mi = mitochondrion, nu = nucleus, v = vacuole


cg
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bb
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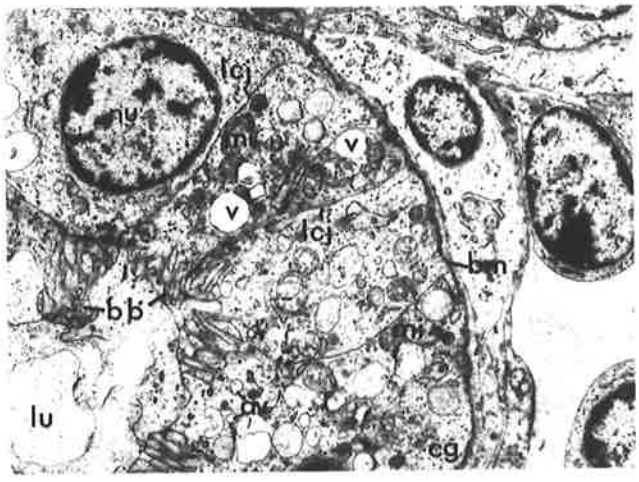
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cg

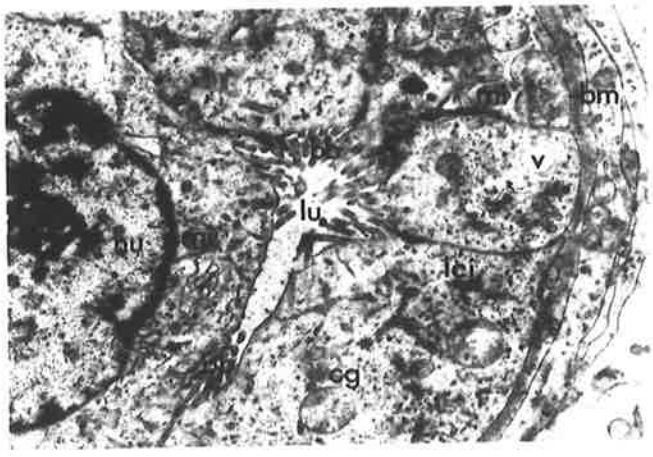
mi
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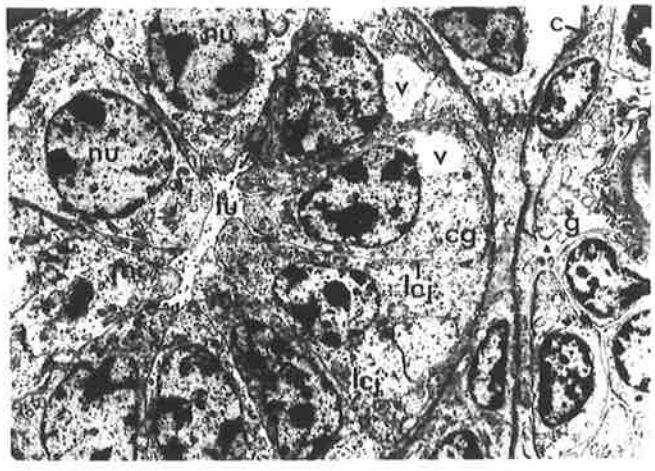
PLATE 14.

A cross section of a distal tubule (123 day-old foetus: x3000).

A longitudinal section of a distal tubule (132 day-old foetus: x6000).

A cross section of a distal tubule (14 day-old foetus: x4000).

Key: bm = basement membrane, c = capsule
cc = cytoplasmic compartment
cg = cytoplasmic granules, g = glomerulus
icm = infolding of the cytoplasmic membrane
lu = lumen, mi = mitochondrion, nu = nucleus
v = vacuole


nu
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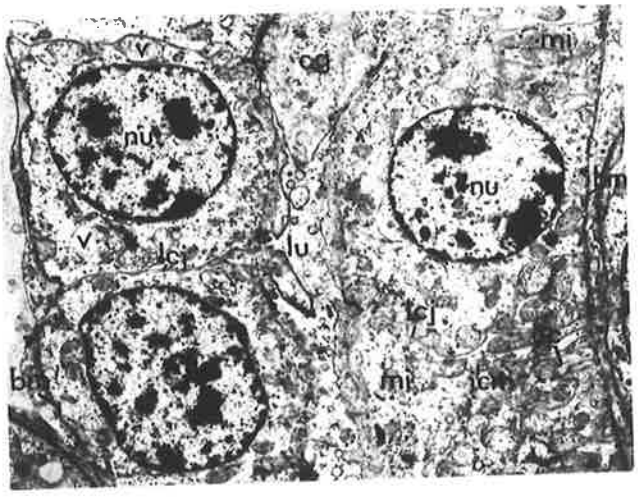
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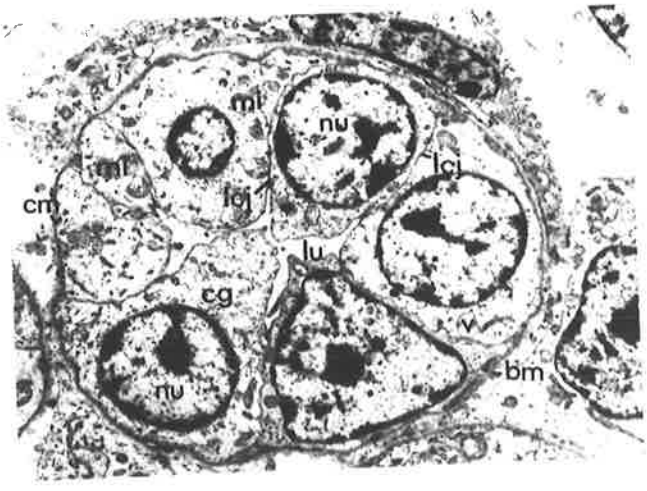
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and 20∝ HP are readily interconvertible. This is due to the activity of 20∝ -hydroxy-steroid oxido-reductasse (20∝ -HSD) in foetal red blood cells (Nancarrow and Seamark 1968). Accordingly, the 20∝ HP in foetal blood may act as a progesterone reservoir. A large number of samples were collected for hormone assay to ensure that a reliable picture of concentration changes could be established. It was hoped that his could then be related to changes in urinary output or composition.

The second approach to studying hormonal involvement in foetal kidney function involved using individual foetuses as case studies. Simultaneous blood and urine samples were collected from these foetuses and the blood was analysed for hormone content while the urine was subjected to the usual analysis of composition. Sampling was conducted each day between 9.30 a.m. and 10.30 a.m.

The third approach was to infuse various hormones into foetuses and to determine what changes occurred in urinary output and composition. The results of these hormone administration experiments are presented in section 5.5.

4.13.1 Hormone concentrations

a. Progesterone The progesterone concentration of foetal blood was positively correlated with foetal age ($r = 0.280$, $0.05 > P > 0.01$, $n = 78$). (See fig. 14). The average value for the period from day 115 to 120 was 2.8ng/ml ($n = 11$) compared with an average of 8.2ng/ml ($n = 10$) for the period from day 135 to day 140. However, from day 140 until term there was a fall in progesterone concentration and the average value for the last 4 days sampled, was 5.4ng/ml ($n = 4$) (appendix table 15).

b. 17∝ -hydroxy-progesterone (17∝ HP) The blood concentration of this hormone was not correlated with foetal age ($r = 0.091$, NS, $n = 78$), (See fig. 14). For 4 successive 5-day periods, beginning on day 115, the average concentration of 17∝ HP varied between 0.94ng/ml and 0.58ng/ml

while the average for the period between days 135 and 140 was 2.21ng/ml. This rise was shortlived since the average concentration over the last 5 days sampled was 0.10ng/ml. Thus the most obvious feature of these results was the rise in the average concentration of 17∝HP between days 130 and 140. An examination of the ungrouped daily averages, reveals that there were in fact, 2 sharp rises in concentration between days 133 and 139 separated by low averages on days 137 and 138 (appendix table 15). However, the standard error for each of these daily averages was so large that the significance of these observations is questionable.

c. 20∝-hydroxypregn-4-en-3-one (20∝HP) Figure 14 illustrates the relationship between 20∝HP concentration and foetal age. Although the overall relationship was significant ($r = 0.219$, $0.05 > P > 0.01$, $n = 78$), it is apparent that this correlation relates primarily to the period between days 115 and 140 after which there was a decrease in 20∝HP concentration. The average value for 20∝HP concentration, between the 115th and 120th days of gestation was 5.34ng/ml ($n = 12$). After day 120 the concentration rose erratically reaching an average of 21.10ng/ml in the period between days 135 and 140 and then falling to an average of 7.94ng/ml during the last 5 days of the study(appendix table 15). In view of the biochemical association between progesterone and 20∝HP it is interesting to note that the concentration of each hormone showed a similar relationship to foetal age.

d. Cortisol Unlike the other hormones which were measured in whole blood, cortisol was measured in plasma. It was found that the concentration of cortisol in foetal plasma was significantly correlated with foetal age ($r = 0.251$, $0.05 > P > 0.01$, $n = 78$) (see fig. 15). In contrast to the hormones discussed above, where a preparturient decline in concentration was usually seen, cortisol concentration increased over the last 10 days of pregnancy. When the cortisol concentrations of those foetuses which delivered normally, were considered alone; the pre-

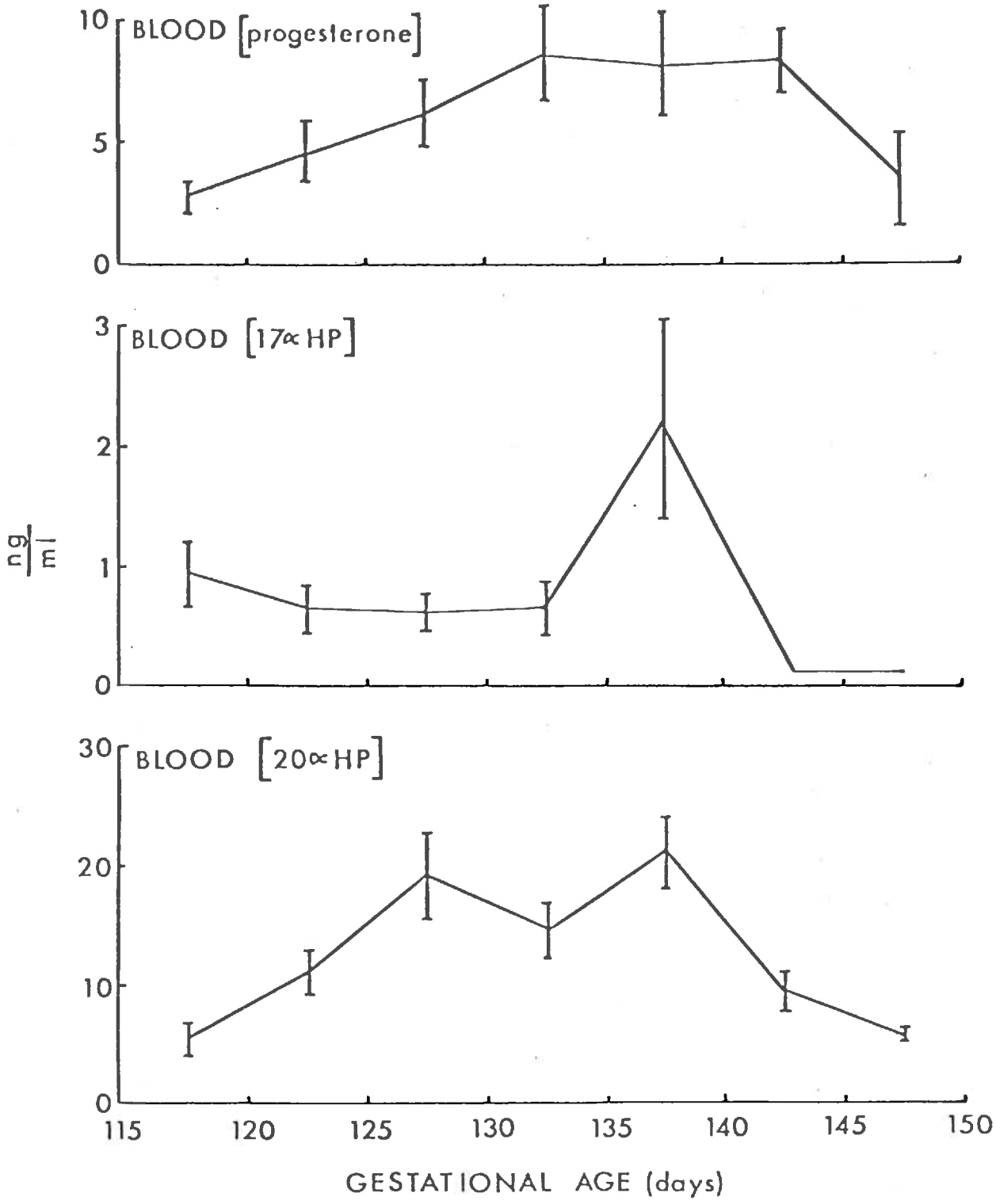

FIGURE 14. The concentration of progestins in foetal blood during the last 35 days of gestation. Samples were assayed using the method of Seamark & Lutwak-Mann (1972). (See appendix table 15 and text pages 88 and 89)

parturient rise was even more apparent and the correlation between plasma cortisol and foetal age was more significant ($r = 0.590$, $P < 0.001$, $n = 38$) (appendix table 16). It can be seen in figure 15 that the relationship was essentially exponential and that the cortisol concentration remained elevated until delivery. Similar changes in the plasma cortisol concentration of foetal lambs have been described by Bassett and Thorburn (1969), Comline et al (1970) and Nathanielsz et al (1972).

e. PRA PRA was measured by incubating foetal plasma in vitro and assaying the amount of angiotensin I generated. Figure 16 shows the average PRA for each 5 day period from day 90 to day 150. The average PRA for days 90 to 95 was 8.5ng/ml/hr, and this increased to averages of 13.18 and 17.7ng/ml/hr. for days 120 to 125 and days 140 to 145 respectively. During the last 7 days of gestation, PRA decreased slightly and the average for days 145-150 was 14.4ng/ml/hr. There was a significant correlation between the individual values for PRA and foetal age ($r = 0.450$, $P < 0.001$, $n = 89$) (appendix table 17).

In a recent study, Fleischman et al (1975) obtained PRA values of a similar order to those obtained in the present work, although the variation between daily averages in their study was greater. Fleischman et al (1975) found that the daily average for PRA varied between 6.6 and 15.5ng/ml/hr. during the period from day 128 to day 140. This included a gradual increase in PRA to 13.0ng/ml/hr. on day 132, followed by a fall of about 50% over the next 2 days. There was then a second increase in PRA to about 15.0ng/ml/hr. on day 139. This biphasic increase in PRA was not evident in the present work.

Earlier studies of the Renin-Angiotensin in chronically catheterised fetuses were conducted by Smith et al (1974) and Broughton - Pipkin et al (1974b). The former study involved only 3 measurements but the latter was a comprehensive study involving 55 measurements of PRA (c.f. 98 in the present work) in foetal sheep aged between 111 and 144 days. The results obtained by

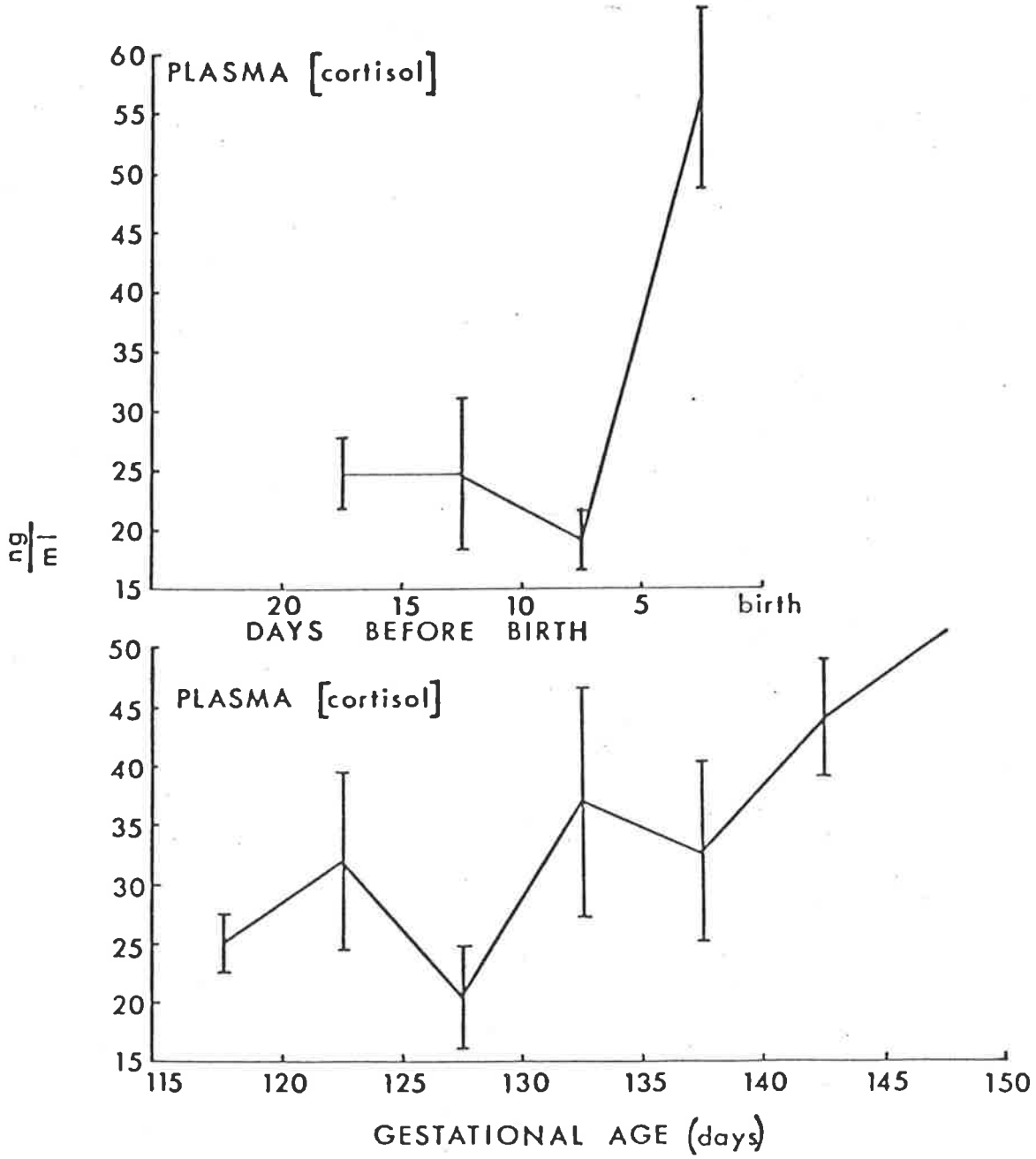

FIGURE 15. The concentration of cortisol in foetal plasma during the last 35 days of gestation. Samples were assayed using the method of Bassett et al (1969). (See appendix table 16 and text pages 89 and 90)

Broughton - Pipkin et al (1974b) are very similar to those of the present work. They obtained an average of 10.7± 1.1 ng/ml/hr for plasma renin in foetal lambs of 111-114 days gestation and evidence of a rise in PRA during gestation was apparent in the fact that lambs less than 120 days had a plasma renin of 9.2 ± 2.7 ng/ml/hr.

Other workers have studied the perinatal renin-angiotensin system by measuring changes in PRC and PRA in response to a variety of stimulii (Trimper and Lumbers 1972; Broughton-Pipkin et al 1971, 1974a). However, these were all acute studies and as such were subject to error induced by stress.

4.13.2 Case studies involving hormone analysis

The results of 6 studies of individual foetuses are presented in this section. In the first three studies, simultaneous plasma and urine samples were collected daily and each plasma sample was divided into 2 aliquots. One aliquot of each plasma sample was analysed to determine the concentration of various steroid hormones, while the remaining aliquots of plasma plus the urine samples, were analysed to determine Na^+, K^+, uric acid and creatinine concentration. In the remaining 3 studies, the sampling procedures were identical; however, one aliquot of each plasma sample was used to estimate PRA. The remaining aliquots of plasma and the urine samples were again analysed to determine Na^+, K^+, uric acid and creatinine concentrations.

a. Foetus 66-345 (appendix tables 18 and 19). Figures 17 and 18 record the data obtained from this foetus inwhich samples were collected for 12 days prior to parturition. On the 13th day (148th day of gestation) a 3.8kg male lamb was delivered normally and further samples were obtained during and after delivery. In this case the total plasma levels of oestradiol 17α ($E_2 17\alpha$), oetradiol 17β ($E_2 17\beta$) and oestrone (E_1) were measured in addition to cortisol and the progestins. The oestrogens were measured using fluorimetry following treatment of the prepared samples with Kober-Ittrich reagents. This technique, as

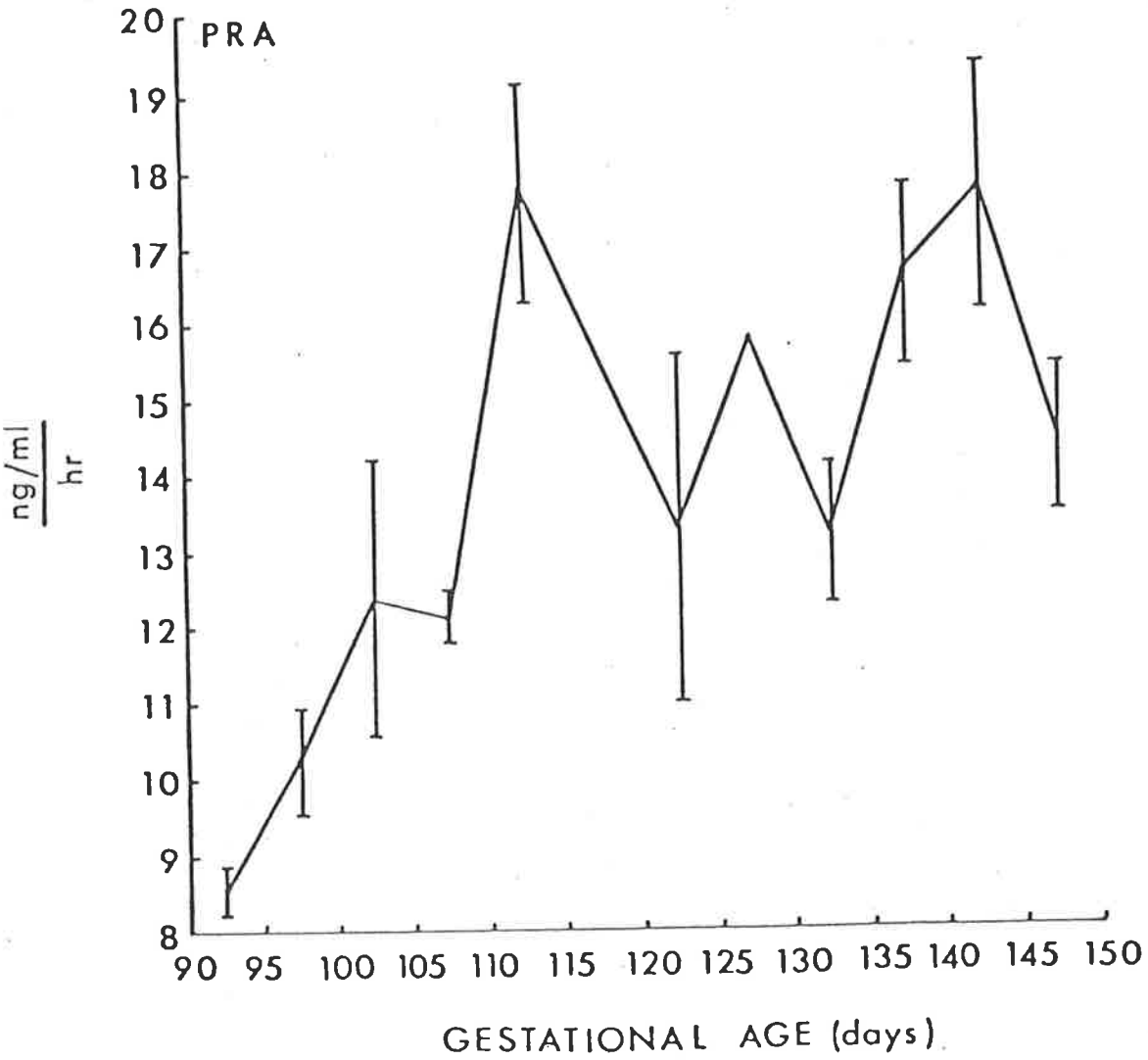

FIGURE 16. Renin activity in foetal plasma (PRA) during the last 60 days of gestation. Samples were assayed using the method of Lumbers, Seamark and Pickles (1971 unpublished).
(See appendix table 17 and text pages 90 and 91)

applied to the measurement of oestrogens in urine, is described by Lunaas(1964) and Brown et al(1968). The method has been modified in this laboratory for use with foetal plasma (Findlay and Seamark 1971).

It can be seen in figure 17 that the concentration of the oestrogens remained relatively constant throughout the perinatal period, with oestradiol 17β present in the highest concentrations, although never exceeding 18ng/ml. However, the 3 plasma samples taken during delivery all had greatly increased oestrogen levels and a sample taken 6 hours after birth revealed a further increase in the concentration of oestradiol 17β and oestradiol 17α . Conversely, the progestins (with the exception of 17α HP) showed quite high concentrations before birth and decreased as term approached. Cortisol rose sharply about 5 days before birth and remained elevated.

When the hormone picture was compared with the concentration of the various solutes in plasma and urine, no definite associations were apparent (see fig. 18). However, the plasma concentrations of uric acid and creatinine rose erratically, beginning about 8 days before delivery. These rises were reflected in similar changes in the urinary concentrations and excretion rates of these substances. However it is perplexing that urinary [K^+] underwent similar concentration changes despite the relative stability of plasma [K^+]. It could be inferred that the rise in the concentration of the urinary solutes (excluding Na^+) was initiated by the fall in progestin level which commenced about 7 days before delivery. Alternatively the rise in plasma cortisol which began 10 days before delivery may have lead to these changes in urinary solute concentration.

Finally, urinary [Na^+] showed a rapid pre-parturient rise beginning 5 days before birth. This rise was not related to the changes in any other urinary solute, nor to plasma [Na^+]; but the increase in Na^+ excretion rate that resulted from a rise in urine flow rate as well as [Na^+] began approximately 8 days before delivery. These changes may have been related to the rise in plasma cortisol or to the decline in plasma progestins or both, although this would appear to be contrary to the reported natriuretic effect of progestins. On the other hand,

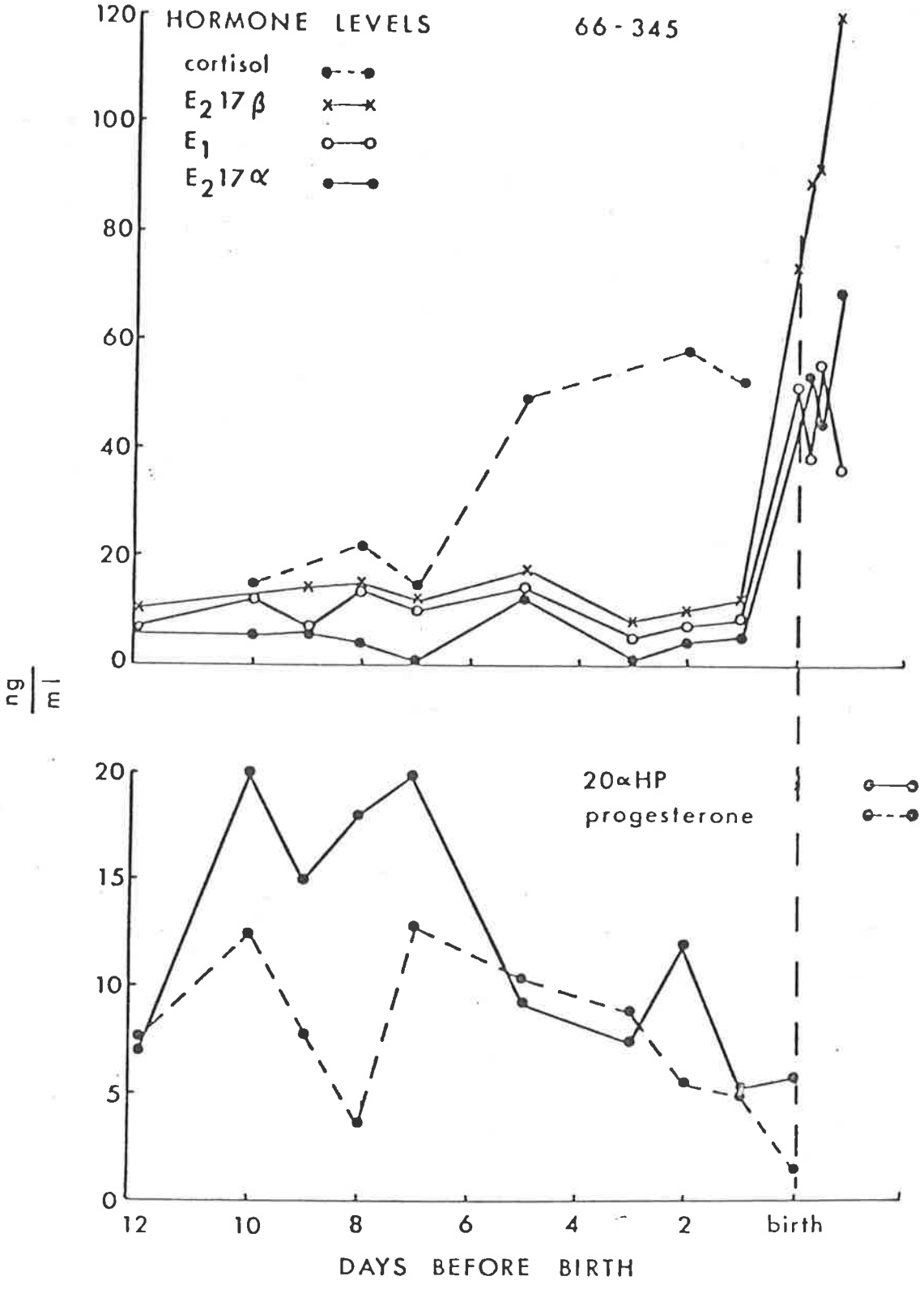

FIGURE 17. The concentration of hormones in the blood and plasma of foetus 66-345 during the 12 days immediately prior to parturition; plus the level of oestrogens during and immediately after delivery. The progestin levels in foetal blood were measured using the method of Seamark & Lutwak-Mann (1972), plasma cortisol by the method of Bassett et al (1969) and total plasma oestrogens by the method of Findlay & Seamark (1971). (See appendix table 18 and text pages 91 and 92)

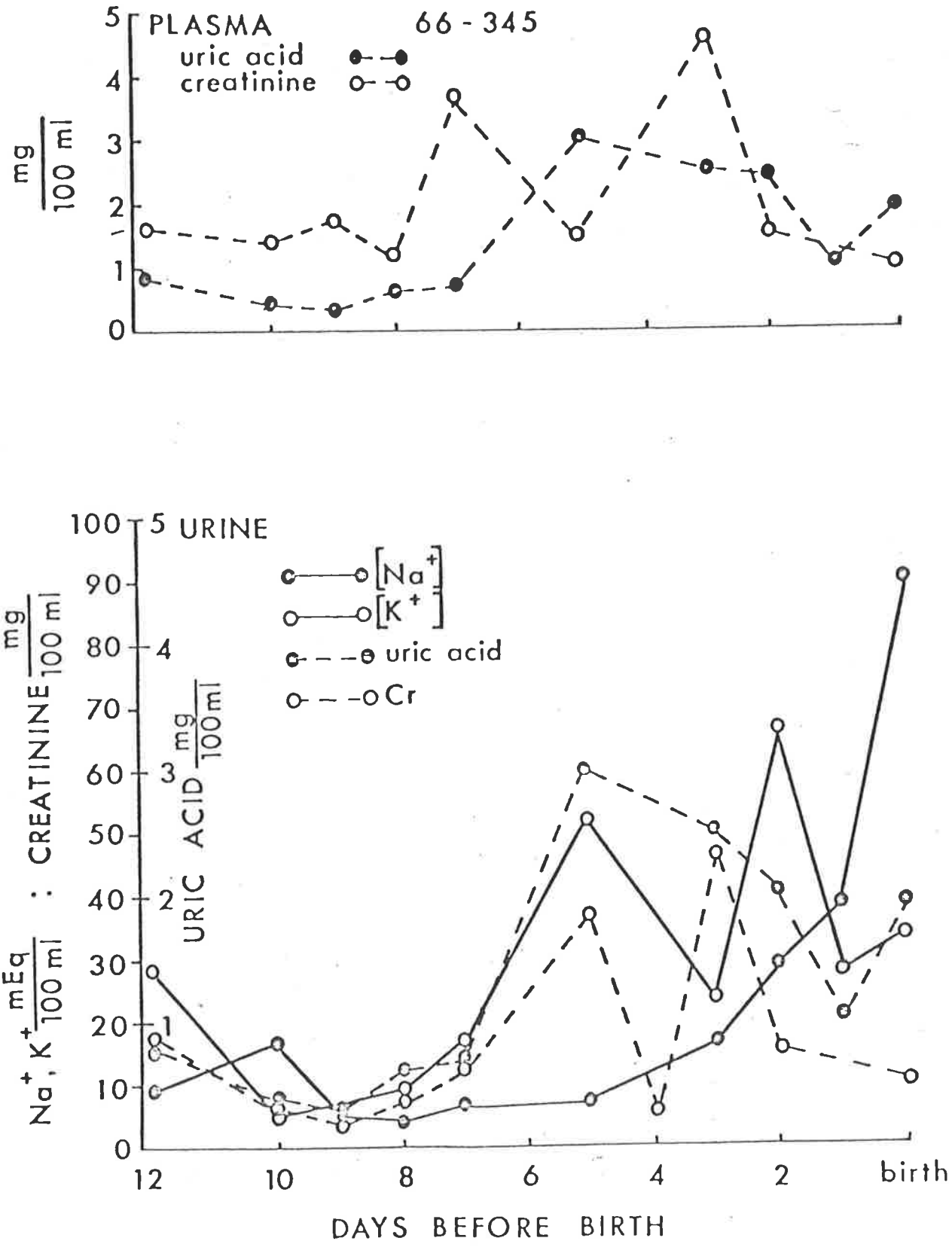

FIGURE 18. The concentration of electrolytes and non-ionic solutes in the urine and plasma of foetus 66-345 during the 12 days immediately prior to parturition.
(See appendix table 19 and text pages 91 and 92)

cortisol may induce natriuresis by increasing GFR.

b. Foetus W177 (appendix tables 20 and 21). In this study, plasma progestin and cortisol levels were measured. Changes in the concentration of progresterone and 17∝HP were similar to those observed in the preceding foetus (see fig. 19). Progesterone was generally lower in concentration than previously, but again there was a decrease as term approached. The most striking changes occurred in the concentration of 20∝HP which began to rise about 10 days before birth, from concentrations of between 1.0 - 1.5ng/ml and reached a maximum of 39ng/ml 3 days before birth. The concentration then declined over the remaining 2 days to levels which were about 1/3 of the maximum. Cortisol concentration showed a typical pattern of change, rising sharply in the final 3 days of pregnancy to reach levels 4 times the basal levels. It was noticeable that the increase in 20∝HP concentration began about the time that the progesterone level began to fall, while the period of rapid decline of 20∝HP concentration corresponded to the time of cortisol increase.

The concentrations of Na^+, K^+, uric acid, and creatinine in foetal plasma, showed no changes that could reliably be associated with the hormonal changes described. The concentration of these substances in foetal urine showed patterns of change similar to those noted in the preceding foetus. There appeared to be 2 peaks in the concentration of the urinary solutes, one occurring between the 10th and 4th days before delivery and the other during the last 3 days of gestation. Again the $[Na^+]$ of foetal urine did not follow the pattern of the other urinary solutes, but showed a large increase immediately before birth. Finally, in both this and the preceding study, the pH of foetal urine reamined relatively constant throughout and there was no pre-parturient decline in pH. The excretion rates of the various urinary solutes showed changes which paralleled the corresponding concentration changes, although relatively high excretion rates were recorded in the last 3 days of the study due to increases in urine flow rate. This case study was terminated following the delivery of a 3.9kg male lamb on the 149th day of gestation.

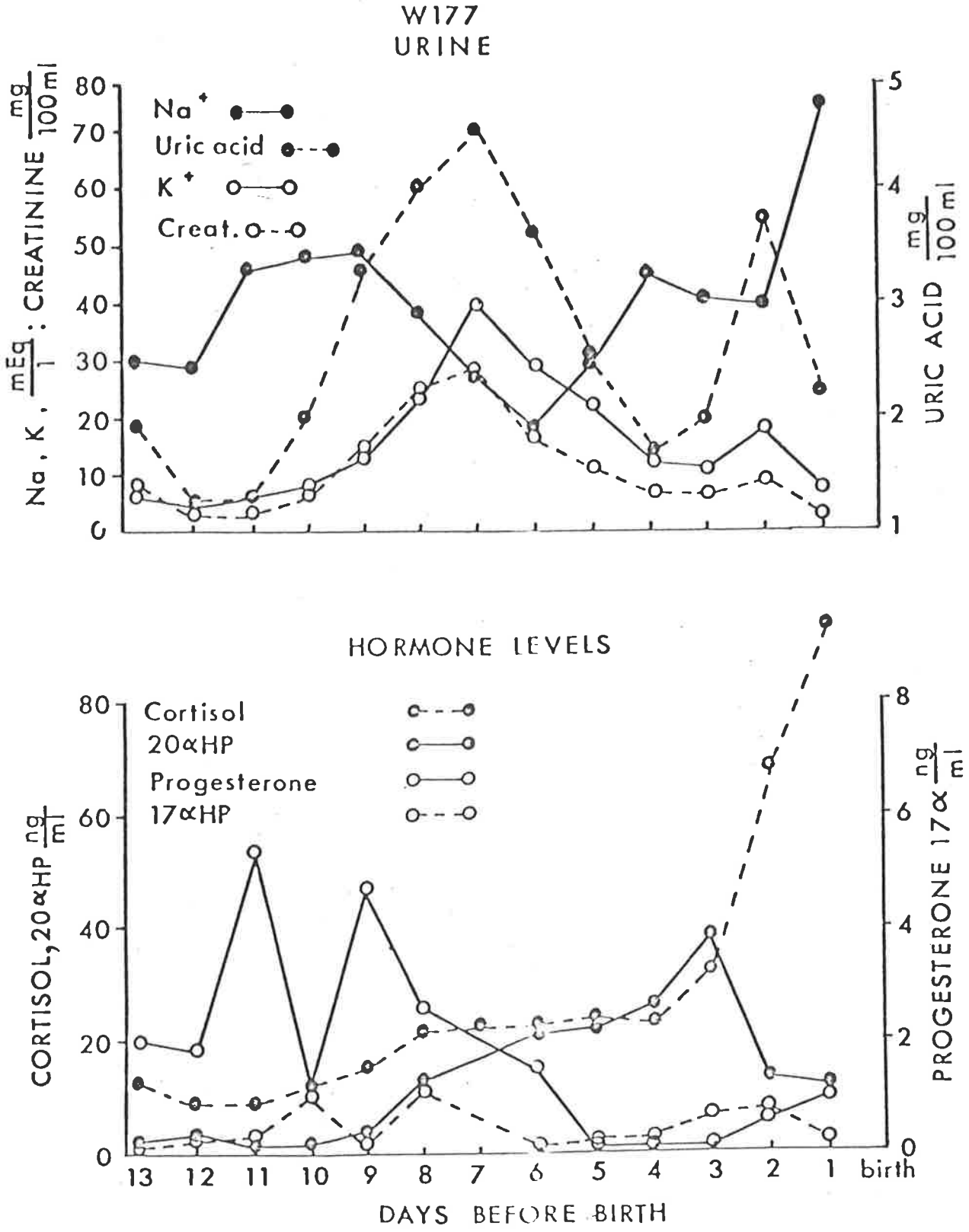

FIGURE 19. The concentration of plasma hormones and urine solutes in foetus W177 during the 13 days immediately prior to parturition. The progestin levels in foetal blood were measured using the method of Seamark & Lutwak-Mann (1972) and plasma cortisol by the method of Bassett et al (1969). (See appendix tables 20 and 21 and text page 93)

c. Foetus W.38 (appendix tables 22, 23 and 24). In this case a normal foetus (3.1kg - female) was retrieved when the mother was slaughtered on day 133 of gestation. The ewe was slaughtered because of the development of paralysis in the hind limbs. However, samples of foetal plasma and urine had been obtained daily from the 115th day of pregnancy to the time of slaughter, thereby providing information concerning a period of gestation prior to that examined in the previous case studies (see fig. 20).

Of the progestins, only 20∝HP concentration showed any major fluctuation reaching a peak of about 12ng/ml on days 122 and 123 and then decreasing toward the end of the study. Progesterone levels varied only slightly and were of a similar order to those in the preceding cases. Similarly, 17∝HP concentration was low and regular, while cortisol concentration rose gradually from about 10ng/ml to 20ng/ml during the study period.

The concentrations of uric acid and creatinine, in foetal plasma, increased after day 124 and reached maximum values on day 128 which were about 4 times their basal levels. Thereafter, the concentration of these solutes fell to about ½ of their peak values. The levels of plasma Na^+ and K^+ increased erratically from 137mEq/L and 3.9 mEq/L respectively on day 116, to 149.5 mEq/L and 4.6 mEq/L on day 133. The concentrations of the urinary solutes showed no major fluctuations and certainly none that could be related to changes in progestin or cortisol levels.

d. Foetuses 66-323, 66-478 and 70-409 (Appendix tables 25, 26 and 27). Figures 21 and 22 show PRA and the concentration of Na^+ and K^+ in the daily plasma and urine samples collected from these 3 foetuses. Urine flow rate is also shown. Foetuses 66-478 and 66-323 were delivered normally and had birth weights of 4.8 and 5.1kg respectively. The third foetus (70-409) was delivered by caesarian-section

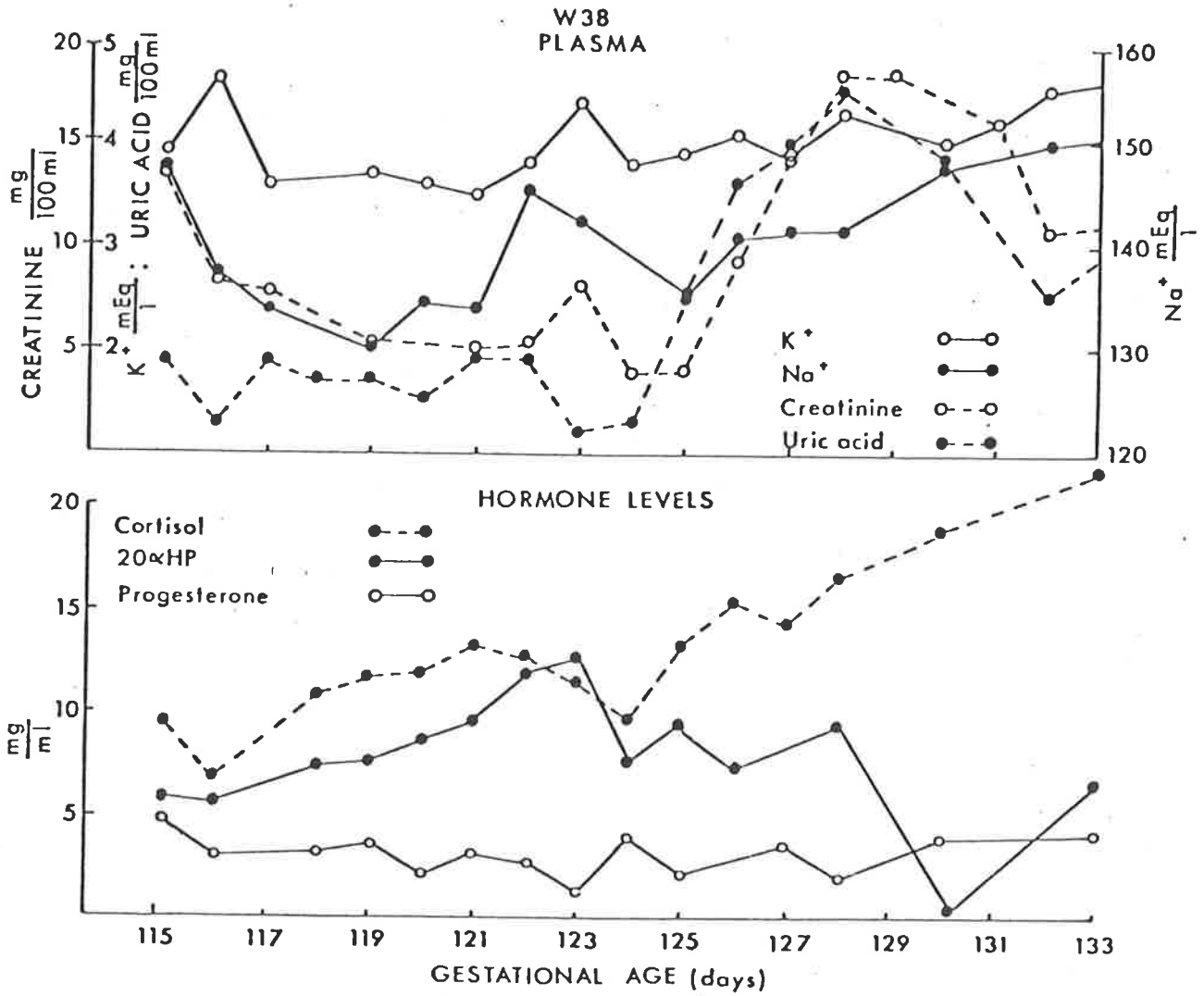

FIGURE 20. The concentration of plasma hormones and plasma solutes in foetus W38 between the 115th and 133rd days of gestation. The progestin levels in foetal blood were measured using the method of Seamark and Lutwak-Mann (1972) and plasma cortisol by the method of Bassett et al (1969).
(See appendix tables 22-24 and text page 94)

on the 153rd day of gestation. It weighed 5.3kg and was apparently normal.

In foetus 66-478 (see fig. 21) plasma [Na^+] increased sharply, about 15 days before delivery and this rise was followed by an increase in PRA. At the time of maximum PRA(11 days before birth) plasma [Na^+] began to fall and this was followed by a decline in PRA. A second rise in plasma [Na^+] during the last 6 days of foetal life was also followed by a rise in PRA.

It is difficult to envisage a physiological relationship in which elevated plasma [Na^+] would initiate an increase in PRA. Urinary [K^+] reached a peak 7 days before birth and this corresponded to a peak in plasma [K^+]. Thereafter, both declined as term approached, although plasma [K^+] increased slightly on the day before delivery. Urinary [Na^+] showed little variation until 5 days before delivery when a sharp rise began and persisted for 2 days. Such pre-parturient increases in urinary [Na^+] have been observed previously (foetuses 66-345 and W177), but on this occasion, the pre-parturient rise was followed by a drop in urinary [Na^+] during the last 2 days of pregnancy. The most significant fall in urinary [Na^+] was on the last day of gestation and in that period there was a 50% decrease in PRA. Urine flow rate showed typically wide fluctuations that were not related to any other parameter measured.

In foetus 66-323 (see fig. 21), there was no discernible relationship between the plasma and urine electrolyte concentrations and PRA. In foetus 70-409 this was again the case. However it is noteworthy that there was a rise in urinary [Na^+] as gestation proceeded and this rise persisted until delivery, despite a 40% decrease in PRA on the day before delivery. This is in contrast to the results obtained with foetus 66-478.

In each of these three studies, there was an increase in foetal PRA which began about 2 weeks before birth and reached a maximum in 2 to 3 days. From then until term the PRA declined erratically. In 2 of the foetuses (70-409 and 66-478) there was some suggestion of a lesser peak in PRA during the last week of gestation but the values decreased sharply during the last day or 2 days before birth.

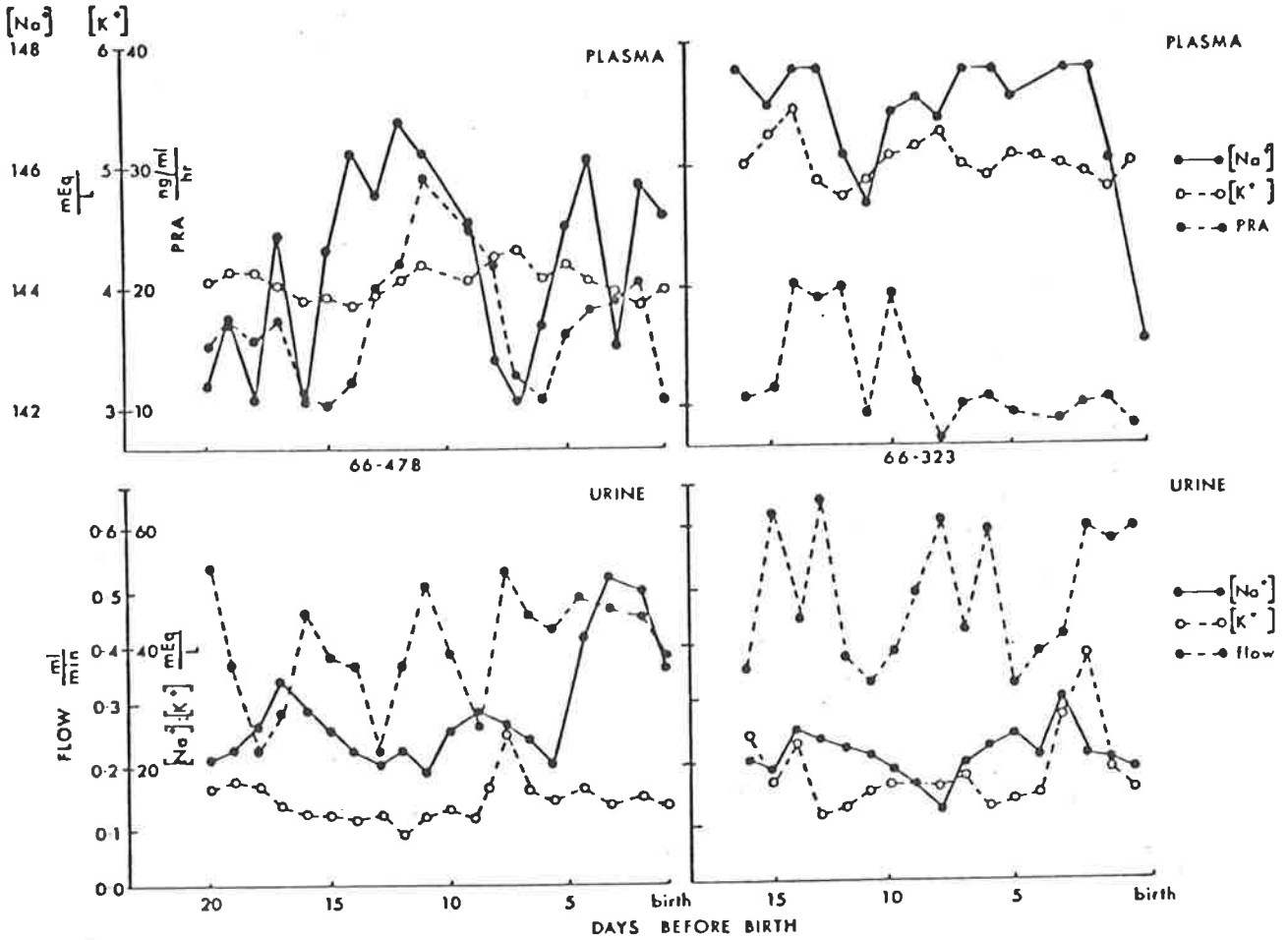

FIGURE 21. The concentrations of Na^+ and K^+ in the plasma and urine of foetuses 66-478 and 66-323, compared with plasma renin activity (PRA) during the last 20 days of gestation. PRA was measured using the method of Lumbers, Seamark and Pickles (1971 unpublished).
(See appendix tables 25 and 26 and text pages 94 and 95)

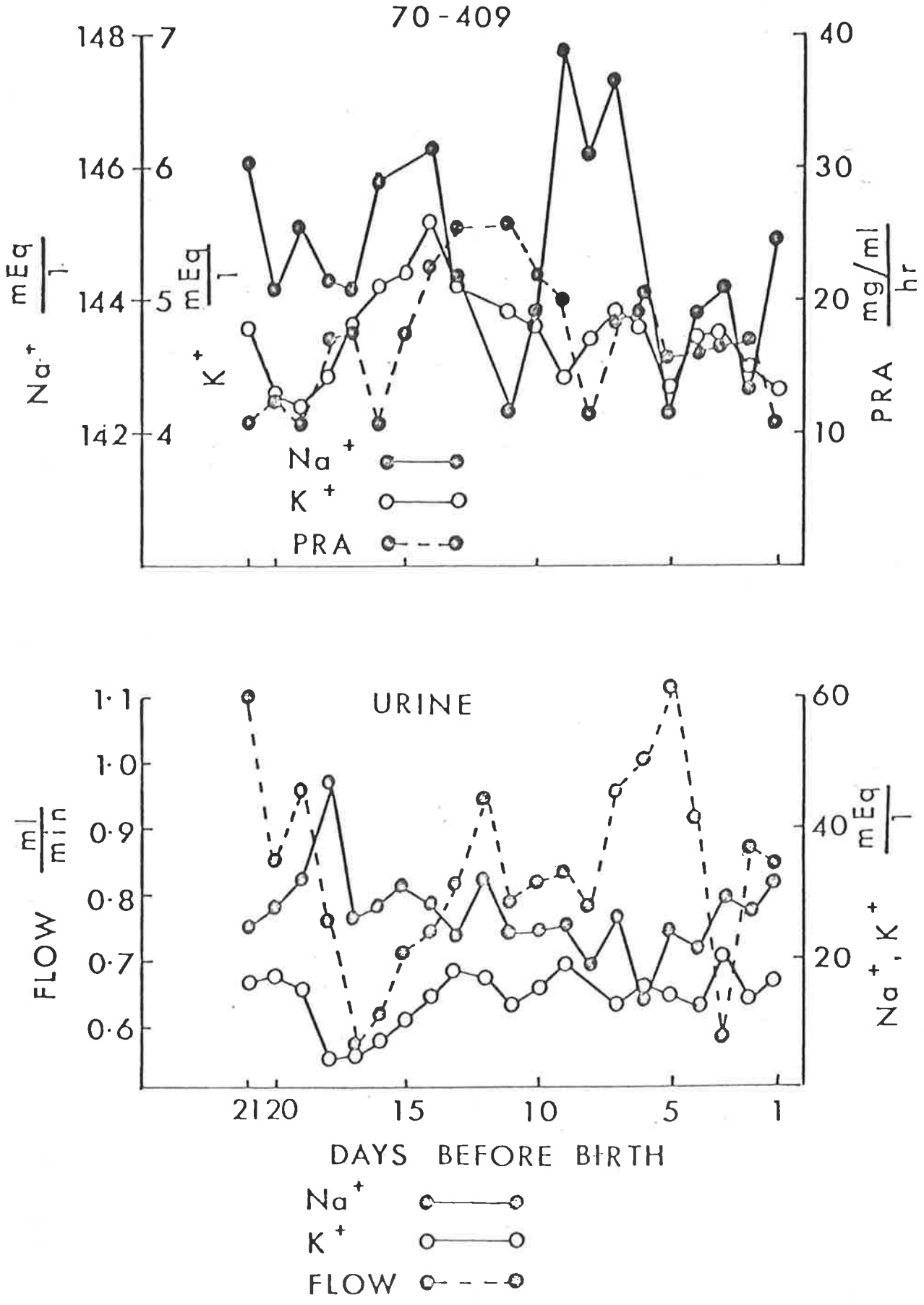

FIGURE 22. The concentrations of Na^+ and K^+ in the plasma and urine of foetus 70-409, compared with plasma renin activity (PRA) during the last 21 days of gestation. PRA was measured using the method of Lumbers, Seamark and Pickles (1971 unpublished). (See appendix table 27 and text pages 94 and 95)

5. *RESULTS FROM FOETAL EXPERIMENTS*

In all of the experiments described in this section the protocol involved continuous observation of various indices of foetal kidney function before, during and after the application of an experimental treatment. Generally the control periods were about 2 hours and the overall length of the experiments 6-12 hours. Basically it is then assumed that any substantial changes that occured during or after the experimental manipulation are attributable to the manipulation.

However there are difficulties in applying this approach to the study of foetal kidney function. Short-term fluctuations in the composition and output of urine by individual untreated foetuses is seen in most published work in this field (Alexander et al 1958a, b, Bernstine 1970, Mellor and Slater 1972b). This is particularly true of sheep foetuses even where stabilised chronic preparations are used. Such control variation has been a complicating factor in the experiments described here. Usually attempts were made to ensure that the urinary parameters were relatively stable before commencing treatment, but for practical reasons this was not always possible and at best only a limited number of factors could be monitored before treatment.

Given that control variability is a feature of foetal renal research, then to draw adequate conclusions, observations must be repeated sufficient times to reliably establish the results. This approach is exhibited in the preceding section where large numbers of daily observations were made so that statistically valid gestational trends could be established. However, adequate repetition is less feasible with experiments. Because of the difficulty of establishing and maintaining the chronic preparations and because of the need to allow several days between successive experiments on the same foetus, the opportunities to conduct experiments are limited.

Accordingly the experimenter must decide whether to intensively study the response to a limited number of treatments or conduct less intensive studies, of a preliminary nature, on a variety of treatments.

In view of the notable lack of data concerning the factors involved in regulating foetal kidney function and because of the need to gather information to assist in interpreting the data reported in the preceding section; it was decided to study a relatively broad spectrum of factors potentially involved in foetal kidney function.

5.1 Foetal Homeostasis

In this section, a series of experiments is described in which the ability of the developing sheep kidney to maintain the stability of the internal environment of the foetus, was examined. Several studies have been carried out on the ability of full-term and newborn human infants to excrete excess salt and water (Ames 1953; Strauss 1960; Theodonius et al 1971 and Barnett et al 1952). Similar experiments have been conducted with rabbits and rats (Yaffe and Anders 1960 and Trimble 1970). No comparable studies of sheep foetuses have been reported.

In the present work, foetal salt-loading experiments were carried out and attempts were made to water-deplete foetuses by establishing osmotic gradients across the placenta and foetal skin. In addition, maternal-foetal relationships were explored by examining foetal kidney activity during salt-loading and water depletion of the ewe.

5.1.1 Foetal saline infusions

Seven experiments were conducted in which foetuses aged between 111 and 146 days were infused with various quantities of Na Cl. For ease of comparison, all infusion rates have been corrected for foetal weight and expressedas mEq/kg/hr. Foetal weight was estimated from a standard age-weight relationship that has been established in this laboratory using a large number of foetuses of known gestational age (see fig. 2). To minimise the effect of bleeding a maximum of four 1.0ml blood samples was taken from each foetus. Usually only one blood sample was taken before the

end of the saline infusion.

a. Foetuses 69-464 and 69-539 (111 days) (appendix tables 28 and 29). The results of these experiments are illustrated in figures 23 and 24. Both foetuses were infused with 0.39 mEq/kg/hr. of Na^+ and Cl^- for one hour (total Na^+ 0.43 mEq) (1.4ml of 1.8% NaCl) and in both cases there was an increase in urine flow rate, urinary [Na^+] and urinary [K^+]. With foetus 69-464 (fig. 23) urinary Na^+ and K^+ concentrations both increased to levels about twice the average pre-treatment levels and the corresponding excretion rates rose to about four times the control averages. These responses which commenced during, or soon after, the infusion period, were still evident 3½ hours after treatment and further rises may have occurred if the experiment had been prolonged. Plasma [Na^+] in this experiment did not change markedly even during the NaCl infusion. In contrast, plasma [K^+] increased despite the fourfold rise in K^+ excretion rate.

In foetus 69-539 (fig.24) urine flow rate rose to a maximum which was 4 times the pre-treatment average while urinary [Na^+] and [K^+] were approximately doubled. The Na^+ and K^+ excretion rates increased 7.5 and 9 times respectively. Most of these changes began during the infusion period and reached a peak at the end of the experiment (four hours after the NaCl infusion was stopped). The most obvious change in the concentration of the plasma solutes was the steady decrease in [Na^+] during the period of increased Na^+excretion. Plasma [K^+] again showed an overall increase.

b. Foetus 69-443 (114 days) (appendix table 30)

This foetus was infused with 0.12 mEq/kg/hr of Na^+ and Cl^- for three hours (1.4ml of 1.8% NaCl). The total dose of electrolyte administered was similar to that used in the preceding experiment, but the rate of infusion was slower. No changes occurred, in the concentration or excretion rate of the urinary electrolytes that would suggest that the foetus had responded to the salt load. During the infusion of NaCl the [Na^+] and [K^+] of foetal plasma remained unaltered. (See fig. 25).

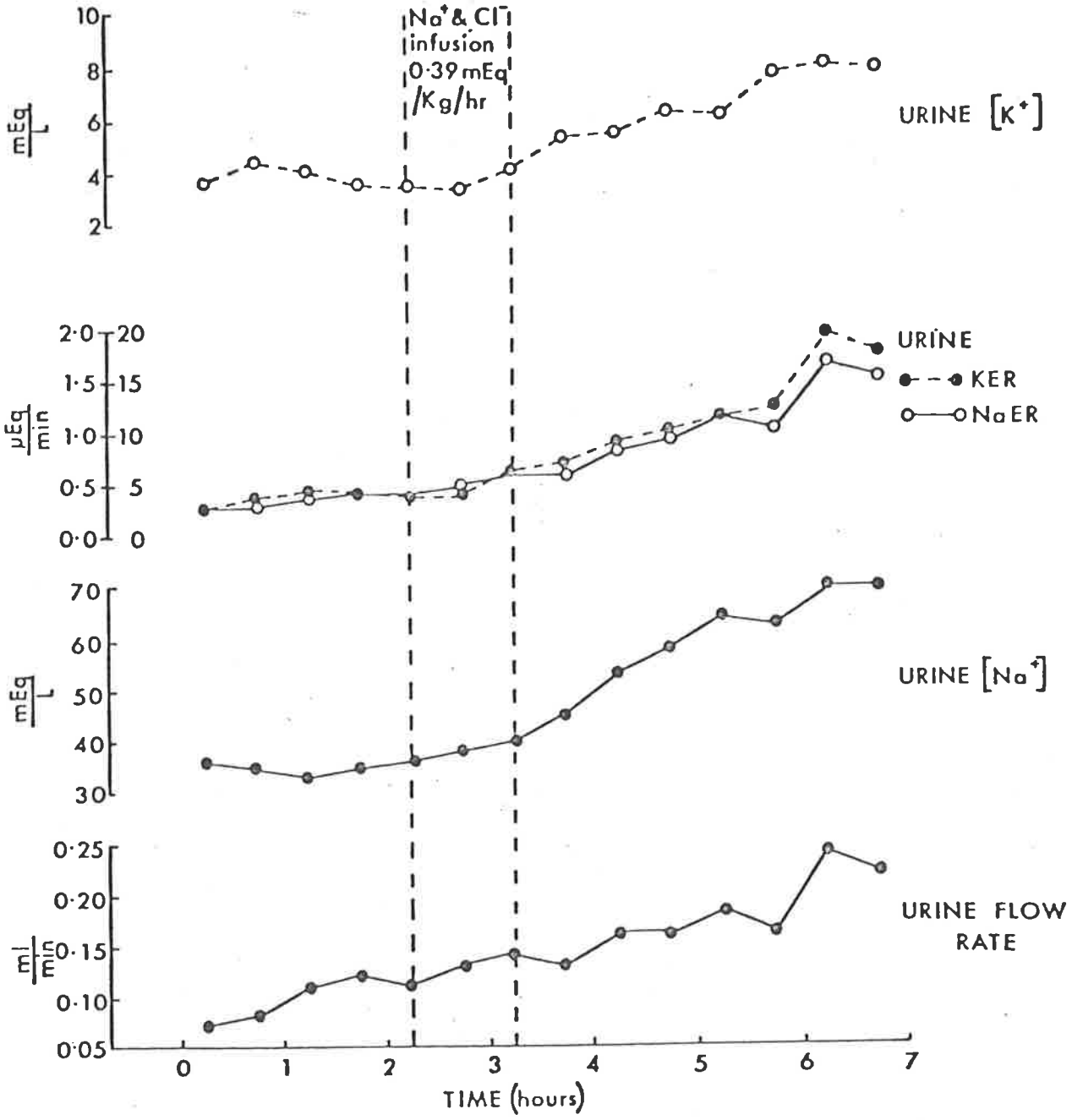

FIGURE 23. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 69-464 (111 days old). (See appendix table 28 and text page 98)

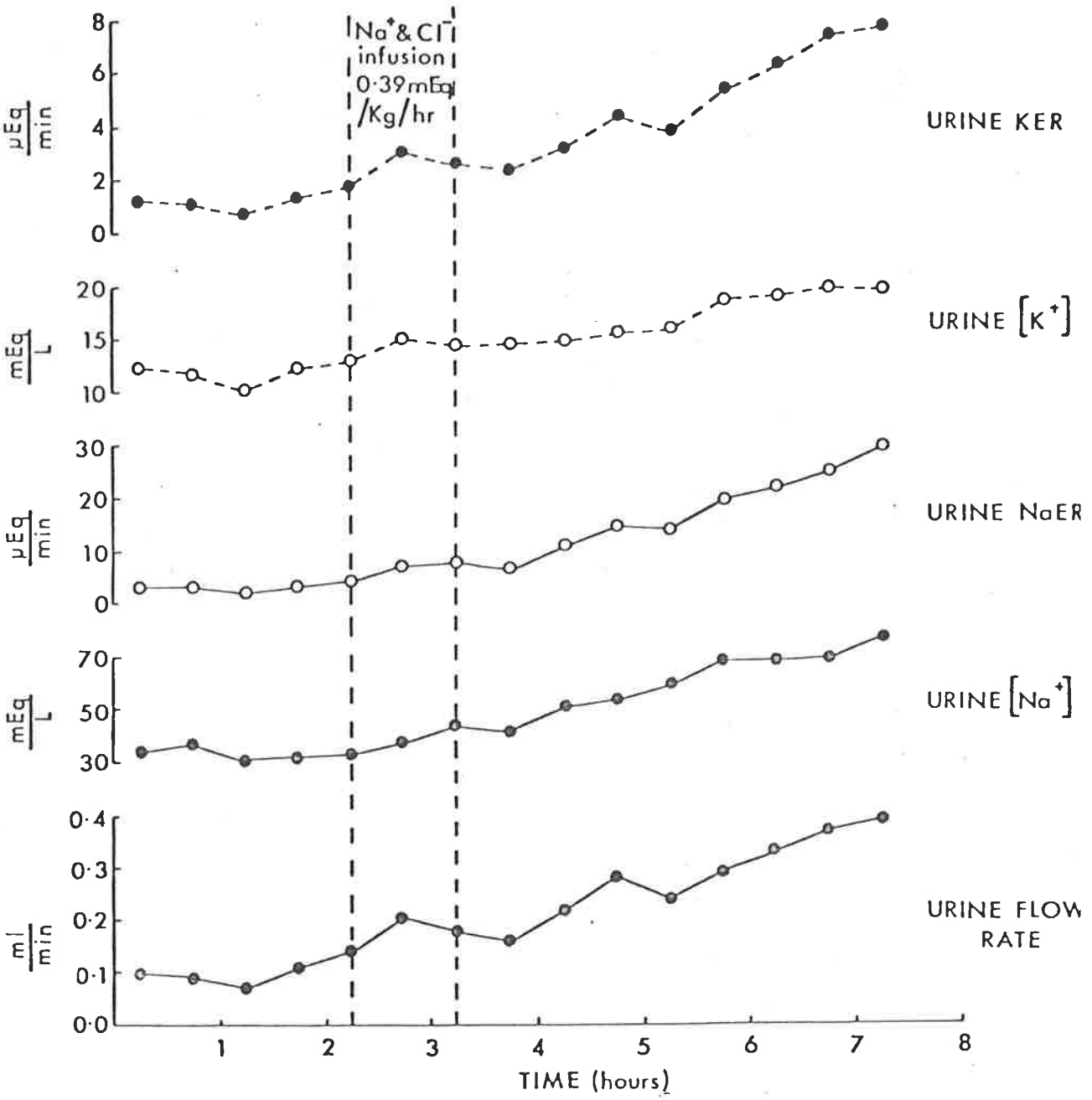

FIGURE 24. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 69-539 (111 days old).
(See appendix table 29 and text page 98)

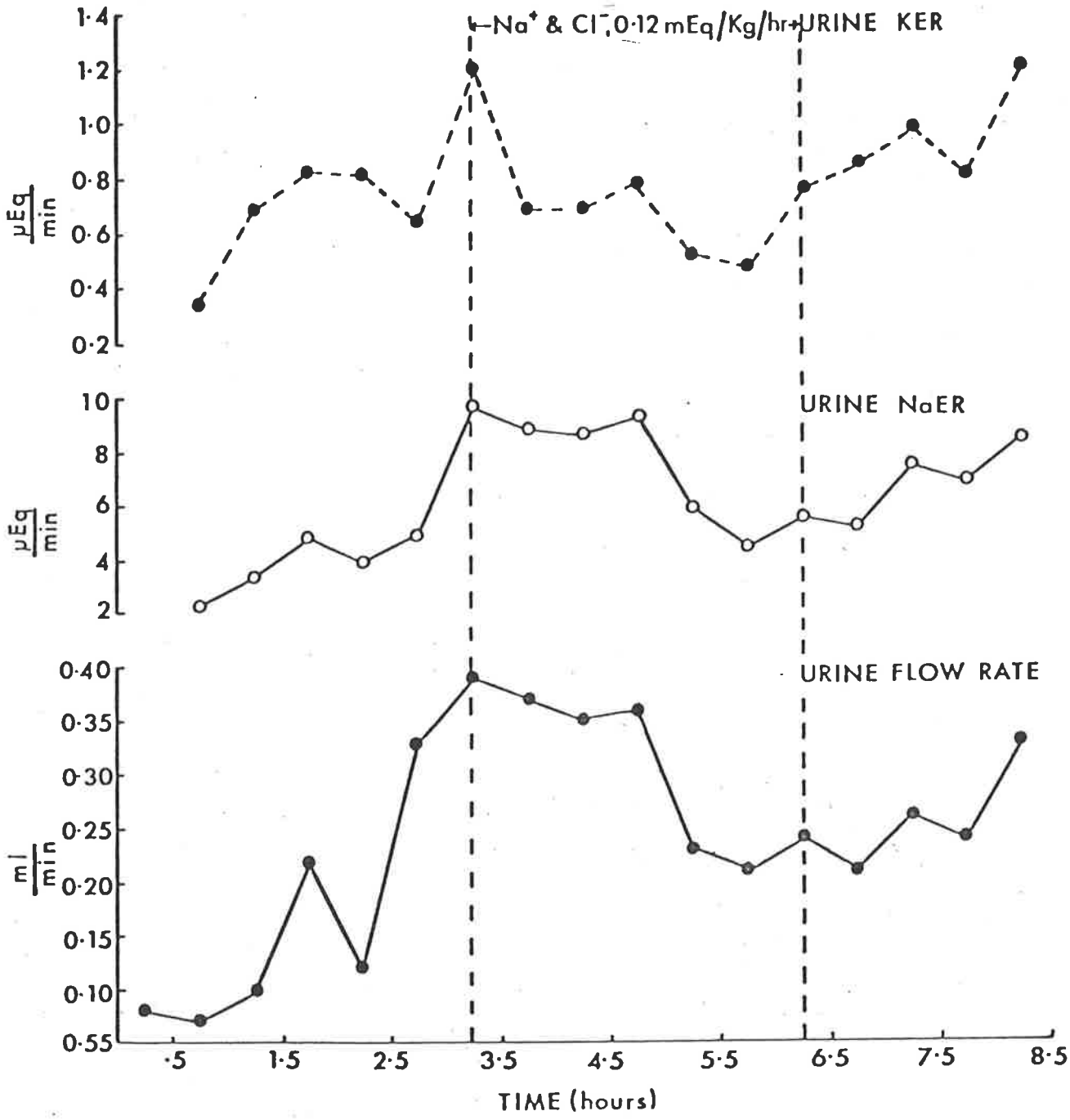

FIGURE 25. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 69-443 (114 days old).

(See appendix table 30 and text page 98)

c. Foetus 69-467 (116 days) (appendix table 31)

On this occasion 0.87 mEq/kg of Na^+ and cl^- was infused in our hour (total Na^+ 1.05 mEq) (3.4ml of 1.8% NaCl). This was 2¼ times the dose given to the 111 day-old foetuses. Compensatory changes in foetal urine volume and composition were evident in this experiment. Urine flow rate reached a maximum (1.73ml/min.) 2½ hours after treatment, which was 1.9 times the average flow rate for the last 5 samples of the control period. The $[Na^+]$ of foetal urine reached a peak at the same time as flow rate. The maximum $[Na^+]$ was almost double the average pre-treatment $[Na^+]$. Urinary $[K^+]$ also increased, but it began to rise earlier than $[Na^+]$ and the magnitude of the rise was not as great (1.6 times). It is noticeable that neither urine flow rate nor urinary $[Na^+]$ began to increase until an hour after the saline infusion was completed. Accordingly, the threefold increase in Na^+ excretion rate which resulted, did not commence until the second hour after treatment. Both urine flow rate and Na^+ excretion rate had begun to decline before the end of the experiment. The excretion rate of K^+ showed a small increase (3.0 times) (see fig. 26).

Plasma $[Na^+]$ increased during the control and treatment periods but then decreased substantially during the time of high Na^+ excretion. The $[K^+]$ of foetal plasma showed no consistent changes during the experiment.

d. Foetus 69-613 (118 days) (appendix table 32).

This foetus received 0.33 mEq/kg/hr of Na^+ and Cl^- for 3 hours (total Na^+ 1.29 mEq) (4.2ml of 1.8% NaCl). After the first hour of the infusion, urine flow rate began to increase and continued to do so for the next 5½ hours by which time the flow rate was 5½ times the pre-treatment average. Urinary $[Na^+]$ began to rise at the same time and had increase 2 3/4 times by the end of the experiment while urinary $[K^+]$ doubled over the same period. The electrolyte excretion rates increased in accordance with the parallel changes of flow rate and electrolyte concentration (see fig. 27).

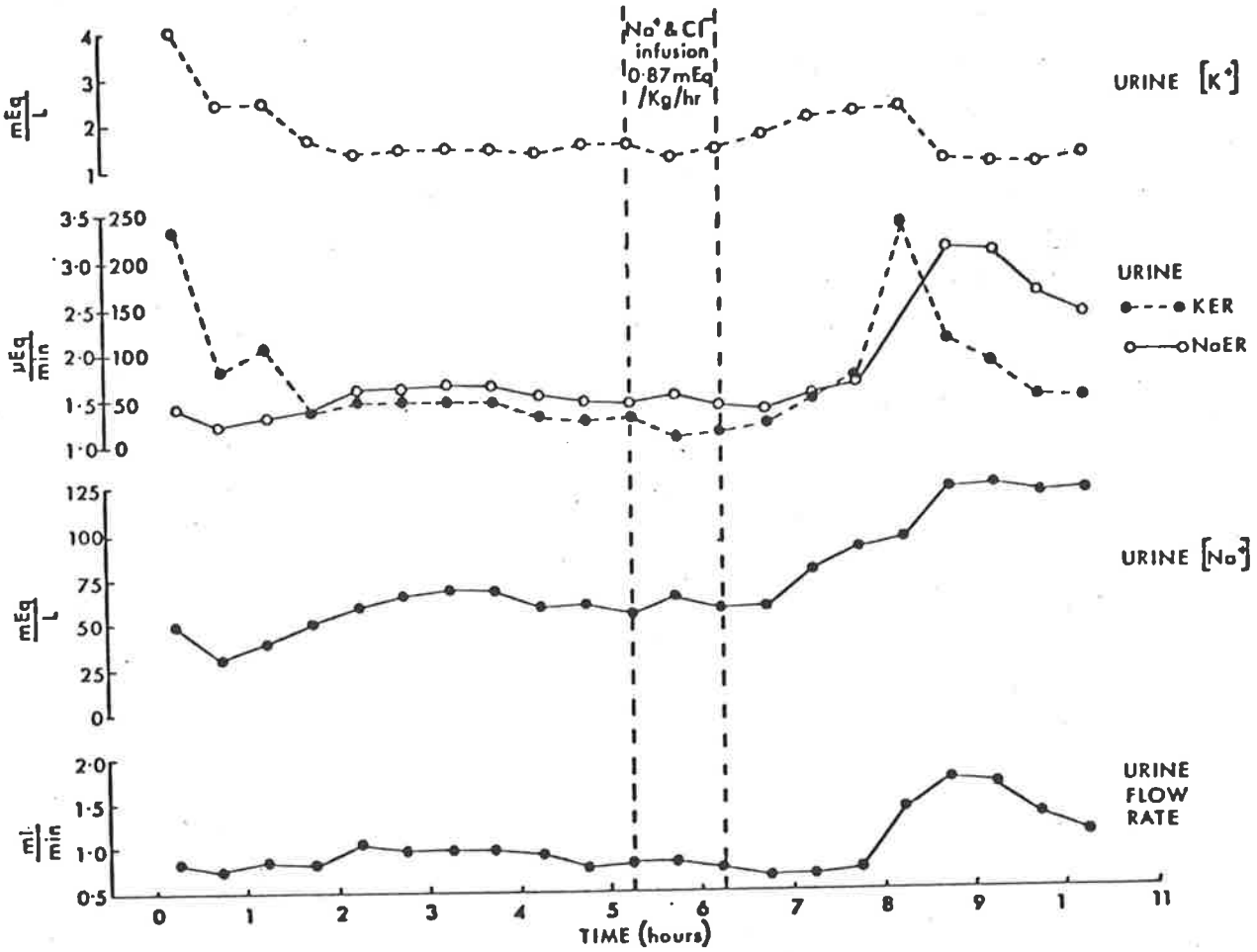

FIGURE 26. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 69-467 (116 days old).
(See appendix table 31 and text page 99)

The remaining urinary solutes analysed; creatinine and uric acid, showed changes in concentration that were inversely related to urine flow rate. Although the excretion rate of these solutes showed some increase.

The $[Na^+]$ of foetal plasma fell throughout the experiment, but most noticeably during the period of increased Na^+ excretion. In contrast, plasma $[K^+]$ increased during the experiment.

e. Foetus 107 (125 days) (appendix table 33)

In this experiment the initial urine flow rates were low, with the pre-treatment average being only 0.03ml/min. Nevertheless, when the NaCl was infused (3.08 mEq/kg/hr for ½ hour; total Na^+ 2.77 mEq) (6.0ml of 2.7% NaCl) the flow rate increased to a maximum of 0.18ml/min. and then returned to control values 2 hours after the infusion. The $[Na^+]$ of the foetal urine also increased by about 60% and at the end of the experiment (3 hours after treatment) it was still at that level. The remaining urinary parameters were not significantly effected by the NaCl treatment. (See fig. 28).

Of the plasma solutes, Na^+ reached a peak in concentration at about the time of maximum Na^+ excretion but declined thereafter. Plasma $[K^+]$ showed no consistent changes.

f. Foetus 66-310 (146 days) (appendix table 34)

This was the oldest foetus used in this series of experiments and accordingly it was given the largest dose of NaCl (0.85 mEq/kg/hr of Na^+ and Cl^- for 3½ hours; total Na^+ 11.32 mEq) (24.5ml of 2.7% NaCl). The response obtained was typical of that obtained in the preceding experiments; although the flow rate increase, which began in the last ½ hour of the infusion period, was comparatively short-lived. Flow rate reached a maximum about 1 hour after treatment and over the following 1½ hours it returned to control levels. The maximum flow rate was about 3¼ times the pre-treatment average. The $[Na^+]$ of foetal urine began to rise at the same time as urine flow rate and remained at a level about 1¼ times the average control level until the end of the experiment. Consequently, Na^+ excretion rate reached a peak within an hour of the end of the

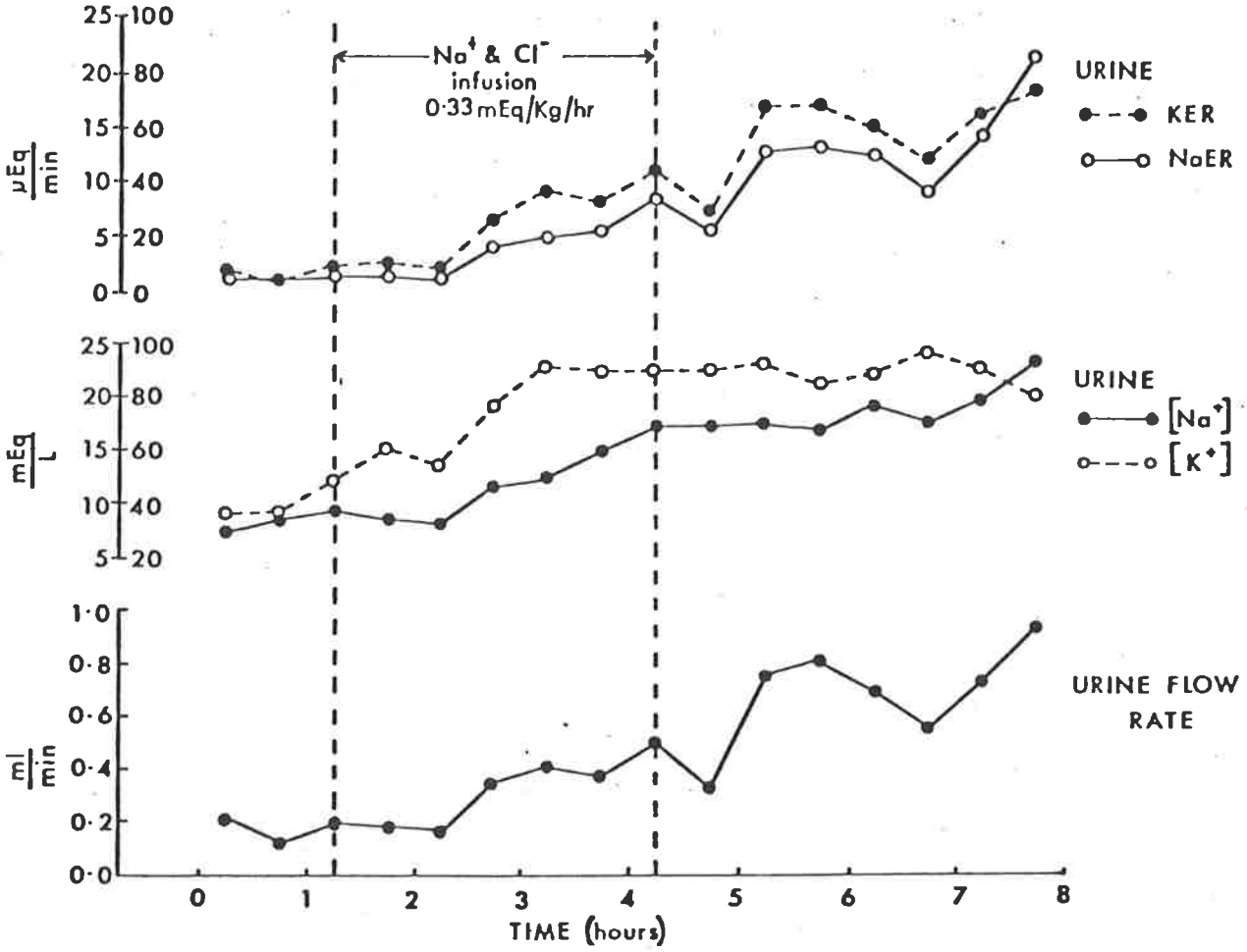

FIGURE 27. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 69-613 (118 days old). (See appendix table 32 and text pages 99 and 100)

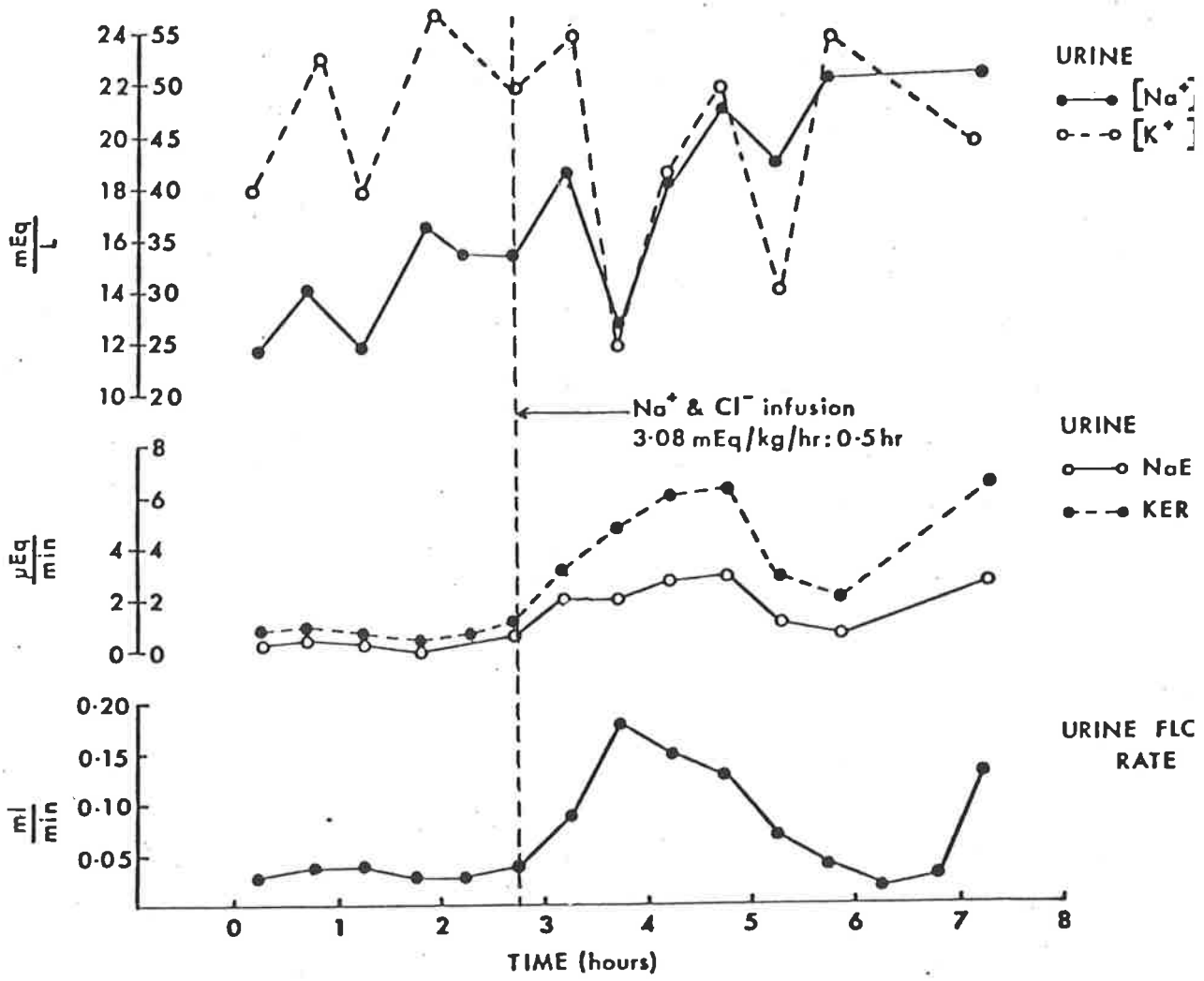

FIGURE 28. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 107 (125 days old).
(See appendix table 33 and text page 100)

infusion, but did not return to control levels because of the elevated $[Na^+]$. In this experiment, urinary $[K^+]$ decreased as did K^+ excretion rate. This decrease in urinary $[K^+]$ was peculiar to this foetus. The remaining urinary solutes, creatinine and uric acid, underwent concentration changes that paralleled those of K^+ (see fig. 29).

In foetal plasma, there was a general rise in $[Na^+]$ with the highest concentration occurring during a period of substantial Na^+ excretion. Plasma $[K^+]$ decreased throughout the experiment.

In summary, it was seen that with the exception of foetus 69-443, all foetuses responded to NaCl infusion by increasing both urine output and urinary $[Na^+]$ and thereby raising Na^+ excretion rate. There was no well defined relationship between dose and response. The variability in the observed responses is probably due in part to developmental changes in the foetal kidney. However much of the variability would be due to the influence of factors which are independent of the foetus, such as the state of maternal hydration and maternal osmotic balance.

In most cases the increase in Na^+ excretion rate following saline infusion was accompanied by an increase in K^+ excretion rate. In those foetuses aged between 111 and 118 days, the magnitude of these changes were similar. However, with the 125 day-old foetus, the rise in K^+ excretion rate was small compared with the rise in Na^+ excretion rate; while with the 146 day-old foetus there was a decline in K^+ excretion rate. These differences may reflect changes in the activity of the renal tubules, but this could not be stated reliably on the basis of these experiments. Nevertheless the ability of the foetal kidney to respond to NaCl loads by increasing the excretion of Na^+ has been established.

Bernstine (1970) reported that in near-term foetal dogs, infused with physiological saline, the increased fluid output of the foetus during the 2 hours following treatment accounted for about 1/3 of the saline infused. In the present work, comparable results were obtained with the two oldest foetuses. The 125 day-old foetus excreted 34% of the Na^+ load in the 2 hours following treatment, while the 146 day-old foetus, which received a

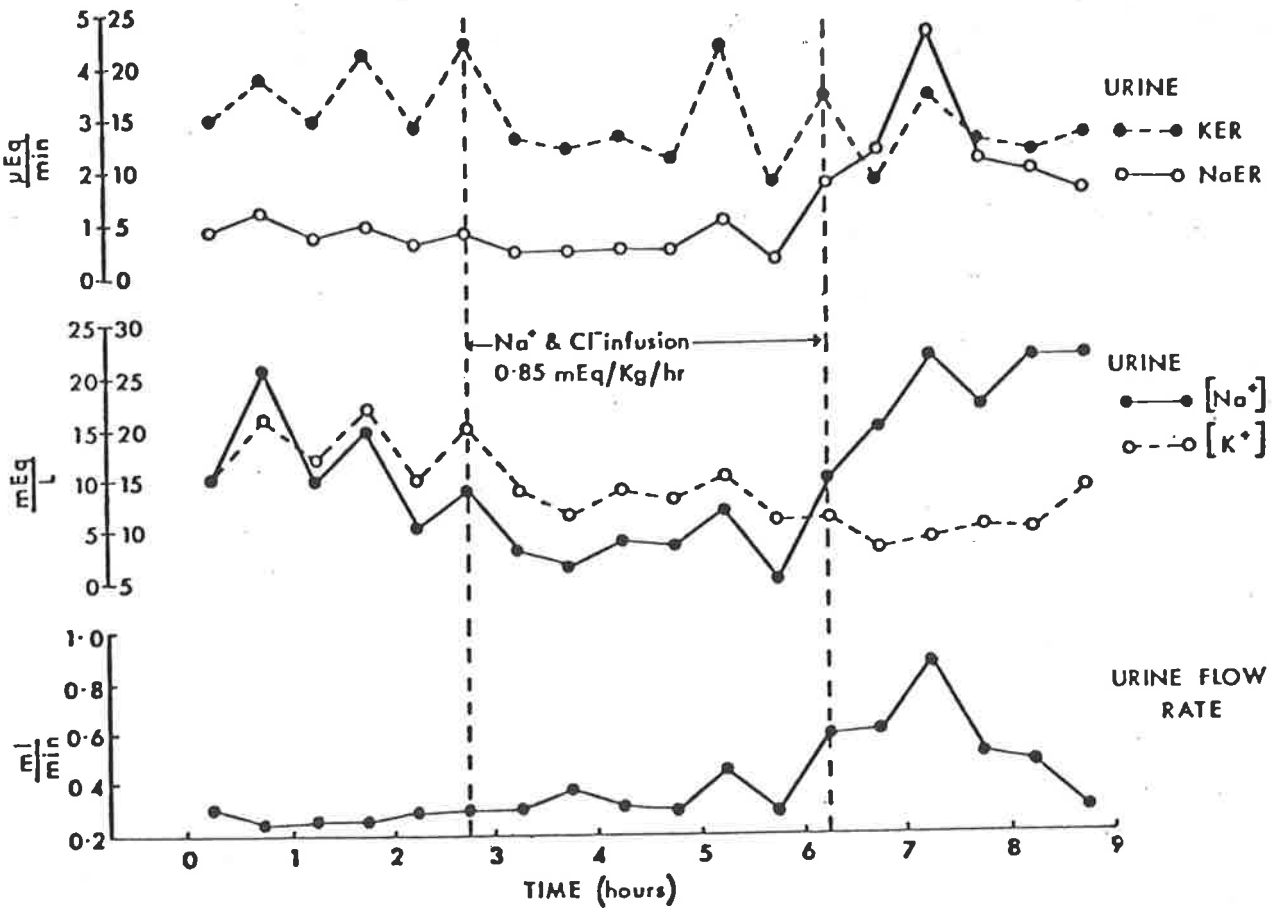

FIGURE 29. Changes in foetal urinary parameters following the infusion of hypertonic saline into foetus 66-310 (146 days old).
(See appendix table 34 and text pages 100 and 101)

large Na^+ load excreted only 11% in the 2 hours following treatment. It may be that in these foetuses the renal mechanisms for handling excess plasma Na^+ were saturated and presumably the remaining Na^+ crossed the placenta into the maternal circulation. In contrast the younger foetuses (111-118 days) which showed natriuretic responses, after saline infusion, excreted more Na^+ in the 2 hours following treatment, than was infused. In these foetuses the Na^+ loads were only a fraction of those administered to the older foetuses and the homeostatic capacity of the foetal kidney was probably not exhausted. However, the Na^+ excreted in the 2 hours following treatment was between 2.6 and 3.7 times the quantity of Na^+ infused which indicates a lack of precision in the regulatory mechanisms of these less mature foetuses.

5.1.2 Foetal Haemorrhage (appendix table 35)

One experiment was carried out in which foetal renal activity was examined following foetal haemorrhage. The foetus used (foetus 12) was 125 days old and had an estimated blood volume of 450ml. (Barcroft 1946). Therefore the 40ml of blood which was withdrawn from the foetus was approximately 9% of its total blood volume. The blood was withdrawn over 10 minutes at the end of a 3 hour control period. Within 1 hour of the blood loss, the foetal haematocrit had fallen to 27 from a ctontrol value of 33 and the haematocrit remained between 25 and 27 for the rest of the experiment. In the control period, foetal MAP was 45mm Hg but fell to 42mm Hg within 10 minutes of the blood loss. The MAP returned to 45mm Hg 30 mins. after the haemorrhage.

With respect to kidney function, changes in urine volume and composition did occur after the bleeding. Urine flow rate, which had a pre-treatment average of 0.82ml/min. fell by 43% immediately after the bleeding and remained low for 2½ hours. Urinary $[Na^+]$ also fell following the blood loss, reaching a level which was about 25% of the average control concentration. The $[Na^+]$ remained low for 2½ hours and then began to rise; however at the end of the experiment (3½ hours after the bleeding)

urinary [Na^+] was still only about 50% of the pre-treatment average. The [K^+] of foetal urine showed similar changes to [Na^+] falling by 50% following the haemorrhage, but unlike [Na^+], the [K^+] had returned to normal by the end of the experiment. As a result of the changes in electrolyte concentration and urine flow, the excretion rates of Na^+ and K^+ fell by 87% and 71% respectively in the ½ hour after the haemorrhage. (See fig. 30).

Alexander et al (1971) reported that foetal sheep respond to haemorrhage by secreting both ADH and ACTH which would explain the results obtained in this experiment.

The foetal plasma samples collected during this experiment revealed that both the [Na^+] and [K^+] of plasma fell immediately after the bleeding but increased toward control levels during the rest of the experiment.

5.1.3 Osmotic Gradients and Foetal Kidney Function

a. Intra-amniotic sucrose infusion (appendix tables 36 and 37).

Two experiments were performed in which sucrose was injected into the amniotic fluid. The use of sugars and other osmotically active substances to water-deplete foetuses has been described by Bruns et al (1963 and 1964).

In the first of the present experiments, 20ml of 2.5M sucrose (2 injections of 10ml) was injected into the amniotic fluid of a 124 day-old foetus (foetus 220). It has been demonstrated that foetal skin is permeable to water at that stage of gestation. Therefore, it was anticipated that by increasing the osmotic pressure (OP) of the fluid surrounding the foetus, water could be removed from the foetal tissues and body fluids via the foetal skin. Measurements of the haematocrit of foetal blood and the OP of foetal plasma suggest that water loss did occur. Each sucrose injection was followed by a fall in urine flow rate, but these changes did not occur until about 30 minutes after treatment.

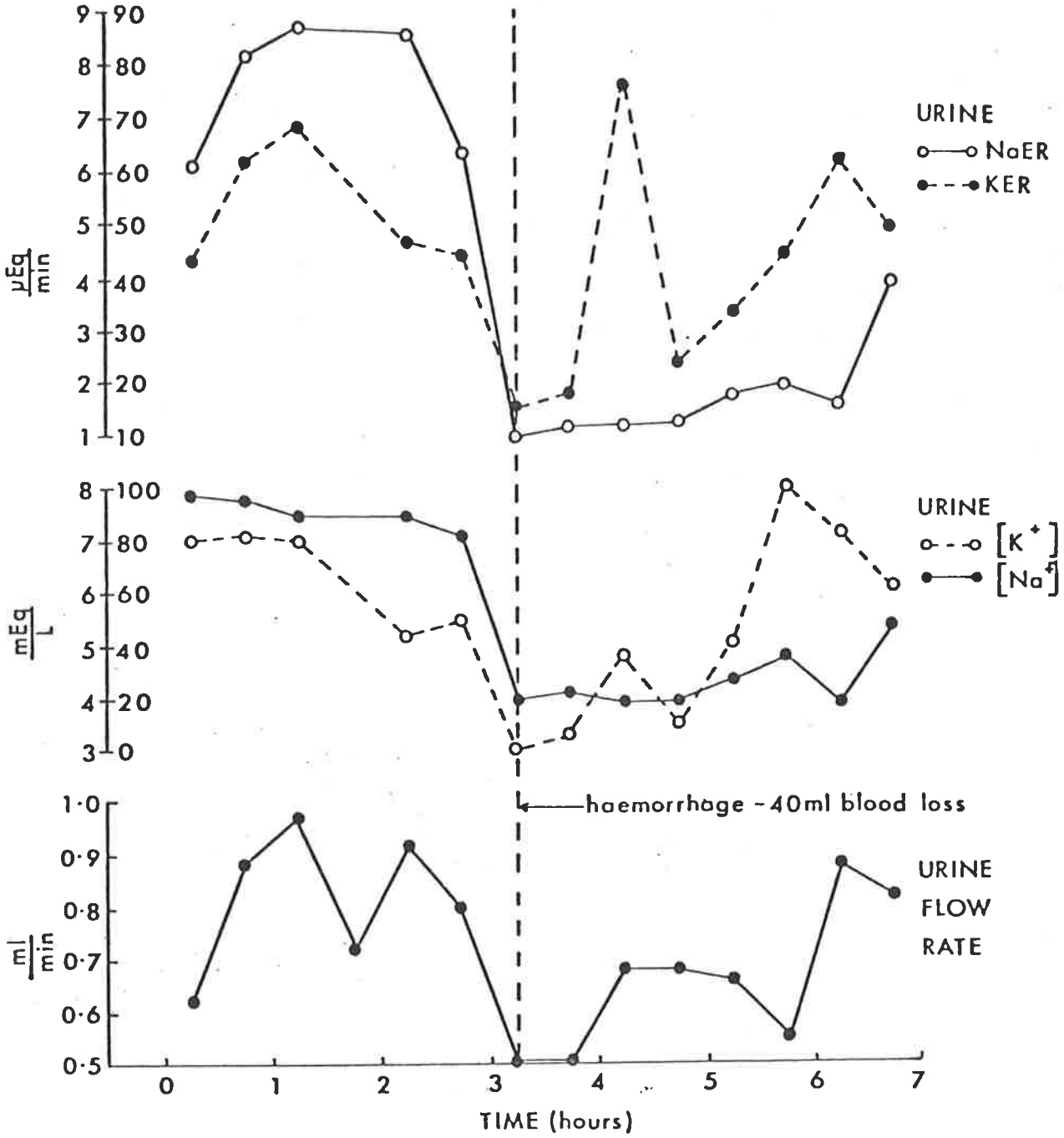

FIGURE 30. Changes in foetal urinary parameters following the removal of 40 ml of blood from foetus 12 (125 days old).
(See appendix table 35 and text pages 102 and 103)

The delayed response probably reflects the time necessary for the increased OP of the amniotic fluid to have an effect on foetal fluids and for the foetal kidney to respond to the water loss. Ninety minutes after the first injection of sucrose, a minimum flow rate which was 68% of the control average was recorded. Two hours after the second sucrose injection a minimum flow rate which was 53% of the control average was reached. It is probably significant that the highest values for plasma OP corresponded with the periods of lowest urine flow and that plasma electrolyte concentrations increased following each sucrose injection. (See fig. 31).

The changes in urinary $[Na^+]$ and $[K^+]$ were less dramatic than the flow rate changes and, accordingly, the relationship between electrolyte concentration and sucrose treatment is less clear. The $[Na^+]$ of foetal urine remained largely unaltered for 2 hours following the first sucrose injection, after which it fell by about 50%. After the second sucrose injection there was an initial increase in urinary $[Na^+]$ but after 1½ hours it began to decline and continued to do so for the rest of the experiment. The urinary concentrations of K^+, uric acid and creatinine showed patterns of change almost identical to that of $[Na^+]$ while urine pH was not significantly affected. The combined effect of the reduced urine flow rates and decreased solute concentrations was a progressive decrease in the excretion rate of all the urine solutes analysed.

Prior to treatment endogenous creatinine clearance was 5.38 ml/min. but following the sucrose injection it fell to a minimum of 3.07 ml/min.

A second experiment of this type was carried out using a 116 day-old foetus (foetus 253). The skin of this foetus could be assumed to be even more permeable to water than that of the foetus used in the preceding experiment. Following the injection of 25ml of 2.5M sucrose into the amniotic fluid there was a large reduction of foetal urine output. The average urine flow during the control period was 0.17ml/min. yet 1½ hours after the sucrose treatment the flow rate was 0.07ml/min. and it decreased further to 0.01ml/min. 5 hours after treatment. The flow rate

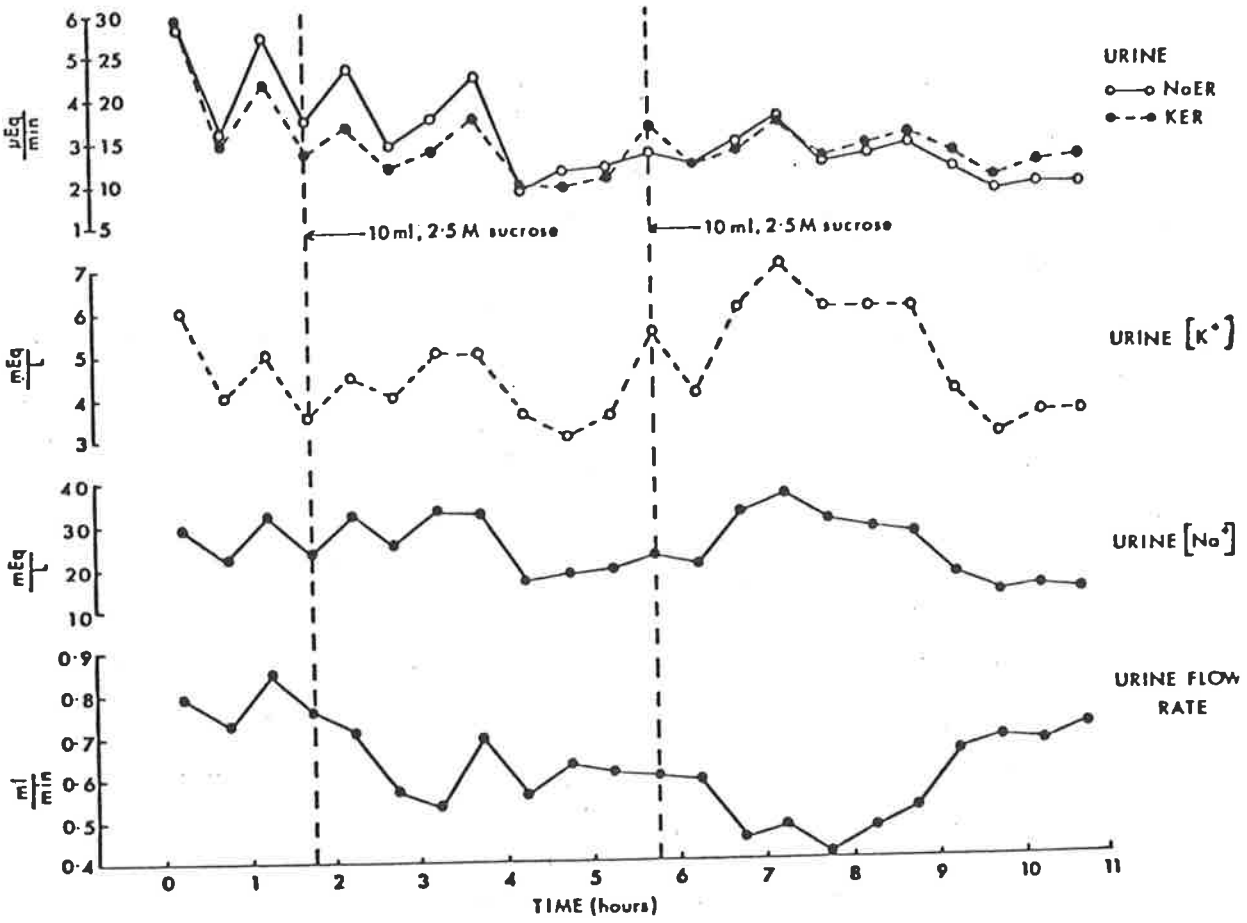

FIGURE 31. Changes in foetal urinary parameters following injections of sucrose into the amniotic fluid surrounding foetus 220 (124 days old).
(See appendix table 36 and text pages 103 and 104)

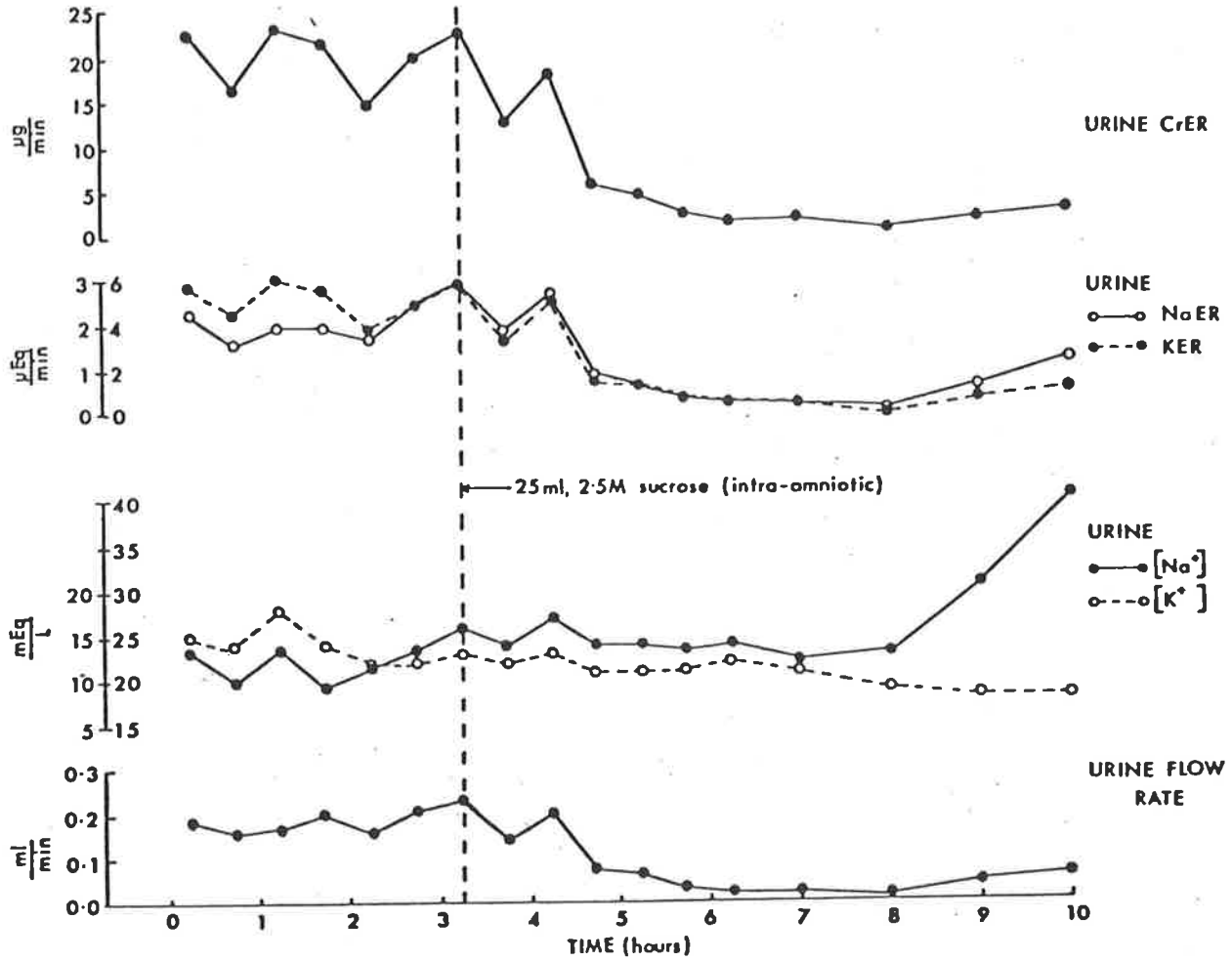

FIGURE 32. Changes in foetal urinary parameters following an injection of sucrose into the amniotic fluid surrounding foetus 253 (116 days old).
(See appendix tables 37 and text pages 103 and 104)

began to rise during the last hour of the experiment but remained far below the control value. The [Na^+] of foetal urine was constant throughout the experiment apart from an increase in the last hour, but Na^+ excretion rate reflected the flow rate changes. The excretion rates of the remaining urinary solutes all decreased substantially, not only because of the reduced output of foetal urine, but also because the urinary concentration of the individual solutes decreased following treatment. (See fig. 32 and appendix table 37).

Because of the nature of the experiment and the immaturity of the foetus, only 3 plasma samples were taken and these did not show haematocrit, OP or electrolyte concentration changes similar to those observed in the previous experiment. On this occasion all of the plasma parameters decreased following treatment but since the first plasma sample of the treatment period was not taken until 2 hours after the injection, it is possible that transient rises occurred but went unobserved. As in the first experiment endogenous creatinine clearance was reduced following treatment. Initially creatinine clearance was 3.42 ml/min. but after treatment values of 2.29 and 2.11 ml/min. were recorded.

b. Maternal sucrose infusion (appendix tables 38 and 39).

In this experiment an attempt was made to water-deplete a foetus by establishing a trans-placental osmotic gradient. To accomplish this, 100ml of 2.5 M sucrose solution was injected intravenously into a ewe which was carrying a 120 day-old foetus (foetus 271). This initial injection of sucrose was followed by the infusion of an additional 60ml over 3 hours. The osmolality of foetal and maternal plasma samples are shown in table 8. It can be seen that initially there was a small osmotic gradient favouring the movement of water into the foetus. During sucrose treatment, the OP of maternal plasma increased sharply and midway through the infusion period there was a nett OP of 19 mOsm/L favouring the movement of water from foetus to mother. By the end of the infusion period, the difference in favour of the ewe was only 5 mOsm/L and 1 hour later the gradient was lost completely. A small gradient was re-established at the end of the experiment due to a belated rise in the plasma

TABLE 8: PLASMA, OSMOTIC PRESSURE OF MOTHER AND FOETUS DURING MATERNAL SUCROSE INFUSION (FOETUS 271, 120 DAYS)

TIME AFTER COMMENCING EXPERIMENT (Hours)	PLASMA OSMOTIC PRESSURE FOETAL (mOsm/L)	MATERNAL (mOsm/L)	
½	295	289	
2	293	287	
			100 ml 2.5 M Sucrose
2½	-	310	
3½	-	314	
			2 ml/min. 2.5 M Sucrose
4	295	304	
5	-	301	
5½	-	295	
6	296	292	
8	296	296	

(See text page 105)

OP of the ewe.

When viewed in the context of the changing osmotic gradient, the fluctuations in foetal urine flow are not entirely as anticipated. While the maximum osmotic gradient in favour of the ewe existed, there was a decrease in the flow rate of foetal urine. However, this decrease was small and its significance questionable. In the post-infusion period, urine flow rate continued to fall despite the elimination of the osmotic gradient. This may have been due to the persistence of renal water-conserving activities, suggesting a lack of precision in the homeostatic ability of the immature kidney. Alternatively, the continued water retention may have been due to the belated increase in maternal plasma OP and its effect on foetal OP..

The $[Na^+]$ of foetal urine followed closely the changes in flow rate; so closely in fact that it suggests that Na^+ and water excretion were linked. The urinary $[K^+]$ was more independent of flow rate and the influence of the maternal sucrose treatment on urinary $[K^+]$ was obscure. Similarly, the concentration of creatinine and uric acid in foetal urine and urinary pH appeared to be unaffected by the maternal sucrose treatment. (See fig. 33). However, endogenous creatinine clearance did appear to be affected by the sucrose treatment. The pre-treatment value was 3.61 ml/min. but 30 minutes after the infusion the clearance rate was 2.25 ml/min.

The OP of maternal plasma increased following the sucrose injection and the urine output of the ewe increased, presumably because sucrose was acting as an osmotic diuretic. Once the OP of the maternal plasma had returned to control levels the flow rate of maternal urine decreased. Therefore during the majority of the experiment, foetal urine flow was decreasing while maternal urine flow was increasing. Also the $[Na^+]$ and $[K^+]$ of maternal plasma decreased during the treatment period and there was a corresponding decrease in the $[Na^+]$ of foetal plasma. However, the plasma $[K^+]$ of the foetus increased. It may have been that the fall in plasma $[Na^+]$ of the foetus

was due to loss of Na^+ across the placenta following the fall in the plasma $[Na^+]$ of the ewe. Alternatively, it may have been due to the simultaneous passage of Na^+ and water across the placenta in response to the artificially induced osmotic gradient.

5.2 Maternal-Foetal Relationships

5.2.1 Simultaneous analysis of maternal and foetal kidney output

To study the relationship between the flow rate and composition of maternal and foetal urine, samples were obtained simultaneously from a ewe and the 122 day-old foetus it carried. Both maternal and foetal urine was collected continuously, in 30 minute fractions, for 24 hours. Urine flow rate was determined for each 30 minute fraction and the $[Na^+]$ and $[K^+]$ of each sample was measured. Apart from the continuous drainage of urine from both mother and foetus, no treatment was applied. The ewe was allowed free access to food and water during the experiment and it drank 3.5 L of water and consumed 0.8kg of lucerne chaff. The results of this study are presented in the appendix tables.

a. Flow Rates (appendix table 40)

There was a significant relationship between the flow rates of maternal and foetal urine ($r = 0.370, 0.01 > P > 0.001$ $n = 48$). Both maternal and foetal flow rate varied considerably from sample to sample but there was an overall decrease in maternal flow rate during the study. For the first three hours of the study the maternal flow rate was 1.5 to 1.7ml/min. compared with 0.3 to 0.7ml/min. in the final three hours. With respect to foetal flow rates the values in the first three hours are similar to those recorded in the last three hours (0.24 and 0.28ml/min. respectively).

b. Sodium Excretion Rates (appendix table 41)

The urinary excretion of Na^+ by the ewe and the foetus were significantly correlated ($r = 0.356, 0.01 > P > 0.001$ $n = 48$). During

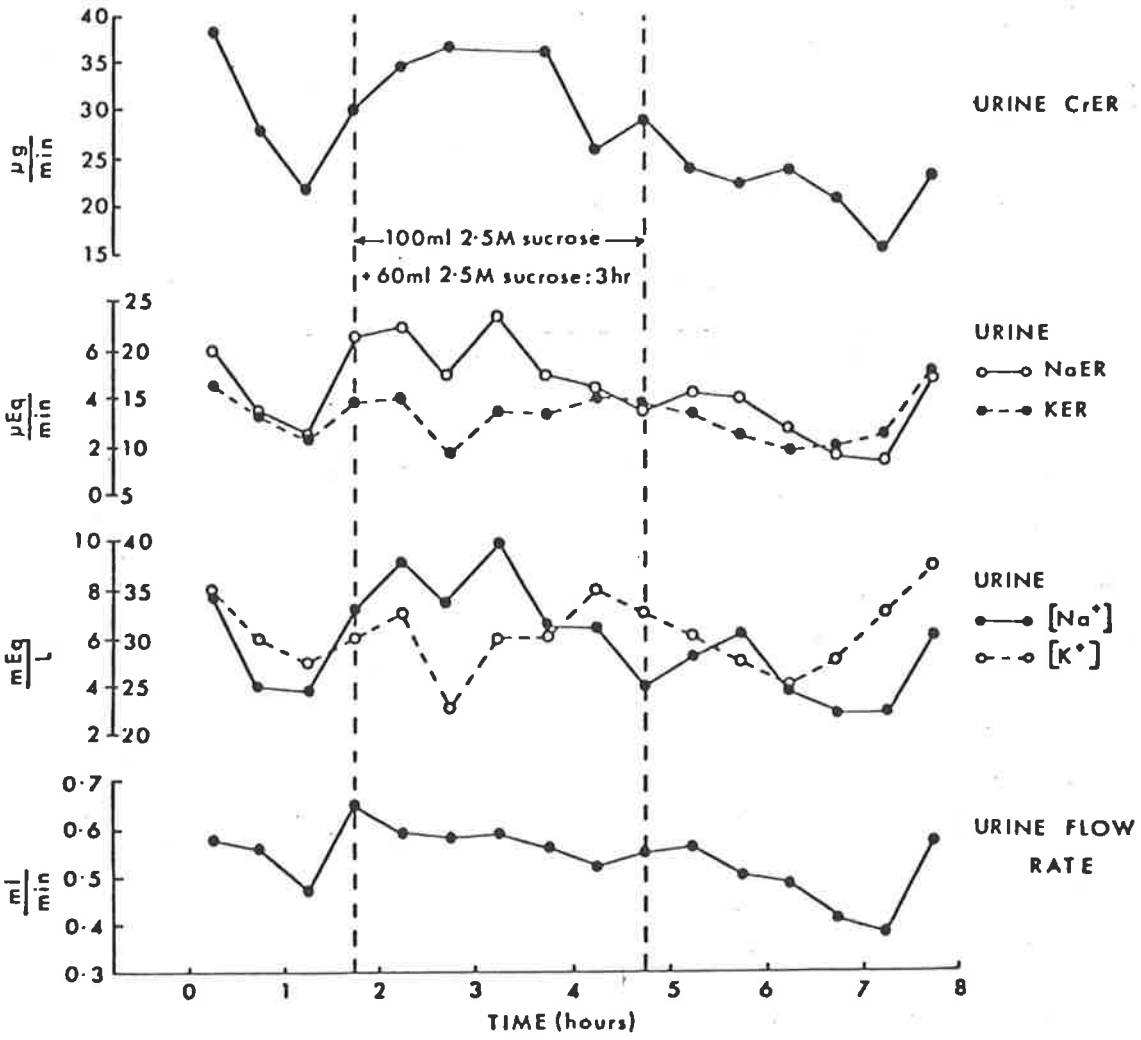

FIGURE 33. Changes in foetal urinary parameters following the intravenous infusion of sucrose into the ewe carrying foetus 271 (120 days old).
(See appendix tables 38 and 39 and text pages 105-107)

the 24 hours of the study, maternal Na^+ excretion showed three peaks with 11 hours between the first and second peaks and six hours between the second and third peaks. Foetal Na^+ excretion followed a similar pattern apart from some divergence between the 14th and 17th hours of the study. However on the basis of this experiment it cannot be concluded that a diurnal pattern of Na^+ excretion exists for mother or foetus. Mellor and Slater (1973) have observed marked changes in the osmolality of foetal urine following the intake of food or water by the ewe. In the present work, the times at which the ewe ate or drank were not noted, accordingly it is not known if the observed fluctuations in Na^+ excretion rate are related to the intake of food or water by the ewe.

c. Potassium Excretion Rates (Appendix table 41)

Maternal and foetal K^+ excretion rates were not significantly correlated ($r = 0.101$ NS $n = 48$). The general pattern of change of maternal and foetal potassium excretion was similar, although during a few periods, the two excretion rates appeared to be between 30 and 60 minutes out of phase.

d. Urinary pH (Appendix table 40)

As with flow rate and Na^+ excretion rate there was a significant correlation between the pH of the simultaneously collected maternal and foetal urine samples ($r = 0.330$ $0.05 > P > 0.01$ $n = 48$). It is noticeable that in all but a few of the paired samples the pH of maternal urine was greater than the pH of foetal urine. There was no recognisable diurnal pattern in the pH change of maternal or foetal urine.

5.2.2 Foetal Kidney output during maternal water depletion

(Appendix tables 42,43 and 44)

Two experiments were carried out in which pregnant ewes were treated with furosemide (Lasix-Hoechst). Furosemide is reported to inhibit renal Na^+ reabsorption in dogs, rat and man (Buchborn and Anastakis, 1964; Deetjen 1965; Berliner et al 1967). More significantly,

furosemide when administered to sheep produces a diuresis, natriuresis, a moderate kaliuresis and an increase in H^+ excretion (Peter, 1969). By withholding drinking water and administering furosemide to the pregnant ewes, their total body water was reduced so that the activity of the foetal kidney could be examined during maternal dehydration.

In the first experiment the ewe, which was carrying a 117 day-old foetus (foetus 253), was given two intravenous injections of 10mg of furosemide. Following the first injection the flow rate of maternal urine increased within ½ an hour from 0.40ml/min. to 4.66ml/min. Also Na^+ excretion rate increased from 46 μEq/min. to 442.7 μEq/min. and K^+ excretion rate from 11.6 μEq/min. to 125.8 μEq/min. Within two hours of the first injection these parameters had fallen to levels which were about 50% greater than their pre-treatment levels. At that point the second injection of furosemide was given and again urine flow rate and electrolyte excretion rates increased substantially. At the end of the experiment, water was offered to the ewe and 2.1 L was consumed in less than 10 minutes.

It can be seen in figure 34 that the urine output of the foetus fell just before the first furosemide injection but then increased steadily throughout the period of maternal diuresis. Following the second furosemide injection there was a small decrease in foetal urine flow, but it was short-lived and again foetal flow rate increased, despite a sixfold increase in maternal urine output. The excretion rates of Na^+ and K^+ fell just prior to the first furosemide injection and then increased steadily during the period of maternal natriuresis and kaliuresis, only to fall again following the second furosemide injection. However, overall the results give no information concerning foetal kidney function because the variation in urinary electrolyte concentrations and excretion rates during the control period prohibits valid interpretation of the experimentally induced response.

In addition to the control variation the results were not as

anticipated. It was thought that the water and electrolyte losses of the ewe would lead to similar depletion of the foetus and thus to a decrease in water and electrolyte excretion by the foetal kidney. However it may have been that the losses induced in the ewe were insufficient to significantly affect the foetuses. By comparing pre-treatment and post-treatment flow rates it was estimated that the water loss induced by furosemide was only 413ml. Also, the total body water (TBW) of the ewe, which was estimated from the dilution of tritiated water (Morris, Howard and MacFarlane 1962), was not greatly altered by the diuretic treatment. In the control period TBW was 30.3 L while one hour after the second furosemide injection it was 29.8 L. This may explain why the anticipated results were not obtained. Nevertheless, in view of the maternal response to furosemide, it is difficult to understand why foetal urine flow increased and why in one instance Na^+ excretion rate increased, unless furosemide crossed the placenta and directly effected the foetal kidney. No information is available on the ability of furosemide to cross the placental barrier in sheep. However, in humans, Wladimiroff (1974) has reported that foetal urine flow rate, as measured by ultrasonic techniques, increases following intra-venous administration of furosemide to the mother.

The second experiment of this type was carried out over 14 hours. After a 3 hour control period, 10mg of furosemide was injected every hour, for 11 hours, into a ewe carrying a 118 day-old foetus (foetus 275). Figure 35 shows that following the first injection there was a large rise in the flow rate of maternal urine but that the response following subsequent injections became progressively smaller. The $[Na^+]$ and $[K^+]$ of maternal urine increased during the furosemide treatment, while the electrolyte excretion rates showed increases which compounded the flow rate and concentration changes. Following the first furosemide injection Na^+ excretion increased from 3.8 μEq/min. to 174.4 μEq/min. while K^+ excretion rate rose from 29.6 μEq/min. to 431.1 μEq/min. Thereafter the excretion rates declined despite the continued administration of furosemide. The OP of maternal plasma which was 279 mOsm/kg at the beginning of the experiment increased to 313 mOsm/kg by the end of the

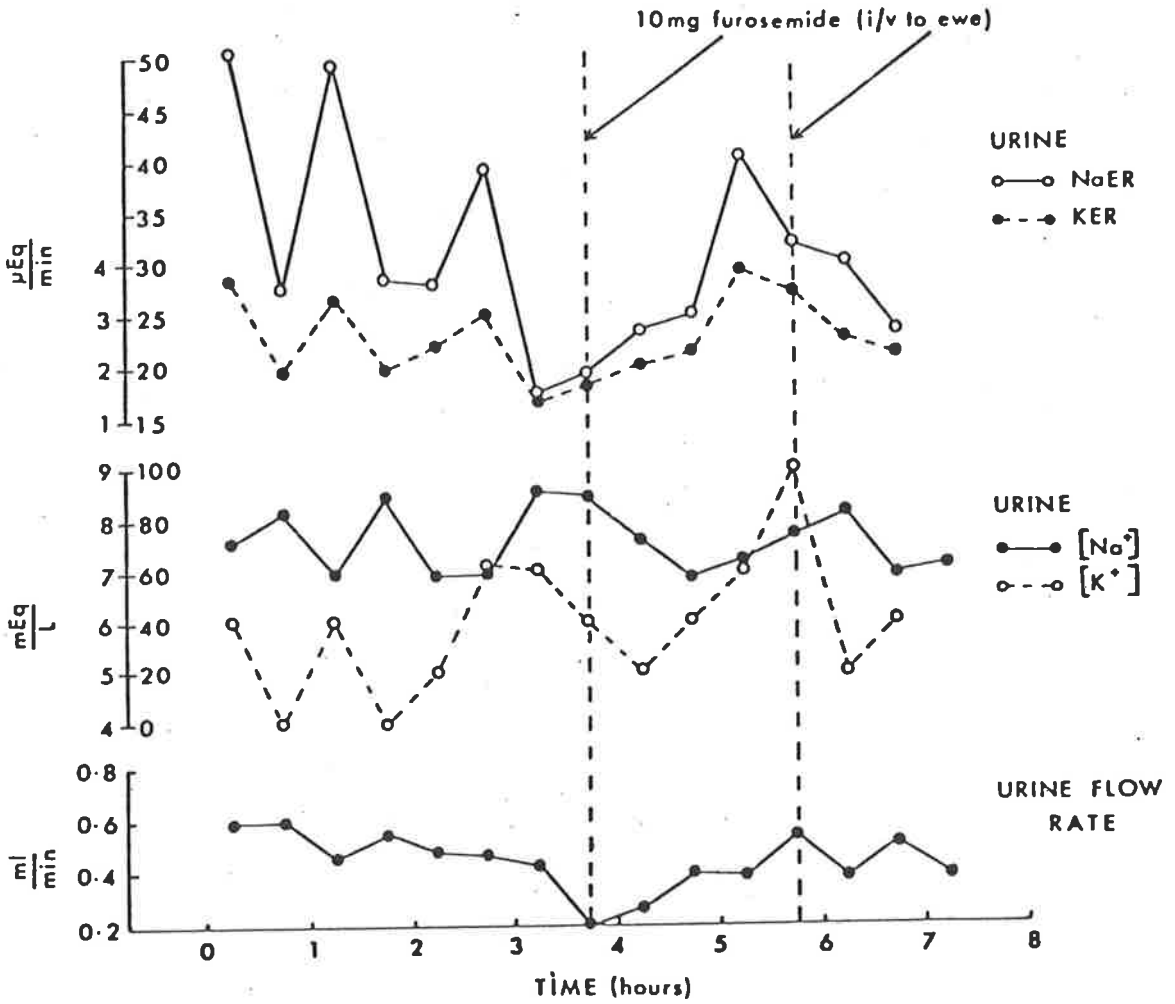

FIGURE 34. Changes in foetal urinary parameters following treatment of the ewe carrying foetus 253 (117 days old) with furosemide. (See appendix table 42 and text pages 108-110)

furosemide treatment. Over the same period plasma Na^+ and K^+ concentrations were not significantly altered despite a total water loss of 1.8 L, of which 680ml was lost in the first 2 hours of treatment. The TBW of the ewe decreased from 32.1 L in the control period to 30.4 L following the last furosemide injection. When the ewe was offered water at the end of the experiment, 1.8 L was consumed within 5 minutes.

The changes in foetal urine composition and flow rate that occurred during the furosemide treatment of the ewe are recorded in figure 36. During the first 3 hours of maternal treatment, there was a fall in foetal urine flow and a corresponding decrease in the excretion rate of all urinary solutes. But after the initial fall, foetal urine flow increased and toward the end of the experiment it exceeded the control values. The $[Na^+]$ and $[K^+]$ of foetal urine also increased after 3 hours of the treatment period and in combination with the rise in flow rate, resulted in natriuresis and kaliuresis. The electrolyte levels in foetal plasma showed no significant changes, but it was noticeable that the minimum $[Na^+]$ occurred at the time of maximum maternal natriuresis. That may indicate that foetal Na^+ crossed the placenta to restore the maternal $[Na^+]$ that was depleted by the natriuresis.

On the basis of these experiments it was not possible to conclude that maternal dehydration and Na^+ loss, similarly depleted the foetus. Accordingly, it could not be established that the foetus responds to Na^+ and water loss by restricting the renal excretion of these substances. In the first experiment it is doubtful whether the water depletion of the ewe was sufficient to effect the foetus. However, in the second experiment the water loss was greater and there was an initial decline in foetal urine flow but thereafter the flow rate increased. At the time of maximum maternal OP, foetal urine output was not reduced.

Although the findings of these experiments were generally inconclusive, the reduced foetal urine flow in the second experiment,

soon after the peak in maternal diuresis, may indicate some homeostatic activity by the foetal kidney. This activity may have been limited because of the immaturity of the foetus. Also, if furosemide crossed the placenta and directly affected the foetal kidney, the anticipated results would have been reversed.

5.2.3 Foetal Kidney output following maternal saline infusion

(Appendix tables 45 and 46)

In this experiment hypertonic saline (2.7% Na Cl) was infused into the jugular vein of a pregnant ewe carrying a 122 day-old foetus (foetus 275). The electrolyte infusion rates were 1.6 mEq/min. of Na^+ and Cl^- for 2½ hrs. Water was withheld from the ewe. As a result of the infusion, the $[Na^+]$ of maternal plasma increased from a pre-treatment average of 142 mEq/L to a maximum of 160.5 mEq/L, while the $[K^+]$ decreased slightly. (See fig. 37).

In foetal plasma, the electrolyte concentrations were variable but overall there was a slight increase in $[Na^+]$ and a slight decrease in $[K^+]$. In fact in the first 7 hours of the experiment, the difference in plasma $[Na^+]$ between mother and foetus changed from a gradient of 1 to 2 mEq/L in favour of the foetus, to 13 mEq/L in favour of the ewe.

As expected, maternal kidney activity was altered as a result of the saline infusion. The average flow rate of maternal urine during the control period was 1.8 ml/min. but within an hour of beginning the infusion it had fallen to 0.17ml/min. and remained at or below that level for three hours. The $[Na^+]$ of maternal urine was not effected as rapidly and it was not until the last ½ hour of the infusion period that $[Na^+]$ increased. Thereafter urinary $[Na^+]$ increased rapidly and reached a maximum which was 24 times the average control concentration. Despite this there was a nett reduction in Na^+ excretion because of the reduced

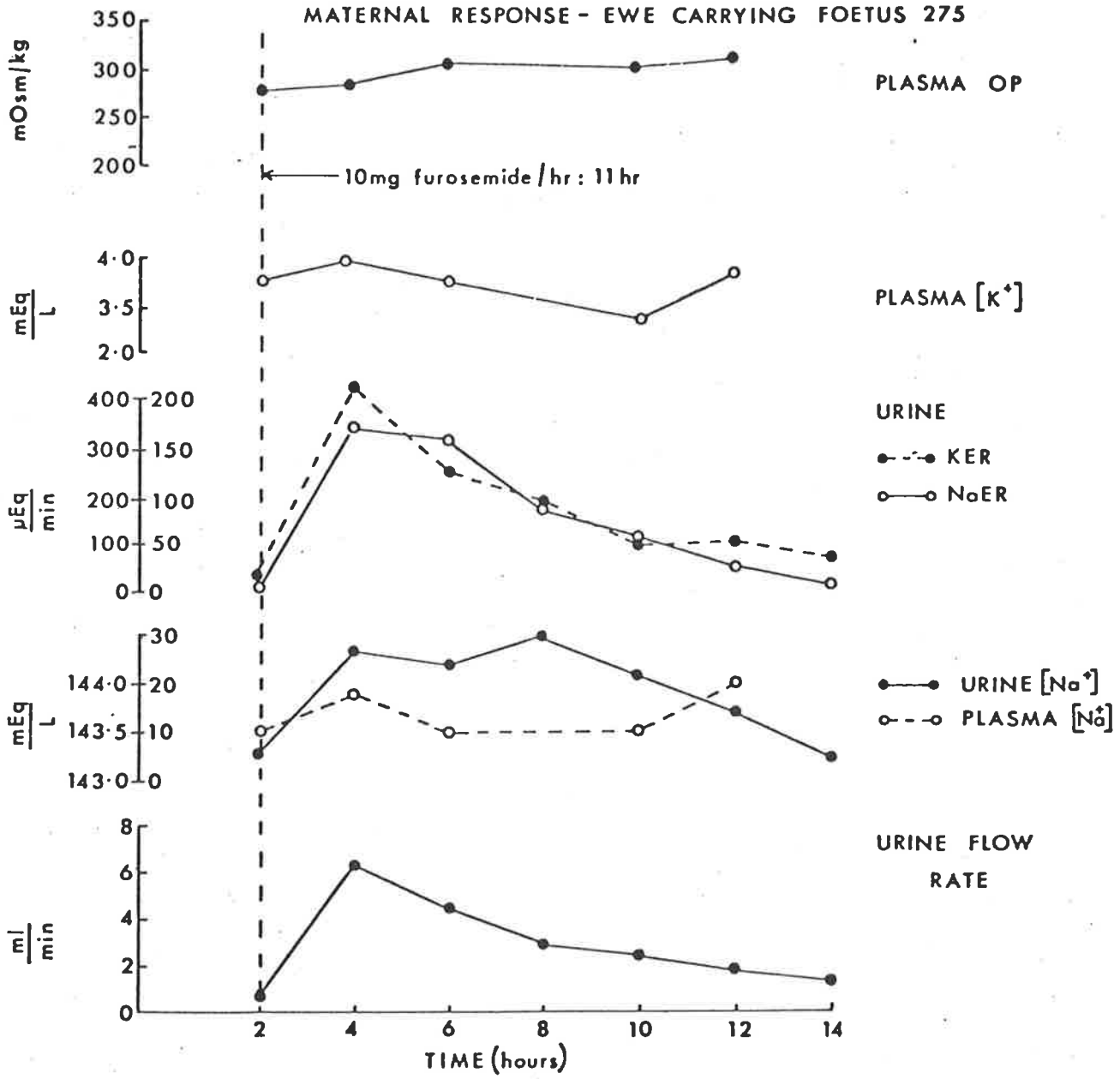

FIGURE 35. Changes in maternal plasma and urinary parameters following treatment of the ewe carrying foetus 275 with furosemide. (See appendix table 43 and text pages 110-112)

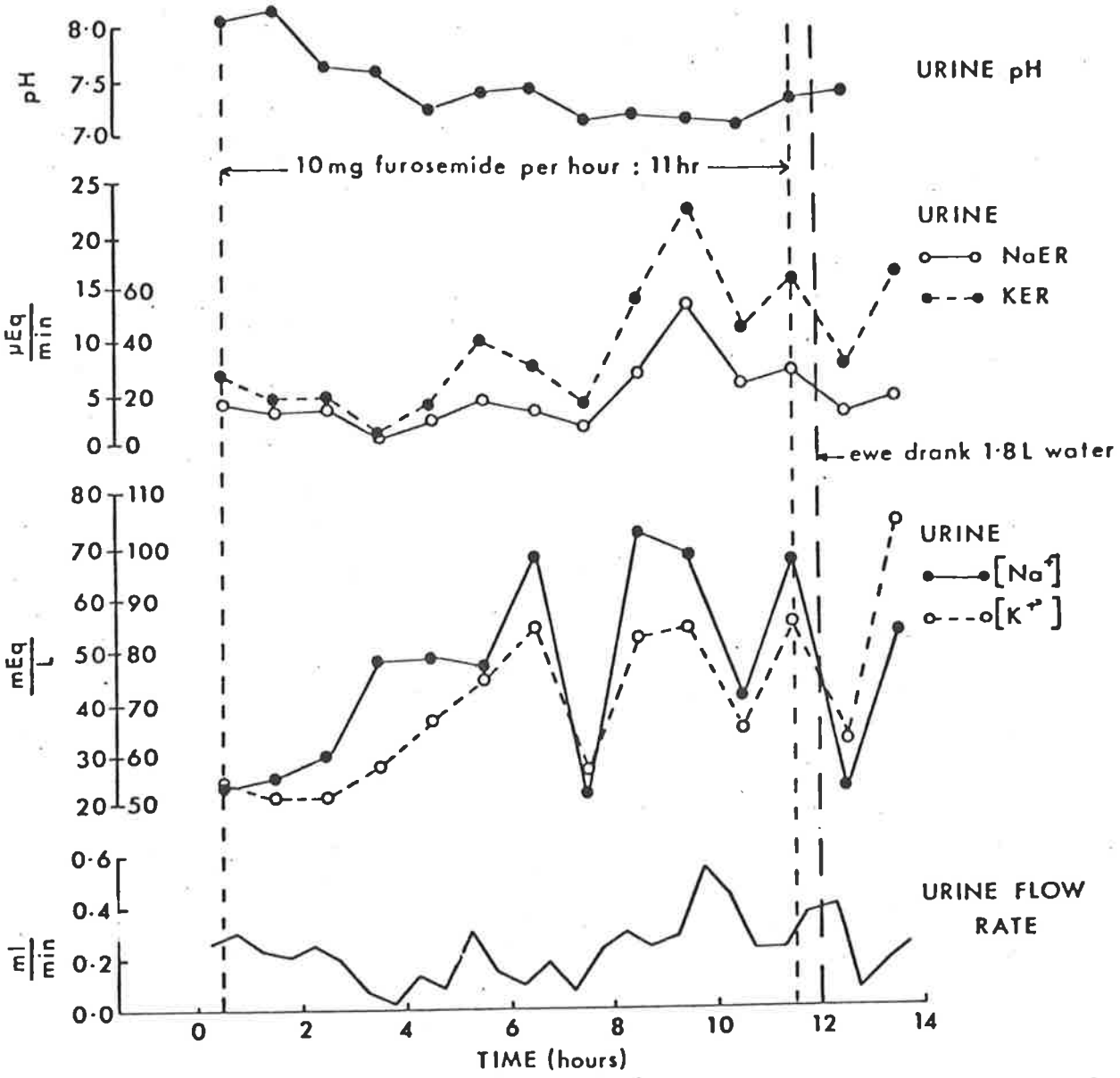

FIGURE 36. Changes in foetal urinary parameters following treatment of the ewe carrying foetus 275 (118 days old) with furosemide. (See appendix table 44 and text pages 110-112)

urine output. However, Na^+ excretion rate increased when the urinary $[Na^+]$ rose and it increased even further when flow rate began to rise. The $[K^+]$ of maternal urine also increased but at its peak it was only 30% greater than the pre-treatment average.

The electrolyte concentrations and flow rate of foetal urine showed patterns of change which paralleled those observed in maternal urine. Although foetal urine output was increasing during the control period, it began to fall within 30 minutes of the start of the saline infusion and it reached a minimum 2½ hours later. The minimum flow rate was about 30% of the pre-treatment average. The $[Na^+]$ in foetal urine did not vary significantly during the control period or during the infusion. However, immediately after the infusion the $[Na^+]$ of foetal urine began to rise and within one hour had reached a maximum concentration which was 2.8 times the pre-treatment average. The $[K^+]$ of foetal urine showed a pattern of change similar to that of $[Na^+]$ although the maximum $[K^+]$ was only 70% greater than its pre-treatment average. Neither Na^+ nor K^+ excretion rate increased substantially during the first seven hours of the experiment because of the converse trends in $[Na^+]$ and $[K^+]$ compared with urine flow rate. This fact, plus the fact that the concentrations of creatinine and uric acid both reached maximums which were about 2.8 times their respective pre-treatment levels implies that the increased concentration of all the solutes is a secondary effect caused by water retention (see fig. 38). The water retention by the foetus is probably a homeostatic response induced by increased $[Na^+]$ in the blood and tissues of the foetus. The additional Na^+ would have entered the foetus as a result of the trans-placental Na^+ gradient established during the maternal saline infusion.

At the 7th hour of the experiment the ewe was allowed access to water and drank 1½ L in five minutes. Within 30 mins. of this maternal water intake, foetal urine flow increased and there was a corresponding

decrease in the [Na^+] and [K^+] of foetal urine. This implies that water crossed the placenta into the foetus, decreased the plasma OP of the foetus and resulted in the return to a normal rate of urine flow. Mellor and Slater (1973) have reported distinct changes in the composition of foetal urine associated with maternal water intake. They found that when a pregnant ewe drank 1.9 L of water, foetal urine osmolality was halved and there was an increase in urine flow rate. Therefore the findings of the present work are consistent with those of Mellor and Slater (1973).

5.3 *Foetal Kidney response to Diuretic Treatment*

A total of 19 experiments were carried out in which 6 different diuretic agents were administered to foetal sheep. The pharmacological activity of the diuretics used, although not precisely defined, have been established with a reasonable degree of reliability in adult animals. Therefore it was anticipated that by studying the effect of these drugs on foetal kidney function, comparisons could be made with the adult responses and conclusions drawn concerning the maturity of the foetal kidney.

The diuretic drugs used included representatives of the major diuretic groups including the benzo-thiazides, carbonic anhydrase inhibitors and the organic-mercurial compounds. Special attention was paid to furosemide and the pharmacologically similar compound sodium ethacrynate, as these are amongst the most potent and widely used diuretics.

5.3.1 Furosemide (4-chloro-N-2-furfuryl-5-sulfamoyl-anthranilic acid) - (Hoechst)

In 7 experiments, foetuses aged between 118 and 140 days received intra-venous injections of furosemide. The doses of furosemide and all other diuretic drugs used have heen expressed as mg/kg of foetal body weight.

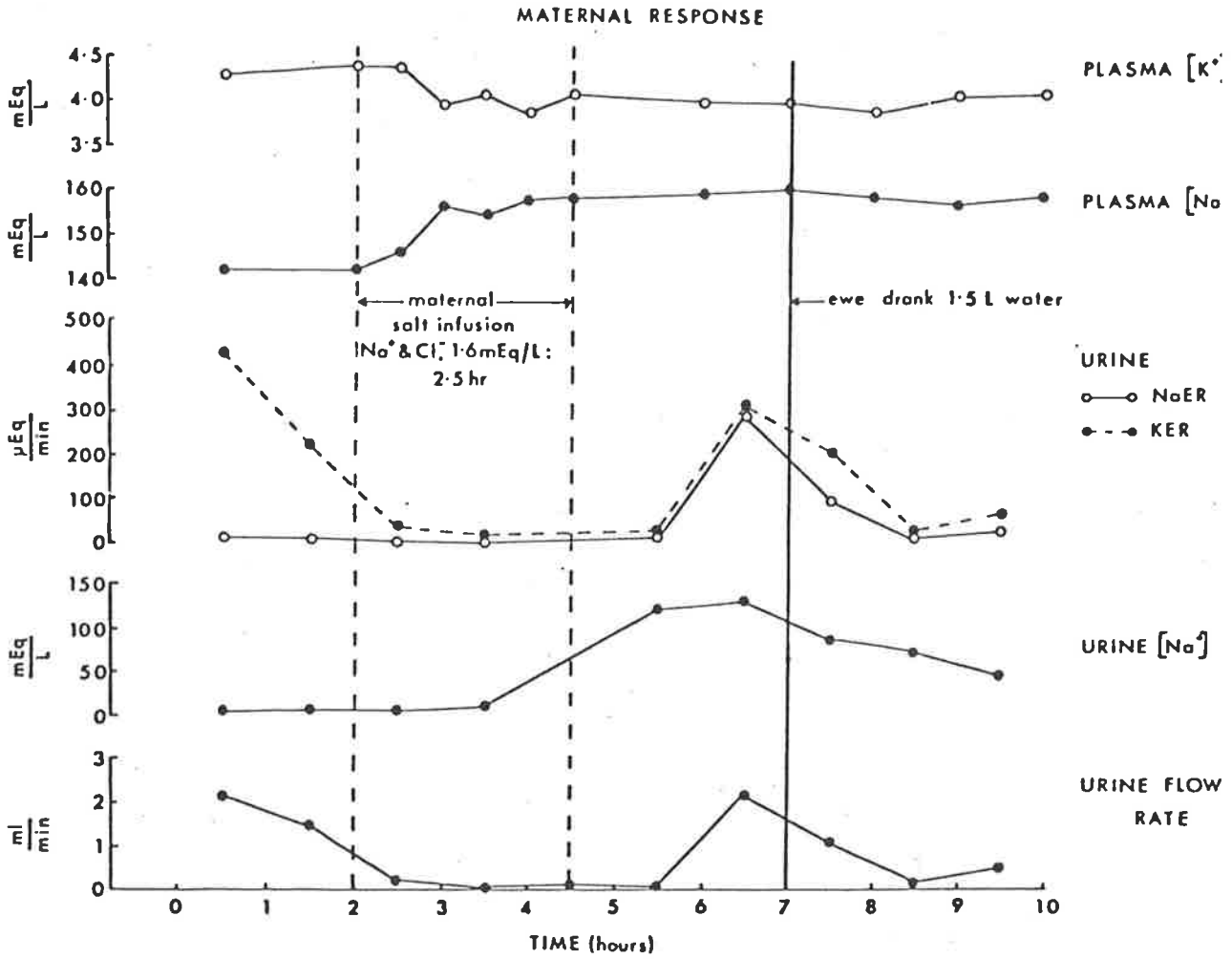

FIGURE 37. Changes in maternal plasma and urinary parameters following the infusion of hypertonic saline into the ewe carrying foetus 275.
(See appendix table 45 and text pages 112-114)

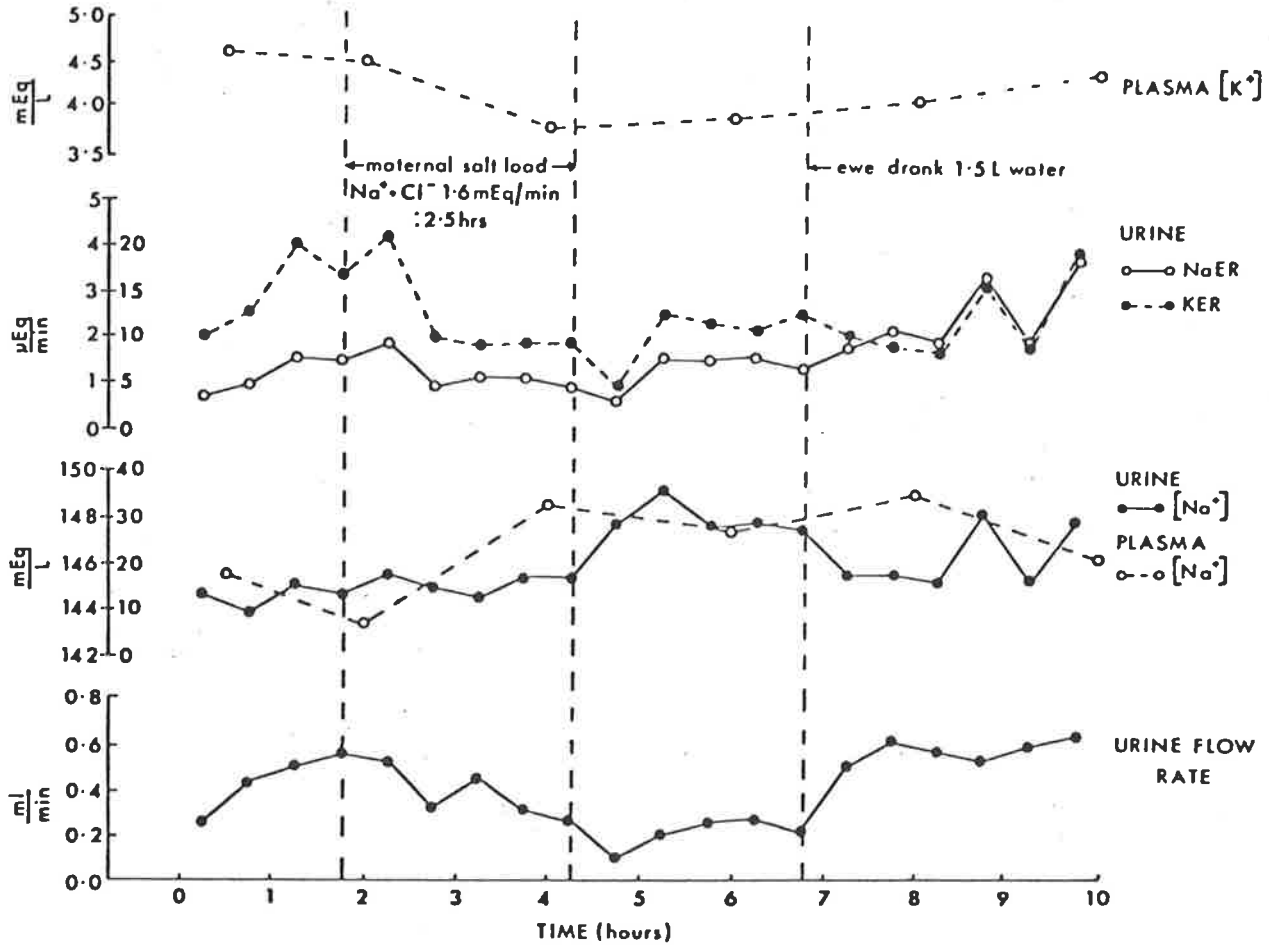

FIGURE 38. Changes in foetal urinary parameters following the infusion of hypertonic saline into the ewe carrying foetus 275 (122 days old).

(See appendix table 46 and text pages 112-114)

a. Foetus 51 (118 days) (appendix table 47)

In this experiment 0.8mg/kg of furosemide was injected into the foetus and resulted in an immediate diuresis. Within ½ an hour of treatment, foetal urine flow had increased 2.7 times and 2½ hours after treatment a maximum flow rate of 1.42ml/min. was recorded which was 3.0 times the pre-treatment average. At the end of the experiment, 5½ hours after treatment, the flow rate was still twice the control value. With respect to urinary electrolytes, there was no reliable evidence that furosemide produced an increase in $[Na^+]$, while $[K^+]$ decreased as the urine flow rate increased. Because of this inverse relationship between flow rate and $[K^+]$ there was no significant alteration of K^+ excretion rate but Na^+ excretion rate did rise in parallel with the flow rate increase. The remaining urinary solutes, creatinine and uric acid, showed changes in concentration which were inversely related to flow rate, suggesting that furosemide had no specific effect on the excretion of those solutes (see fig. 39).

The plasma solutes showed an overall increase in concentration from the time of maximum diuresis until the end of the experiment. This may have been due to foetal water loss during the diuresis although these losses would normally be corrected by an influx of water from the ewe.

b. Foetus 69-559 (121 days) (appendix table 48)

The dose of furosemide used in this experiment was 1.0mg/kg and again diuresis began within ½ an hour of treatment. Three hours after treatment a maximum flow rate of 1.45ml/min. was recorded which was 13.2 times the pre-treatment average. At the end of the experiment, 7½ hours after treatment, the flow rate was still 4.8 times the control average. Again urinary $[Na^+]$ and more particularly urinary $[K^+]$ decreased during the period of maximum diuresis. Nevertheless there was a kaliuresis, &

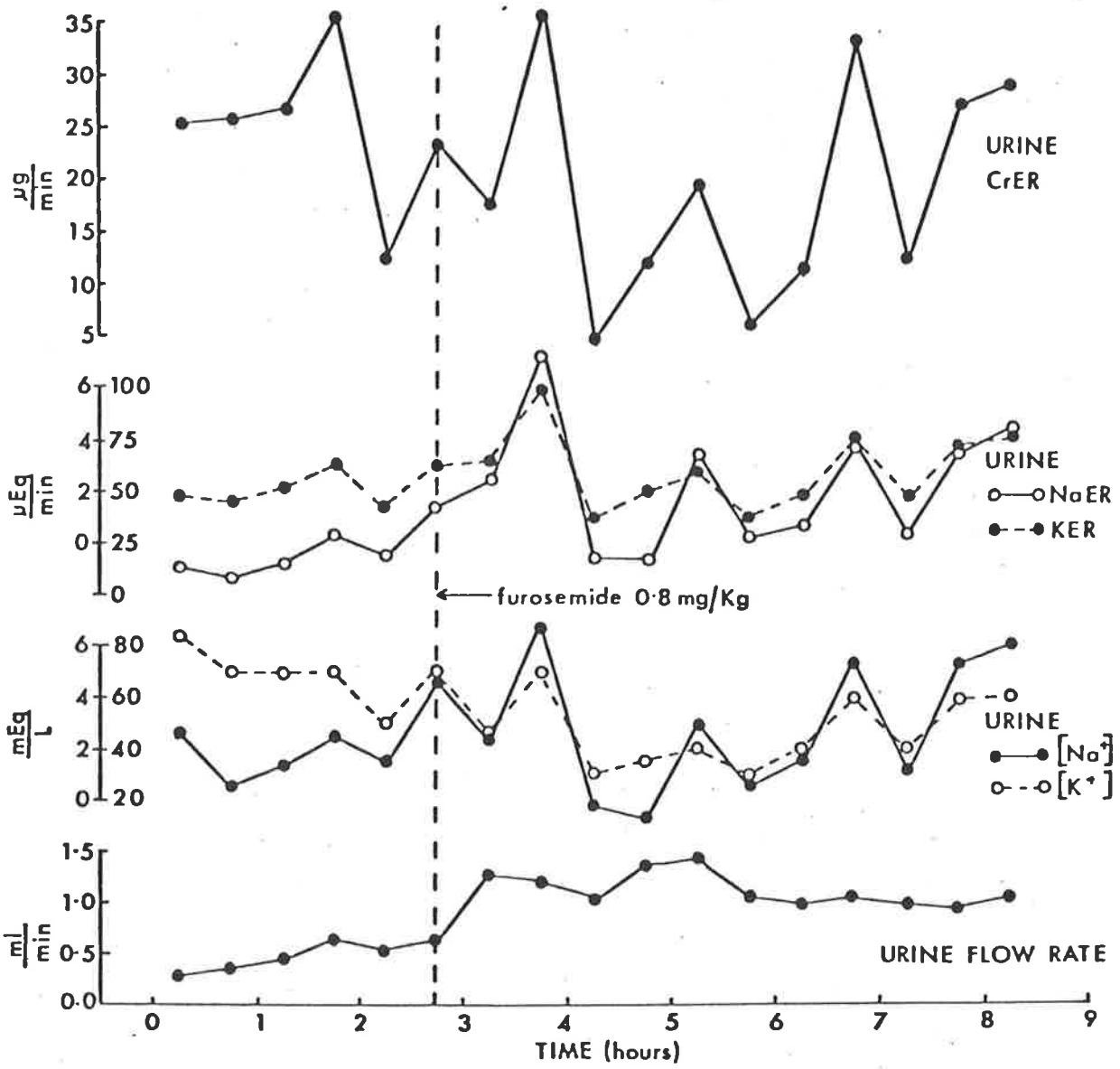

FIGURE 39. Changes in foetal urinary parameters following the injection of furosemide into foetus 51 (118 days old).
(See appendix table 47 and text page 115)

because the fall in $[Na^+]$ did not offset the increase in urine output, there was a nett increase in Na^+ excretion rate. The urinary concentrations of creatinine and uric acid decreased as flow rate increased and there was no significant change in creatinine excretion rate, although there was a nett increase in uric acid excretion. The pH of foetal urine changed erratically during the experiment, however several high pH measurements following treatment, may have resulted from a decrease in H^+ excretion. (See fig. 40).

No changes were observed in the concentration of the plasma solutes that could be related to the diuretic treatment. However changes in MAP occurred which appeared to be related to treatment. Prior to the furosemide injection the MAP varied between 36.5 and 38.0 mmHg, yet immediately after treatment, the MAP increased to 43 mmHg and remained between 40 and 43 for two hours.

c. Foetus 107 (121 days) (appendix table 49)

This foetus was the same age as the preceding foetus and had a similar pre-treatment flow rate (average 1.0ml/min.). The same dose of furosemide was given (1.0mg/kg). Despite these similarities, the maximum urine flow recorded following treatment was only 4.6 times the pre-treatment average and this was about 1/3 of the increase seen with the preceding foetus.

As previously, the $[K^+]$ of foetal urine decreased during the period of maximum diuresis although on this occasion there was a nett increase in K^+ excretion. In contrast, urinary $[Na^+]$ rose almost immediately after the diuretic treatment and reached a peak which was 1.4 times the average control concentration. This peak in $[Na^+]$ corresponded with the maximum urine flow resulting in a maximum Na^+ excretion rate which was 6.7 times the control average (see fig. 41).

The difference in response between this foetus and foetus 69-

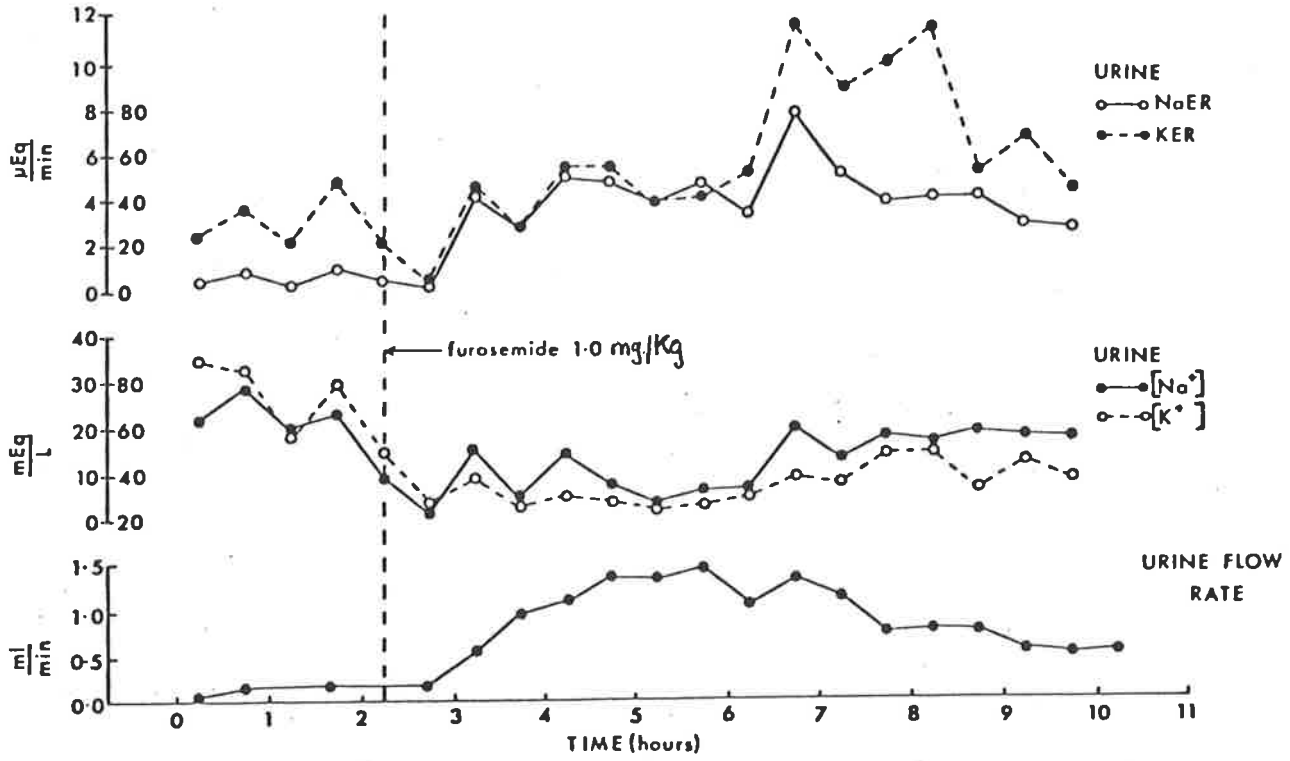

FIGURE 40. Changes in foetal urinary parameters following the injection of furosemide into foetus 69-559 (121 days old).
(See appendix table 48 and text pages 115 and 116)

559, suggests that there is no well defined dose-response pattern in foetal sheep treated with furosemide and that individual variation can moderate the basic response to the drug. This individual variation probably involves not only foetal maturity but also differences in maternal and foetal water and electrolyte balance prior to treatment.

With respect to the plasma solutes in this experiment, $[Na^+]$ decreased and $[K^+]$ increased during the first two hours after treatment. Over the next four hours the reverse trends were noted and the concentration of both electrolytes approached control values. Finally, on this occasion furosemide had no effect on MAP. During the control period MAP was between 37 and 39 mmHg and it remained within that range following treatment.

d. Foetus 150 (122 days) (appendix table 50)

The dose of furosemide used in this experiment was again 1.0mg/kg. Foetal urine flow increased immediately after treatment and reached a peak 1½ hours later at which point the flow rate was 2.4 times the pre-treatment average. The $[Na^+]$ of foetal urine during the control period was variable although it was relatively stable for three hours prior to treatment. In the ½ hour immediately following treatment there was a sharp rise in $[Na^+]$ after which it was again variable, but increased overall. The Na^+ excretion rate reflected the variation in $[Na^+]$ but there was a definite natriuresis with the maximum Na^+ excretion rate being 4.9 times the pre-treatment average. In contrast, urinary $[K^+]$ and K^+ excretion rate showed no significant change following treatment.

During the control period the urinary concentrations of creatinine and uric acid varied so widely that the subsequent influence of furosemide on the concentration and excretion rate of these solutes could not be assessed. Similarly, the effect of furosemide on urinary pH could not be determined. Thus with the respect to foetal urine output and composition, the only significant effect of furosemide in this

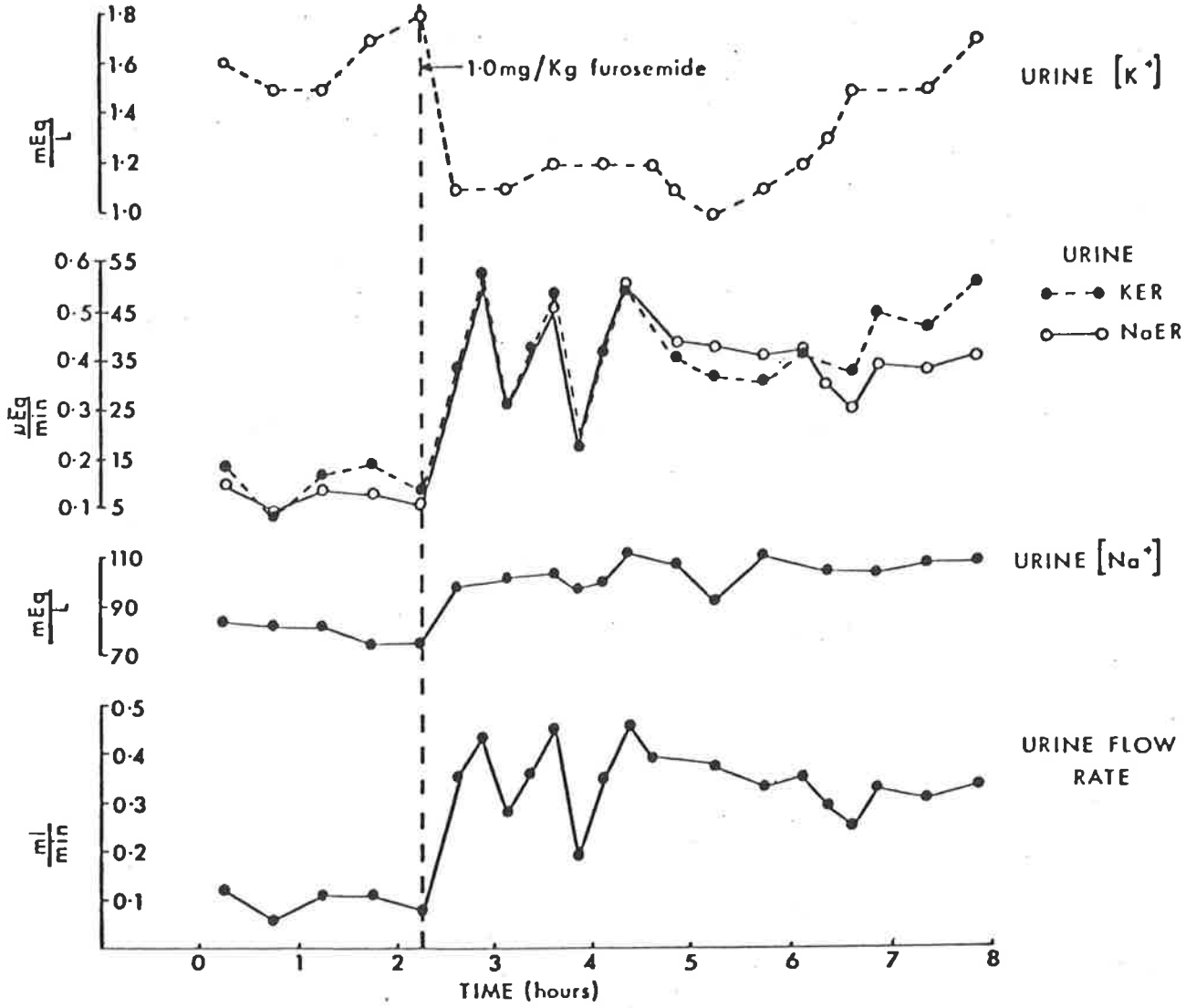

FIGURE 41. Changes in foetal urinary parameters following the injection of furosemide into foetus 107 (121 days old).
(See appendix table 49 and text pages 116 and 117)

experiment was to increase urine flow rate although urinary [Na^+] also appeard to be increased. In foetal plasma the situation was no less complicated, although K^+, uric acid and creatinine all showed overall rises in concentration following furosemide treatment. (See fig. 42).

e. Foetus 230 (122 days) (appendix table 51)

This foetus was the same age as the preceding foetus but received a slightly higher dose of furosemide (1.3mg/kg). In response, the flow rate of foetal urine increased to a maximum which was 2.8 times the pre-treatment average. This occurred within a ½ hour of treatment and 4½ hours after treatment the flow rate had returned to normal. The [Na^+] of foetal urine also began to rise immediately after the furosemide injection, but the highest [Na^+] was recorded between 1 and 1½ hours after treatment, at which point the urinary [Na^+] had increased 2.3 times. As a result of these changes, Na^+ excretion rate increased 4.4 times with the maximum natriuresis occurring one hour after treatment. The [K^+] of foetal urine fell slightly during the diuresis; consequently, there was only a small transient increase in K^+ excretion rate. Creatinine and uric acid concentrations began to fall immediately after the furosemide injection. In both cases the magnitude of the concentration decrease was comparable with the magnitude of the flow rate increase, implying that the concentration changes were the result of increased water excretion and urine dilution. Accordingly, there was no significant change in the excretion rate of these solutes. Urinary pH was also unaffected by the furosemide treatment. (See fig. 43).

In comparing the response of this foetus and foetus 150, following furosemide treatment, only the dynamics of the diuresis can be considered since the concentration of the urinary solutes varied so widely in foetus 150. In relation to the dose of furosemide administered, the diuresis induced in this experiment was greater than in the preceding experiment. A 30% increase in dose resulted in a 15% increase in the maximum urine

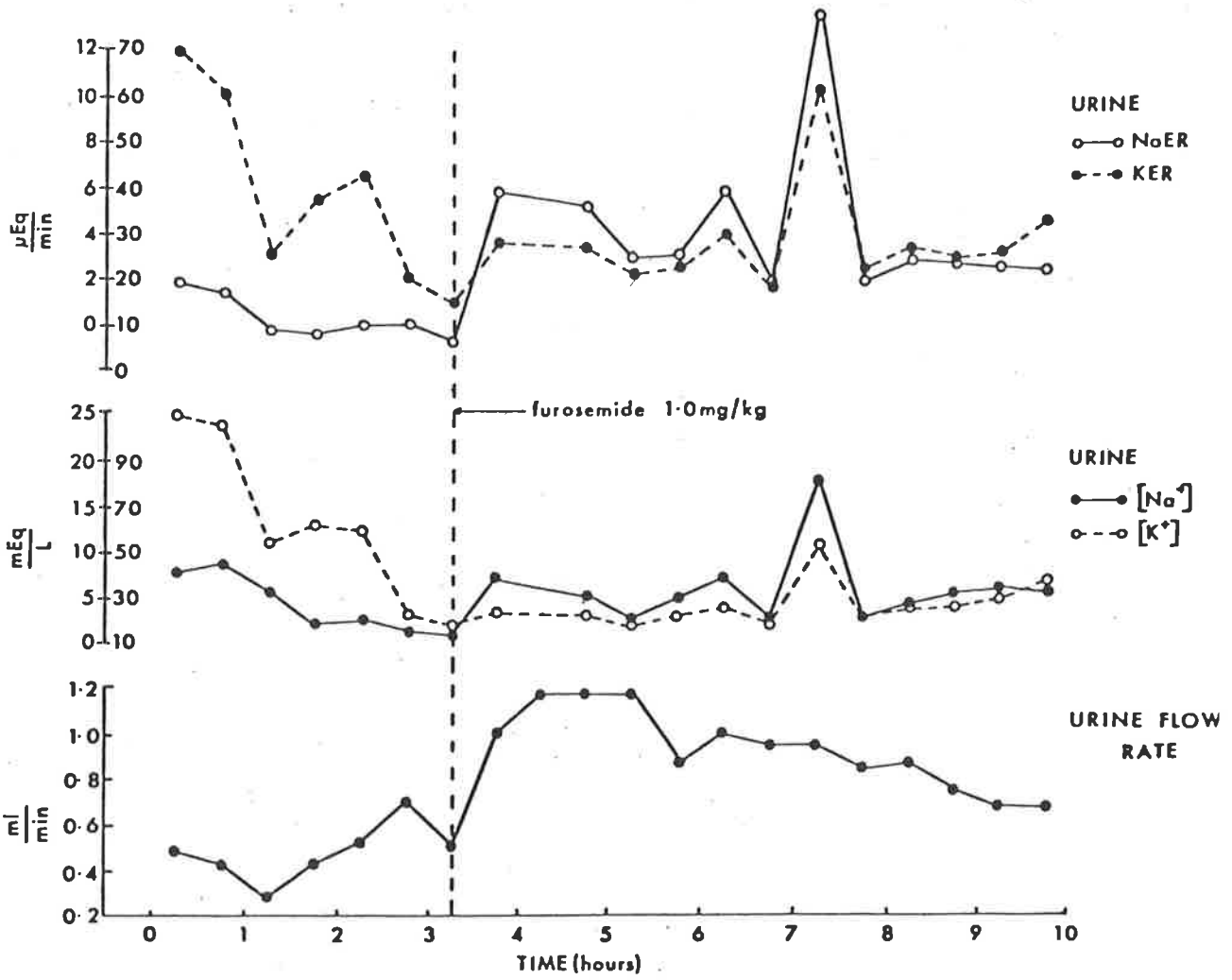

FIGURE 42. Changes in foetal urinary parameters following the injection of furosemide into foetus 150 (122 days old).
(See appendix table 50 and text pages 117 and 118)

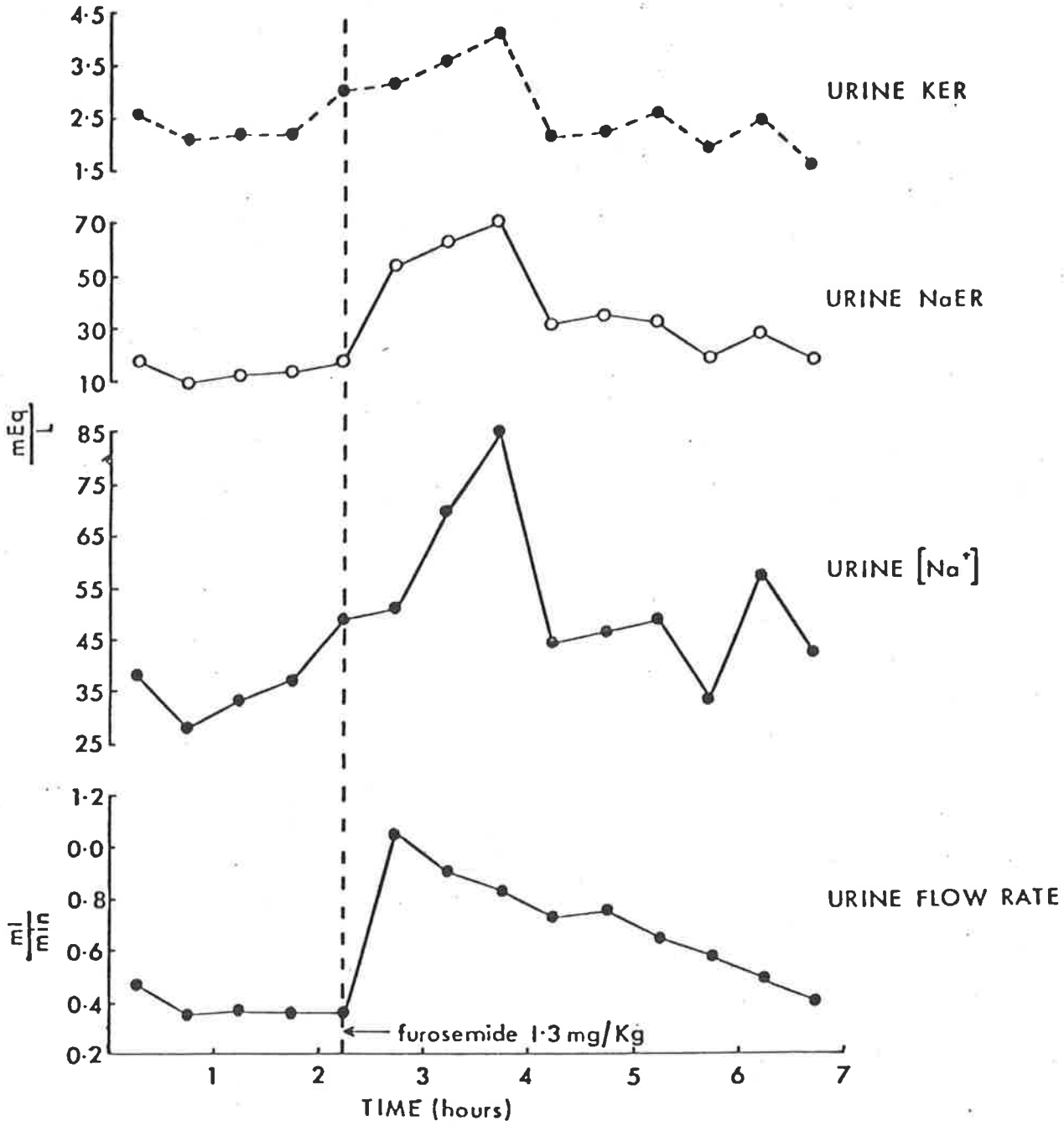

FIGURE 43. Changes in foetal urinary parameters following the injection of furosemide into foetus 230 (122 days old).

(See appendix table 51 and text pages 118 and 119)

flow rate. But in view of the response variation previously noted between foetuses of the same age, this 15% increase in flow rate may not have been dose related.

With respect to the concentration of plasma solutes, in this experiment, $[Na^+]$ increased following the furosemide injection while $[K^+]$ was essentially unaltered.

f. Foetus 66-310 (139 days) (appendix table 52)

On this occasion the pre-treatment flow rate of foetal urine was lower than in any of the younger foetuses (average, 0.26ml/min.). Nevertheless, in response to a 0.8mg/kg dose of furosemide, the flow rate increased to a maximum of 0.62ml/min. within ½ an hour. This maximum was 2.4 times the control average. The $[Na^+]$ of foetal urine was variable during the control period, but after treatment the concentration was always greater than the pre-treatment average. Therefore it can be concluded that the $[Na^+]$ of foetal urine increased in response to furosemide. The maximum $[Na^+]$ was 3.0 times the control average and it coincided with the peak in urine flow rate. However, unlike flow rate, the $[Na^+]$ did not return to control levels until the end of the experiment. Potassium, creatinine and uric acid all decreased in concentration during the control period and continued to decrease following furosemide treatment. Thus, apart from initiating increased water excretion and diluting foetal urine, furosemide had not direct effect on the urinary concentration of K^+, creatinine or uric acid. The pH of foetal urine decreased throughout the experiment but the decline was independent of the diuretic treatment (see fig. 44).

In this experiment, as in most of the preceding experiments, the plasma solutes showed no changes in concentration that could reliably be related to the diuretic treatment. In most cases, the post-treatment concentrations were similar to the control concentrations and where divergences did occur their significance was questionable. This was

largely because the number of blood samples that could be taken without joepardising experimental validity, was small. Accordingly it was not possible to closely monitor plasma composition changes and assess the effect of treatment. This limitation applied to many of the experiments described in this section.

g. Foetus 66-440 (140 days) (appendix table 53).

This was the oldest foetus treated with furosemide and it had the lowest initial flow rates. The pre-treatment average was 0.11ml/min., but following the injection of furosemide (0.9mg/kg) the urine flow rate increased almost immediately and eventually reached a maximum of 0.41ml/min. (3.7 times the pre-treatment average). The urine flow rate was still twice the control average three hours later.

The $[Na^+]$ of foetal urine also increased after treatment, although not as immediately as flow rate. The maximum $[Na^+]$ was 2.8 times the pre-treatment average but was not recorded until 2½ hours after treatment. As a result of these changes there was a parallel increase in Na^+ excretion rate. In contrast to $[Na^+]$, the $[K^+]$ of foetal urine decreased after treatment but because of the diuresis there was a nett increase in K^+ excretion rate. This kaliuresis was short-lived. Creatinine and uric acid excretion rates also increased after the furosemide injection, while the pH of foetal urine was elevated for 1½ hours after treatment. (See fig. 45).

SUMMARY

The data relating to urine flow rate and electrolyte excretion rate changes following furosemide treatment of the 4 foetuses aged 121 and 122 days has been pooled, as has the data for the 2 foetuses aged 139 and 140 days. These results are shown in table 9.

In all of the experiments described, furosemide treatment resulted in an increase in urine flow rate. Usually the increase began within ½ an hour of treatment but the magnitude of the diuresis varied. The maximum flow rates were between 2.4 and 13.2 times the pre-treatment

TABLE 9 CHANGES IN FLOW RATE AND ELECTROLYTE EXCRETION RATES FOLLOWING TREATMENT OF FOETUSES WITH FUROSEMIDE

(The percentage change in urinary parameters was obtained by comparing the maximum value for each parameter following treatment with the corresponding pre-treatment average. The values for foetuses of similar ages have been pooled).

FOETAL AGE (Days)	Percentage change in urinary parameters			
	FLOW	$[Na^+]$	NaER	KER
121-122	575 ± 219 (4)	203 ± 34 (4)	802 ± 133 (4)	284 ± 55 (4)
139-140	306 ± 48 (2)	292 ± 12 (2)	738 ± 16 (2)	196 ± 34 (2)

(mean ± standard error, n in brackets)

(See text page 120)

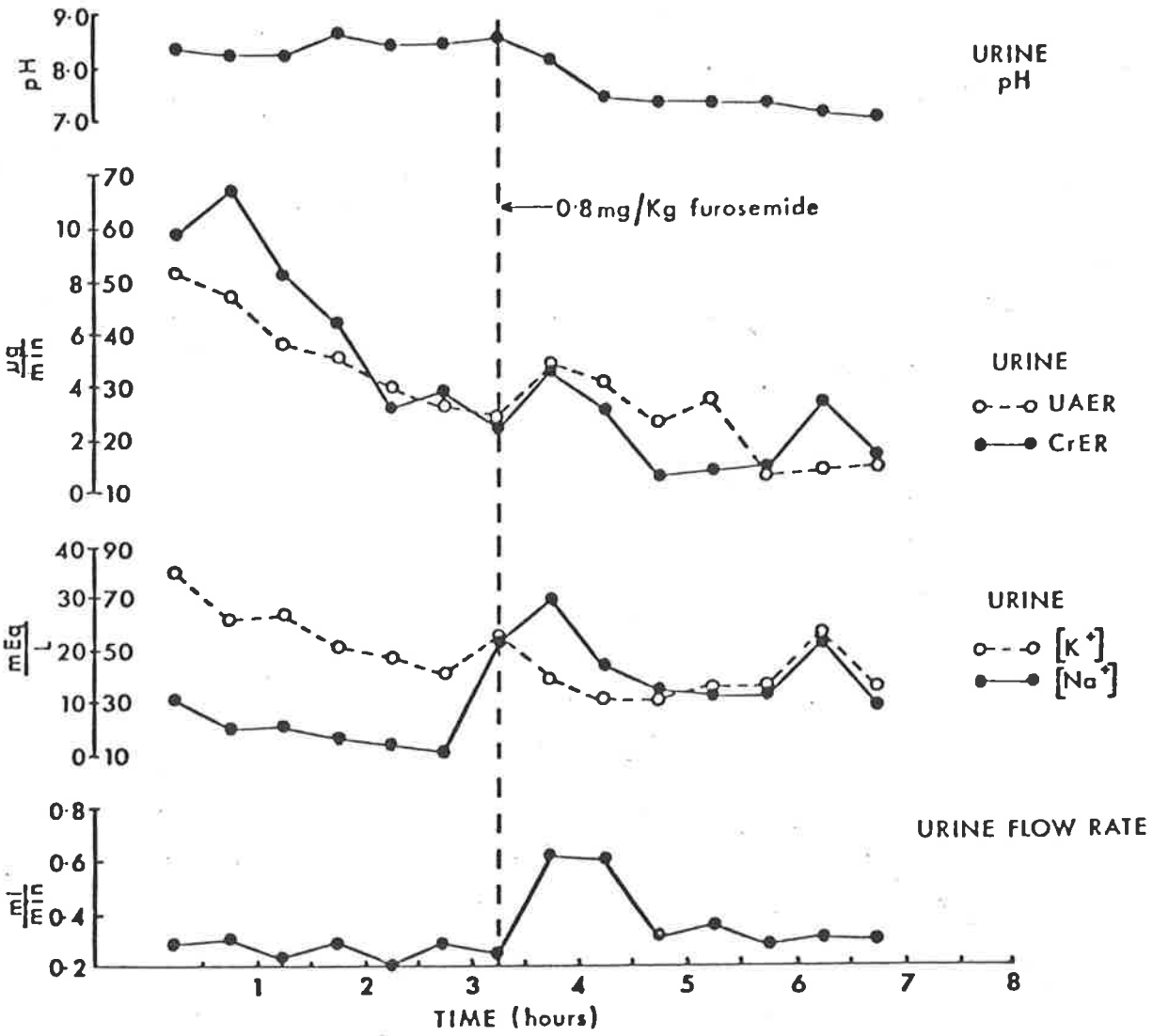

FIGURE 44. Changes in foetal urinary parameters following the injection of furosemide into foetus 66-310 (139 days old).
(See appendix table 52 and text pages 119 and 120)

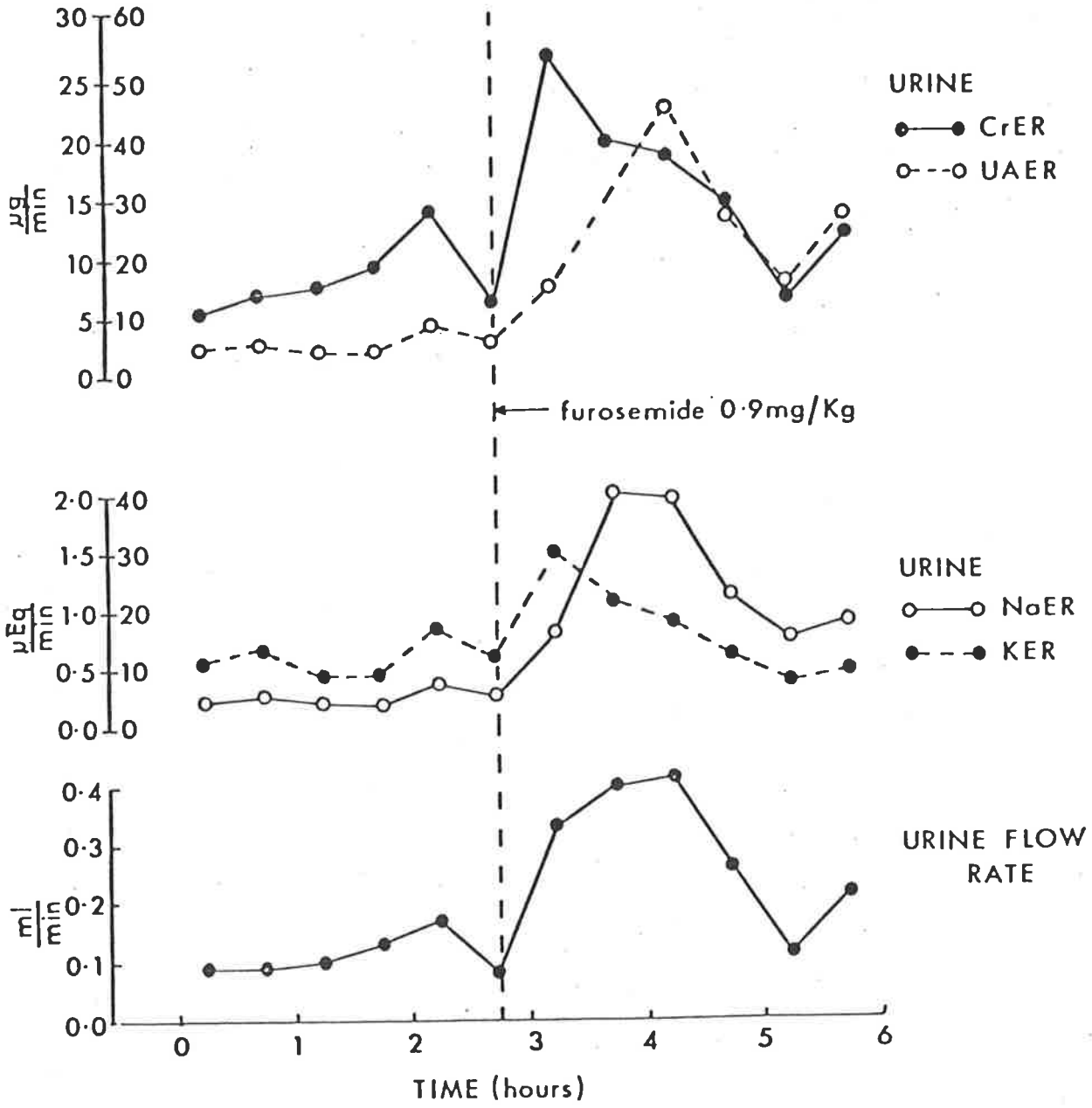

FIGURE 45. Changes in foetal urinary parameters following the injection of furosemide into foetus 66-440 (140 days old).
(See appendix table 53 and text page 120)

averages. Similarly the duration of the diuretic responses varied from 4½ hours to over 8 hours. These variations in the magnitude and duration of the diuretic responses do not appear to be related to the dose of furosemide administered.

In the youngest foetus examined, there was no evidence that furosemide increased the $[Na^+]$ of foetal urine. However in the remaining experiments the drug resulted in an increase in urinary $[Na^+]$ which was greatest in the more mature foetuses despite the use of smaller furosemide doses. Thus it can be concluded that furosemide causes an increase in urinary $[Na^+]$ and that the response may be related to foetal age.

In all cases the nett effect of the changes in urine flow rate and $[Na^+]$ was a large natriuresis. The magnitude of these natriuretic responses was not directly related to foetal age nor to the dose of furosemide administered. Where both urinary $[Na^+]$ and flow rate increased following treatment, the patterns of change were not necessarily parallel. In some experiments the peak in $[Na^+]$ occurred at the same time as the maximum flow rate but in others the electrolyte peak occurred later and the elevated Na^+ level persisted longer than the diuresis.

The $[K^+]$ of foetal urine usually decreased following furosemide treatment. Because the fall in urinary $[K^+]$ was usually inversely related to the rise in urine flow rate, the K^+ change was presumed to be the result of urine dilution rather than any major alteration of K^+ exchange in the nephron. Nevertheless in all experiments there was an increase in K^+ excretion rate, but since it was usually short lived and smaller in magnitude than the corresponding rise in flow rate, it can be concluded that furosemide has a relatively limited affect on the renal excretion of K^+ by foetuses aged between 120 and 140 days. Similarly, furosemide has little effect on uric acid and creatinine excretion. In some experiments the concentration of these solutes decreased during diuresis, but the excretion rate of the solutes was unaffected. Changes in the pH of foetal urine could not be related to furosemide treatment, although in man,

furosemide has been reported to increase H^+ excretion (Fraser et al 1967).

No consistent effect of furosemide on the concentration of plasma solutes was observed. Individual foetuses showed changes in plasma composition that could be related to the diuretic treatment but the lack of consistency between foetuses precludes generalisation. Finally, three experiments in which foetal MAP was measured before and after furosemide treatment yielded contradictory results. In one experiment MAP increased following treatment while in the remaining experiments it was unaltered.

5.3.2 Sodium Ethacrynate (2,3-dichloro-4-[2 - methylene butyrylphenoxy] acetic acid) (Merck Sharpe and Dohme)

Three experiments were conducted in which sodium ethacrynate was injected intra-venously into foetuses aged 114, 123 and 129 days.

a. Foetus 223 (114 days) (appendix table 54)

A dose of 2.1mg/kg was administered to this foetus and within ½ an hour the urine flow rate had increased to a maximum of 1.06ml/min. which was 8.8 times the control average. Thereafter the flow rate gradually declined, although five hours after the ethacrynate injection the flow rate was still 4.2 times the pre-treatment average. The $[Na^+]$ of foetal urine was also effected by ethacrynate but not as dramatically as urine flow rate. The $[Na^+]$ increased slowly to a peak of 135 mEq/L which was 1.5 times the pre-treatment average for $[Na^+]$. This peak was reached 2½ hours after treatment and for the remainder of the experiment $[Na^+]$ was variable. Sodium excretion rate increased in parallel with the change in flow rate although the subsequent rise in $[Na^+]$ contributed even further to the natriuresis. The K^+ excretion rate also increased, but because urinary $[K^+]$ decreased after ethacrynate treatment, the nett rise in K^+ excretion was only ½ that of the flow rate increase. The urinary concentration of creatinine and uric acid also decreased sharply at the onset of diuresis. In the case of creatinine there was no

significant alteration of excretion rate, but with uric acid there was a small rise in excretion rate. The pH of foetal urine was affected by ethacrynate treatment. Prior to treatment, the pH was between 6.5 and 6.7 but within a ½ hour of treatment, the pH increased to 7.1 and about one hour later it reached 7.3. By the end of the experiment, the pH had returned to control levels. (See fig. 46).

Of the plasma solutes analysed, the only one that appeared to be effected by ethacrynate treatment was Na^+. The plasma sample taken immediately after the peak in Na^+ excretion, had a $[Na^+]$ below that of the control plasmas and two subsequent plasma samples had even lower Na^+ levels.

b. Foetus 230 (123 days) (appendix table 55)

This foetus was given a slightly lower dose of sodium ethacrynate than the preceding foetus (1.7mg/kg). Despite this, diuresis began just as promptly but the maximum flow rate was reached about 30 minutes later than inthe first experiment. Secondly, although the maximum flow rate recorded (1.70ml/min.) was greater than in the first experiment it was only 4.3 times the pre-treatment average. In contrast the urinary $[Na^+]$ in this experiment reached a maximum, which was 3.3 times the pre-treatment average and this was a greater increase than that obtained in the preceding experiment. Also on this occasion, the maximum $[Na^+]$ occurred at the same time as the maximum flow rate and although $[Na^+]$ varied thereafter, it remained elevated until the end of the experiment, whereas flow rate returned to normal within five hours of treatment. As a result of these responses, Na^+ excretion rate increased to a maximum which was 14 times the control value and the natriuresis persisted until the end of the experiment. The $[K^+]$ of foetal urine was also effected by ethacrynate, increasing threefold. In combination with the flow rate response, this increase in $[K^+]$ produced a kaliuresis of similar magnitude to the natriuresis. The remaining urinary solutes,

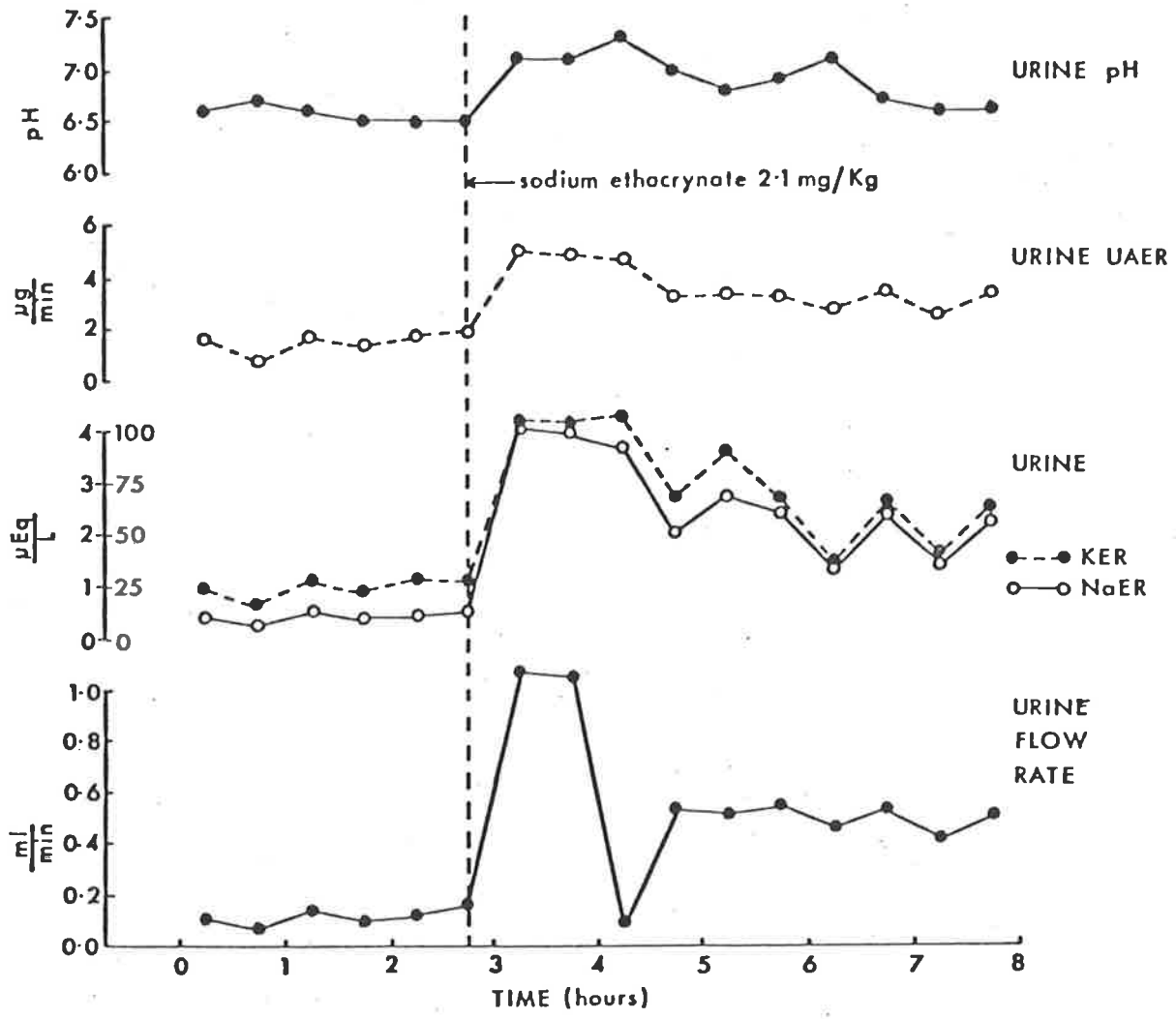

FIGURE 46. Changes in foetal urinary parameters following the injection of sodium ethacrynate into foetus 223 (114 days old).
(See appendix table 54 and text pages 122 and 123)

creatinine and uric acid, decreased in concentration as the urine flow rate increased and the excretion rate of these solutes was essentially unaltered. Finally the pH of foetal urine increased following the ethacrynate injection and the magnitude and duration of the pH change was similar to that seen in the preceding experiment. (See fig. 47).

With respect to plasma composition, the $[Na^+]$ decreased following ethacrynate treatment, as did creatinine concentration; however, the concentrations of K^+ and uric acid showed no regular changes.

c. Foetus 230 (129 days) (appendix table 56)

Foetus 230 was treated, six days after the preceding experiment, with a similar dose of sodium ethacrynate (1.6mg/kg). On this occasion the average pre-treatment flow rate was 0.18ml/min. compared with 0.40ml/min. in the earlier experiment. Following treatment the flow rate reached a maximum of 2.08ml/min. (11.6 times the pre-treatment average). This maximum was achieved in the first hour after treatment and over the next two hours control levels were re-established. The pre-treatment levels for $[Na^+]$ were similar in both of the experiment using foetus 230, but in the present experiment the $[Na^+]$ increased 4.6 times following treatment, compared with 3.3 times in the earlier experiment. Again the peak in $[Na^+]$ coincided with the peak in diuresis resulting in a maximum Na^+ excretion rate which was 41.7 times the control average for that parameter. At the end of the experiment the Na^+ excretion rate was still three times normal due entirely to the persistence of increased urinary $[Na^+]$. The $[K^+]$ of foetal urine decreased after the ethacrynate injection, but the fall in concentration did not offset the rise in urine flow rate and accordingly K^+ excretion rate increased for about two hours. The same occurred with creatinine and uric acid where the maximum excretion rates achieved were 1.6 and 3.3 times the respective pre-treatment averages. Also, as in both of the preceding experiments, ethacrynate treatment was followed by an increase in the pH of foetal urine. During the control

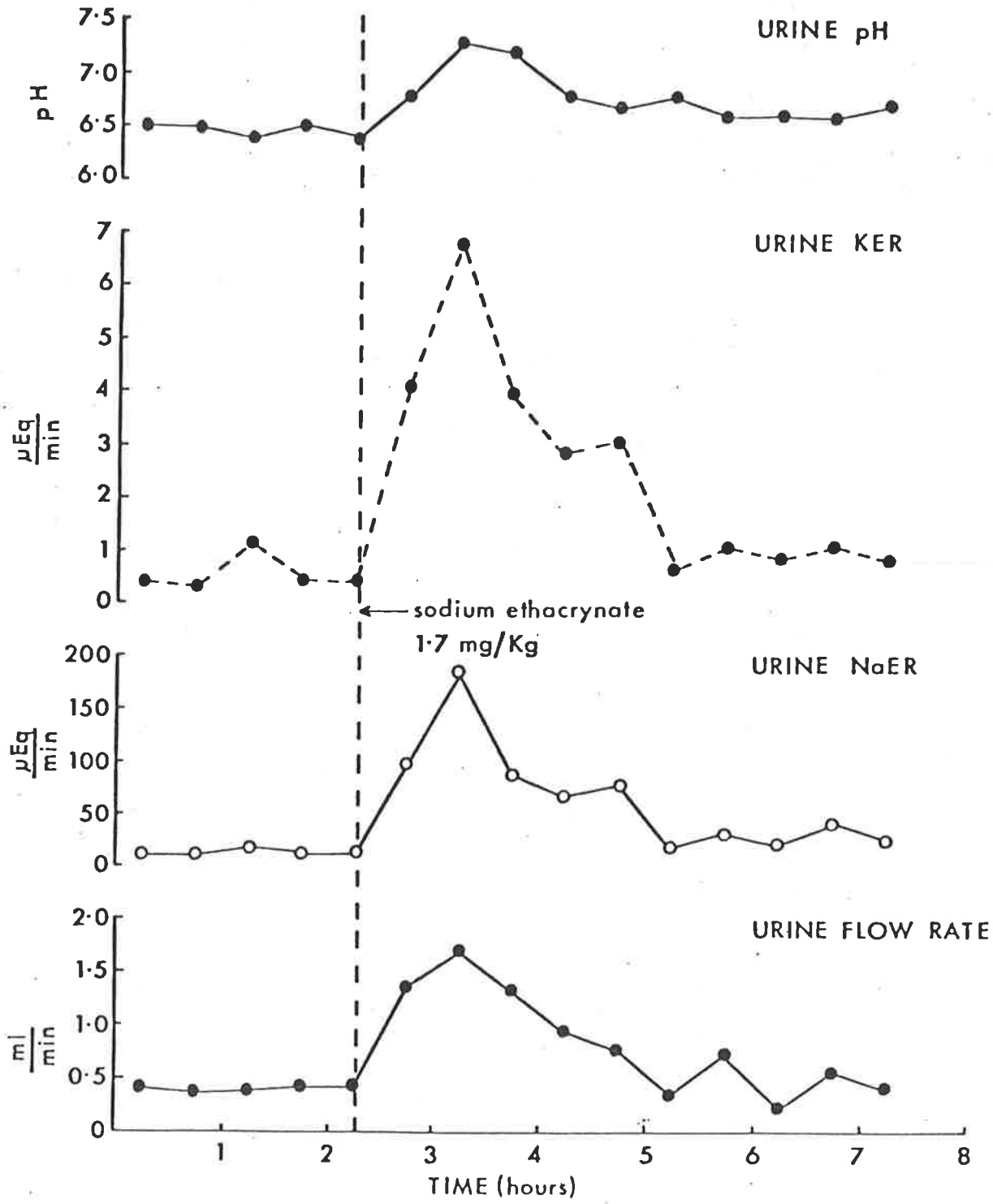

FIGURE 47. Changes in foetal urinary parameters following the injection of sodium ethacrynate into foetus 230 (123 days old). (See appendix table 55 and text pages 123 and 124)

period the average pH was 6.6 but within one hour of treatment the pH reached 7.2 (see fig. 48).

In the foetal plasma the most obvious change was again a decrease in $[Na^+]$, but a small decrease in creatinine concentration also occurred. The K^+ and uric acid levels were too variable to discern any effect that could be related to the treatment.

SUMMARY

Table 10 summarises the percentage change in flow rate and electrolyte excretion rates that were obtained in all foetuses treated with furosemide and sodium ethacrynate.

All foetuses treated with sodium ethacrynate showed a prompt diuresis. Urine flow rate increased within minutes of treatment and peak flow rates were recorded within one hour. In one experiment, the maximum flow rate was 11.6 times the average flow rate during the control period. However in an experiment conducted on the same foetus six days earlier a similar dose of sodium ethacrynate produced a maximum flow rate which was only 4.3 times the pre-treatment average. The absolute value of the maximum flow rate was similar in both experiments, but in the second experiment the initial flow rate was lower. It may be therefore, that there is an upper limit to the diuretic response that can be induced in foetuses of a given age by specific doses of sodium ethacrynate and that this limit is independent of pre-treatment flow rates. In the youngest foetus, the initial flow rate was comparable with that of foetus 230 at 129 days, but a larger sodium ethacrynate dose produced a smaller increase in urine flow. Also, in both experiments with foetus 230 the diuresis had ended three to four hours after treatment but with the youngest foetus (foetus 223) the response persisted for over five hour. This prolonged response was probably due to the higher dose of ethacrynate administered but the relatively small diuresis produced suggests that the sensitivity of the foetal kidney to ethacrynate or its capacity to respond to that drug, increases as the foetus matures.

TABLE 10 CHANGES IN FLOW RATE AND ELECTROLYTE EXCRETION RATES FOLLOWING TREATMENT OF FOETUSES WITH DIURETICS

(The percentage change in urinary parameters was obtained by comparing the maximum value for each parameter, obtained after treatment, with the corresponding pre-treatment average)

FOETUS	FOETAL AGE (Days)	DIURETIC	DOSE (mg/kg)	Percentage change in Urinary Parameters FLOW	NaER	KER
51	118	furosemide	0.8	296	548	262
69-559	121	furosemide	1.0	1318	1220	423
107	121	furosemide	1.0	460	674	363
150	122	furosemide	1.0	244	339	181
230	122	furosemide	1.3	276	442	170
66-310	139	furosemide	0.8	238	715	247
66-440	140	furosemide	0.9	373	760	244
223	114	ethacrynate	2.1	883	1002	429
230	123	ethacrynate	1.7	425	1406	1236
230	129	ethacrynate	1.6	1156	4170	752

(See text page 125)

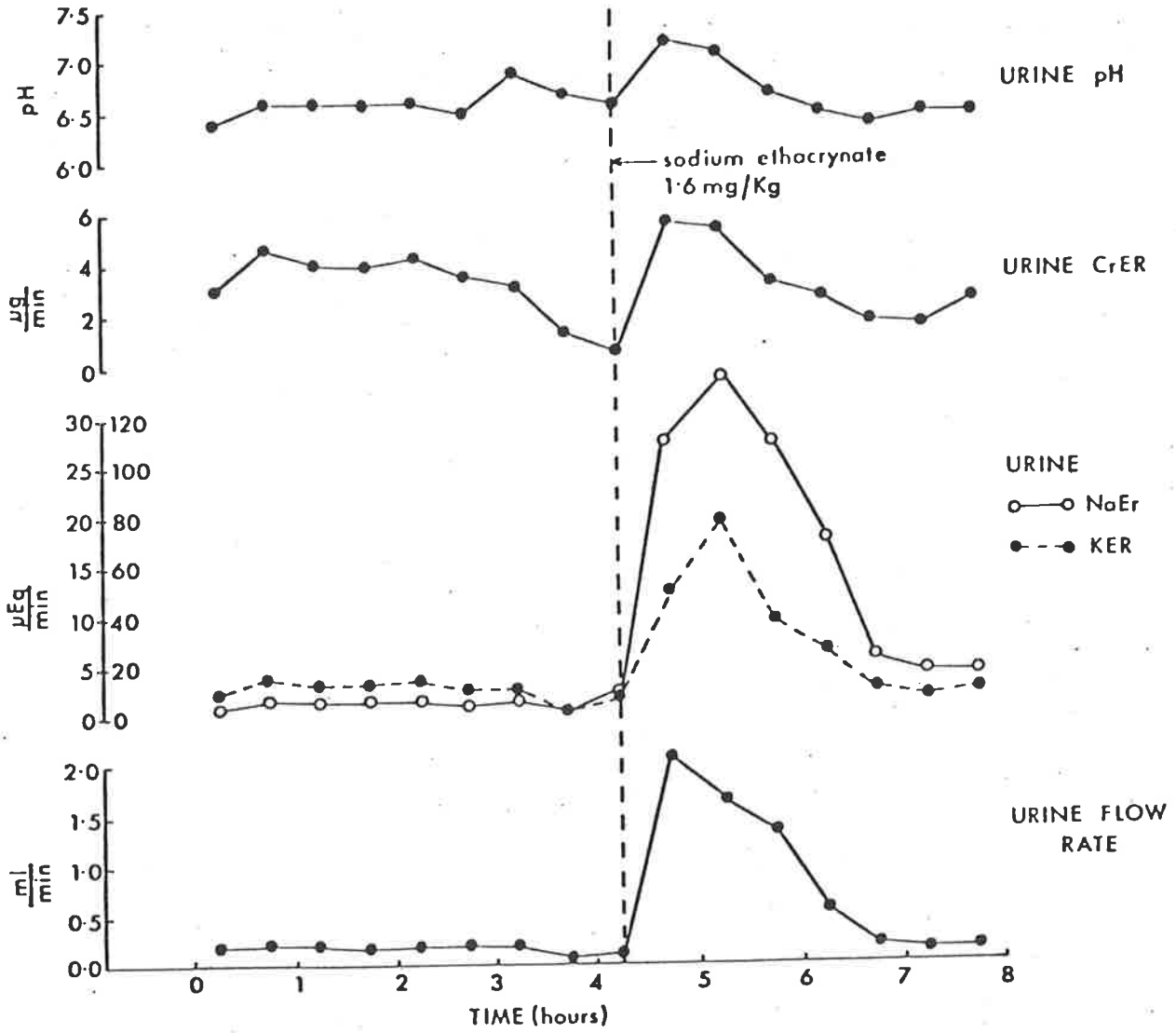

FIGURE 48. Changes in foetal urinary parameters following the injection of sodium ethacrynate into foetus 230 (129 days old).
(See appendix table 56 and text pages 124 and 125)

In all three experiments there was an increase in the $[Na^+]$ of foetal urine soon after the sodium ethacrynate injection. Although these changes began promptly, the maximum $[Na^+]$ was usually recorded about 1½ hours after treatment and in all cases the increase in $[Na^+]$ was more prolonged than the diuresis. The natriuresis evident in all experiments reached a peak within 1 to 1½ hours of treatment but persisted at reduced levels throughout the experiments. Kaliuresis also occurred in each experiment. Although the $[K^+]$ of foetal urine was not obviously effected by sodium ethacrynate treatment, the fact that its level was maintained in the face of large increases in water excretion, indicates a substantial rise in K^+ excretion. In all experiments, the urinary levels of uric acid and creatinine decreased as the urine volume increased but the relative magnitude of these inverse changes was such that there was a nett rise in the excretion rate of these solutes. Finally the pH of foetal urine was invariably effected by ethacrynate treatment. In all cases, urine pH increased within one hour of treatment and the increase generally persisted for about two hours. The urine changed from acid to alkaline.

5.3.3 Acetazolamide (Diamox-Lederle)

Acetazolamide is a sulphonamide possessing a chemical structure and pharmacological activity distinctly different from the bacteriostatic sulphonamides. It is a carbonic anhydrase inhibitor acting specifically on the enzyme which catalyses the reversible reaction involving the hydration of carbon dioxide and the dissociation of carbonic acid.

Two foetuses aged 130 and 145 days were given intravenous injections of 50 and 28mg/kg of acetazolamide respectively. After the first treatment, foetus 130 was given a second injection of 50mg/kg of acetazolamide while foetus 145 was given a second injection of 56mg/kg of acetazolamide. The results obtained in these experiments were as follows.

a. Foetus 223 (130 days) (appendix table 57)

Following the first acetazolamide injection the urine flow rate showed an immediate increase of about 80% but then fell to control levels during the next hour. A second increase began about 1½ hours after treatment and the flow rate reached a maximum of 1.46ml/min. three hours after the injection. This maximum was 3.2 times the average pre-treatment flow rate. Over the next 2½ hours the flow rate gradually returned to pre-treatment levels. The second injection of acetazolamide caused a 90% increase in flow rate within ½ an hour, but within 1½ hours if had returned to control levels and no significant increases occurred thereafter.

The Na^+ and K^+ concentrations of foetal urine were also effected by acetazolamide treatment. The $[Na^+]$ increased steadily after treatment and within two hours reached a maximum which was 3.7 times the pre-treatment average. Following that peak, the $[Na^+]$ varied widely but was still elevated when the second injection of acetazolamide was given. A similar pattern was seen for $[K^+]$, although the maximum $[K^+]$ was reached within a ½ hour of treatment and persisted for two hours. As a result of these changes in flow rate and electrolyte concentration, the excretion rates of Na^+ and K^+ reached peaks which were 9.5 and 6.4 times their respective pre-treatment averages. These maximum excretion rates coincided with the peak in diuresis.

Following the second injection of acetazolamide, the change in the electrolyte concentration of foetal urine was biphasic. For the first 1½ hours, both $[Na^+]$ and $[K^+]$ increased but then they dropped sharply only to rise again as the experiment proceeded. This pattern was reflected in the electrolyte excretion rates. The urinary concentration of creatinine and uric acid increased for about two hours following acetazolamide treatment, but thereafter there was no consistent pattern of concentration change for either solute. Following the second injection of acetazolamide, these solutes showed a biphasic pattern of concentration change, similar to that described for K^+. Before treatment, the average

pH for foetal urine was 7.3 but within a ½ hour of treatment this had risen to 7.6 and within 2½ hours to 7.9. At the time of maximum diuresis and maximum electrolyte excretion rate the urinary pH was 7.9 and it rose even further to a maximum of 8.2 five hours after the first acetazolamide injection. The second dose of acetazolamide did not induce an additional increase in urine pH. (See fig. 49).

With respect to the plasma solutes, the only change of possible significance was the fall in $[Na^+]$ during the natriuresis which followed the first acetazolamide injection. If the natriuresis was responsible for the plasma sodium depletion, it is difficult to understand why plasma $[Na^+]$ increased following the second treatment, despite continued natriuresis. Possibly by that stage of the experiment, foetal plasma $[Na^+]$ was sufficiently depleted to initiate an influx of maternal Na^+.

b. Foetus 271 (145 days) (appendix table 58)

In this experiment the average pre-treatment flow rate was 0.81 ml/min. and within 15 minutes of acetazolamide treatment (28mg/kg) the flow rate had increased to 1.06ml/min. (1.3 times). Following this peak, the diuresis gradually subsided and two hours after treatment, control flow rates were recorded. At that point the second acetazolamide injection was given (56mg/kg) and again urine flow rate increased within 15 minutes. However, on this occasion the maximum flow rate achieved was only 1.00ml/min. which was 1.27 times the average flow rate for the ½ hour before the second injection. Thus, despite the higher dose of acetazolamide used in the second injection, the diuresis induced was not as great and its duration was shorter.

The $[Na^+]$ and $[K^+]$ of foetal urine increased immediately after acetazolamide treatment, however the maximum $[Na^+]$ (2.2 times the pre-treatment average) was reached about 1½ hours after treatment while the maximum $[K^+]$ (1.8 times the pre-treatment average) was reached about 2½ hours after treatment. In the natriuresis and kaliuresis that resulted

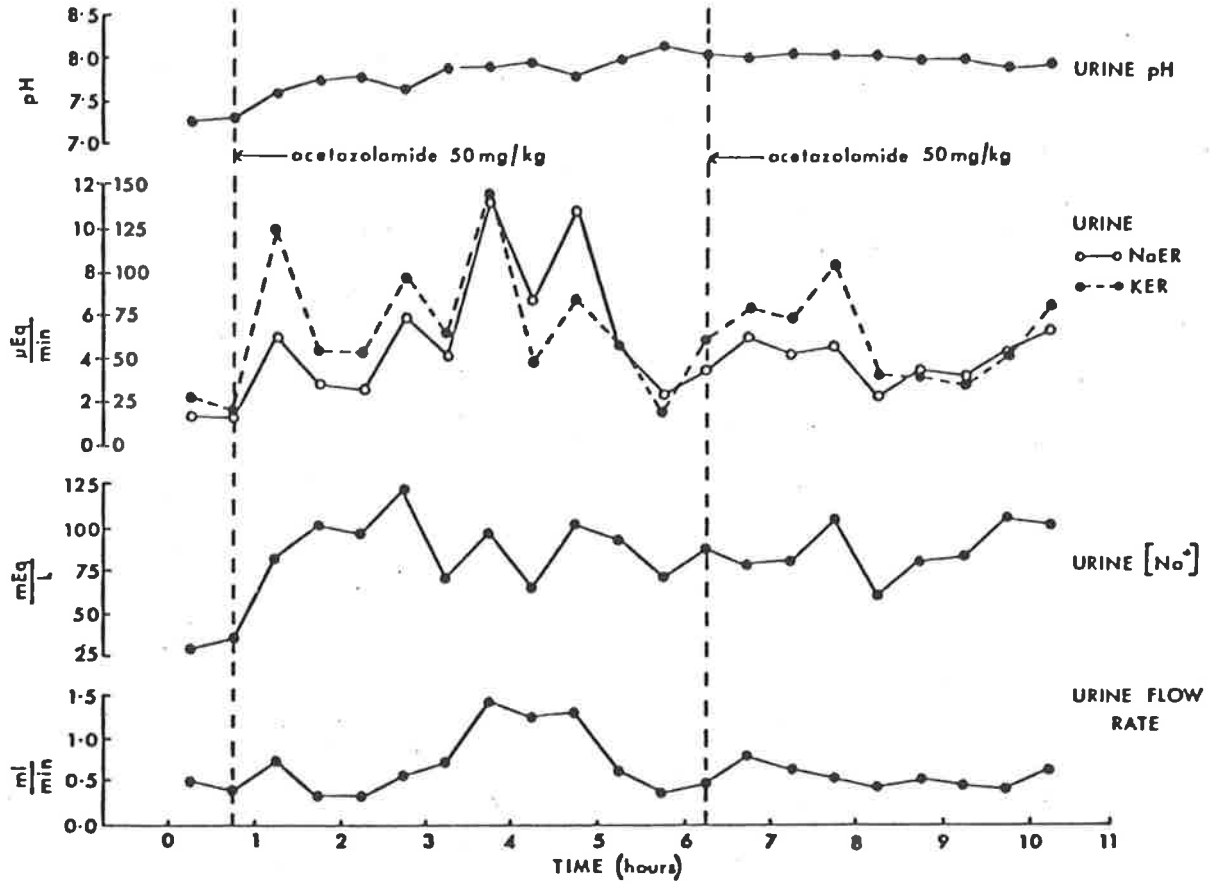

FIGURE 49. Changes in foetal urinary parameters following injections of acetazolamide into foetus 223 (130 days old).
(See appendix table 57 and text pages 126 and 128)

from the simultaneous increases of urinary electrolyte concentration and urine output, the maximum Na^+ and K^+ excretion rates were 3.4 and 2.5 times their respective control averages. In each case the maximum excretion rates coincided with the maximum electrolyte concentration rather than the peak of diuresis.

Following the second injection of acetazolamide there was only a small additional rise in urinary $[Na^+]$. This was not surprising, as unlike flow-rate, $[Na^+]$ had not returned to control levels by the time the second injection was given. It is noticeable, however, that despite the increased dose of acetazolamide administered on the second occasion, the maximum $[Na^+]$ achieved was similar to that achieved after the first treatment. The second treatment had no immediate effect on urinary $[K^+]$ although about 10 minutes after treatment, $[K^+]$ began to rise. The diuresis which occurred immediately after the second acetazolamide injection was associated with simultaneous rises in Na^+ and K^+ excretion rates. (See fig. 50).

In contrast to the preceding experiment, acetazolamide did not induce a rise in the urinary concentration of creatinine and uric acid on this occasion. The concentration of both solutes decreased as urine flow increased and there was no significant change in the excretion rate of either solute. On the other hand, urinary pH was again altered following acetazolamide treatment. The average pH prior to treatment was 7.5 but within 15 minutes of the first injection the pH was 8.0 and 1¼ hours later it was 8.4. The urinary pH immediately prior to the second injection was 8.4 and despite the additional dose of acetazolamide, no further rise in pH occurred.

Only three blood samples were taken during this experiment and although there was a progressive decrease in the plasma concentration of Na^+ and K^+ the significance of this trend was not established.

SUMMARY

In both foetuses tested, acetazolamide induced diuresis. In the youngest foetus the maximum flow rate was recorded about three hours after treatment and was greater, both in terms of magnitude and relative increase than the maximum flow rate obtained with the older foetus. Initially, however, the older foetus received about ½ the acetazolamide dose used with the younger foetus, but even when the dose was doubled no further rise in urine flow rate resulted. In both experiments the $[Na^+]$ and $[K^+]$ of foetal urine increased following acetazolamide treatment, with $[Na^+]$ showing the largest rise. In the younger foetus the maximum electrolyte levels were reached about two hours after treatment, but as with diuresis, the electrolyte response was more prompt in the older foetus where the maximum levels were reached one hour after treatment. In both foetuses the electrolyte response persisted longer than the diuresis. Acetazolamide had no consistent effect on the urinary concentration and excretion rate of uric acid and creatinine, although both foetuses responded to acetazolamide by producing increasingly alkaline urine. When additional doses of acetazolamide were administered during periods of maximum pH no additional rises in pH could be induced.

5.3.4 Sodium Chlorothiazide (Sodium Diuril - Merck Sharpe and Dohme) (6 - chloro - 7 - sulfamyl - 1,2,4 - benzothiadiazine - 1,1 - dioxide)

Three experiments were carried out in this series using foetuses aged 113, 129 and 143 days. In each experiment two injections of chlorothiazide were given.

a. Foetus 275 (113 days) (appendix table 59)

This foetus was given an initial chlorothiazide dose of 83mg/kg. The pre-treatment flow rate was low (average 0.04 ml/min.) but increased

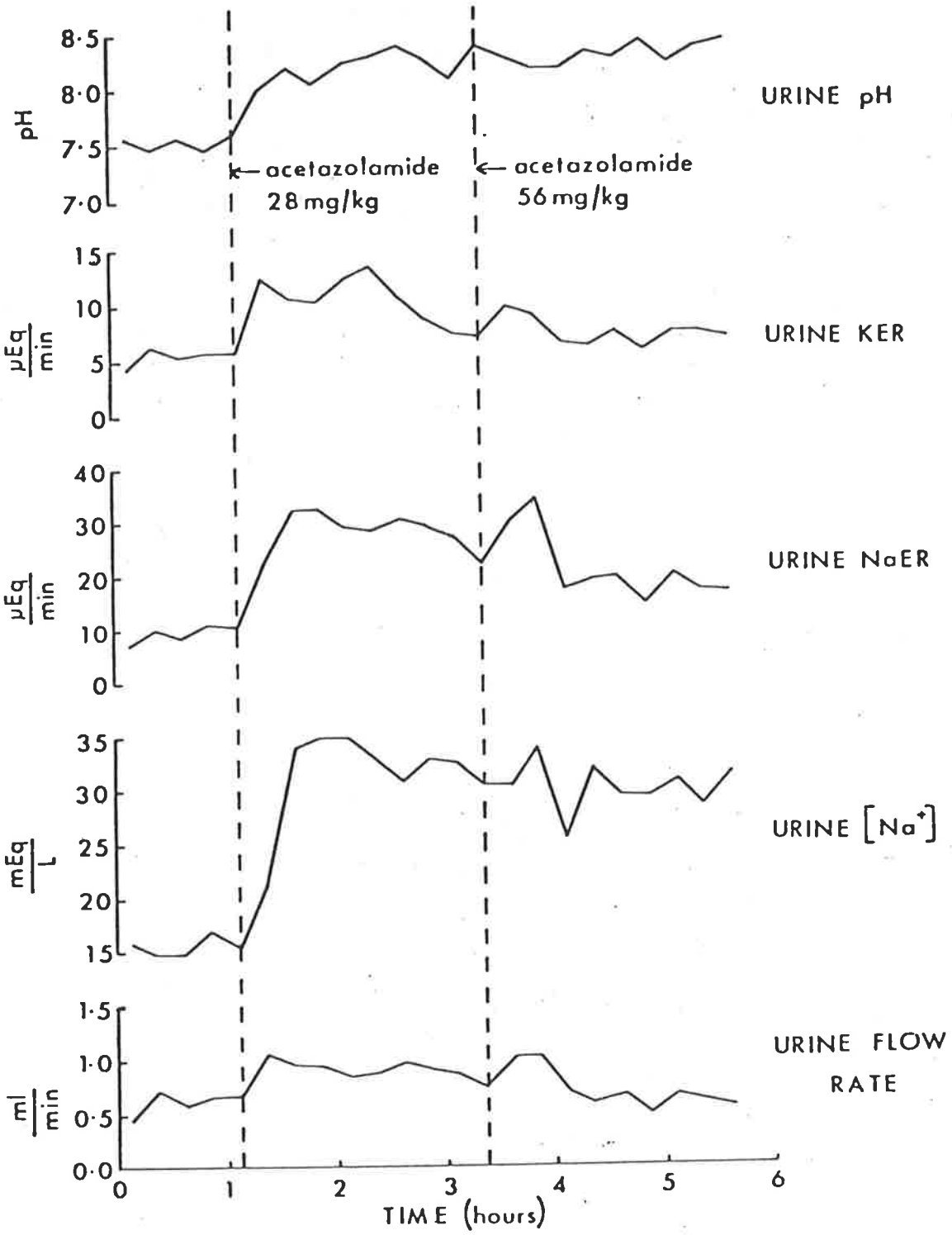

FIGURE 50. Changes in foetal urinary parameters following injections of acetazolamide into foetus 271 (145 days old).
(See appendix table 58 and text pages 128 and 130)

sharply following treatment and reached a maximum of 0.24ml/min. 1 hour after treatment. Over the next hour the flow rate returned to control levels whereupon 166mg/kg of chlorothiazide was injected. This second injection produced a maximum flow rate of 0.35ml/min. within one hour, yet the diuresis faded even more quickly than the first diuresis.

The $[Na^+]$ of foetal urine double within one hour of the first chlorothiazide injection reaching a maximum of 63.5 mEq/L, then control values were re-established over the next 30 minutes. After the second injection of chlorothiazide the $[Na^+]$ of foetal urine reached a maximum which was 2.2 times the pre-treatment average. As a result of these changes the Na^+ excretion rate increased 13.3 times following the first chlorothiazide injection and 17.2 times following the second. In both cases the natriuresis was short-lived.

The $[K^+]$ of foetal urine was not significantly effected by the first dose of chlorothiazide but was reduced following the second. Accordingly there was an increase in K^+ excretion rate following the first treatment but not after the second. The concentration of the remaining urinary solutes, uric acid and creatinine, decreased following the first chlorothiazide injection and continued to decrease after the second, but nevertheless there was a nett increase in the excretion rate of these solutes after each treatment.

The pH of foetal urine was also effected by chlorothiazide treatment. The average pH before treatment was 7.2 but within 30 minutes of the first injection it had risen to 8.0. Immediately before the second injection the urinary pH was 7.6 and despite a slower response the pH increased to 7.8 following the injection. (See fig. 51).

No significant concentration changes were observed in foetal plasma.

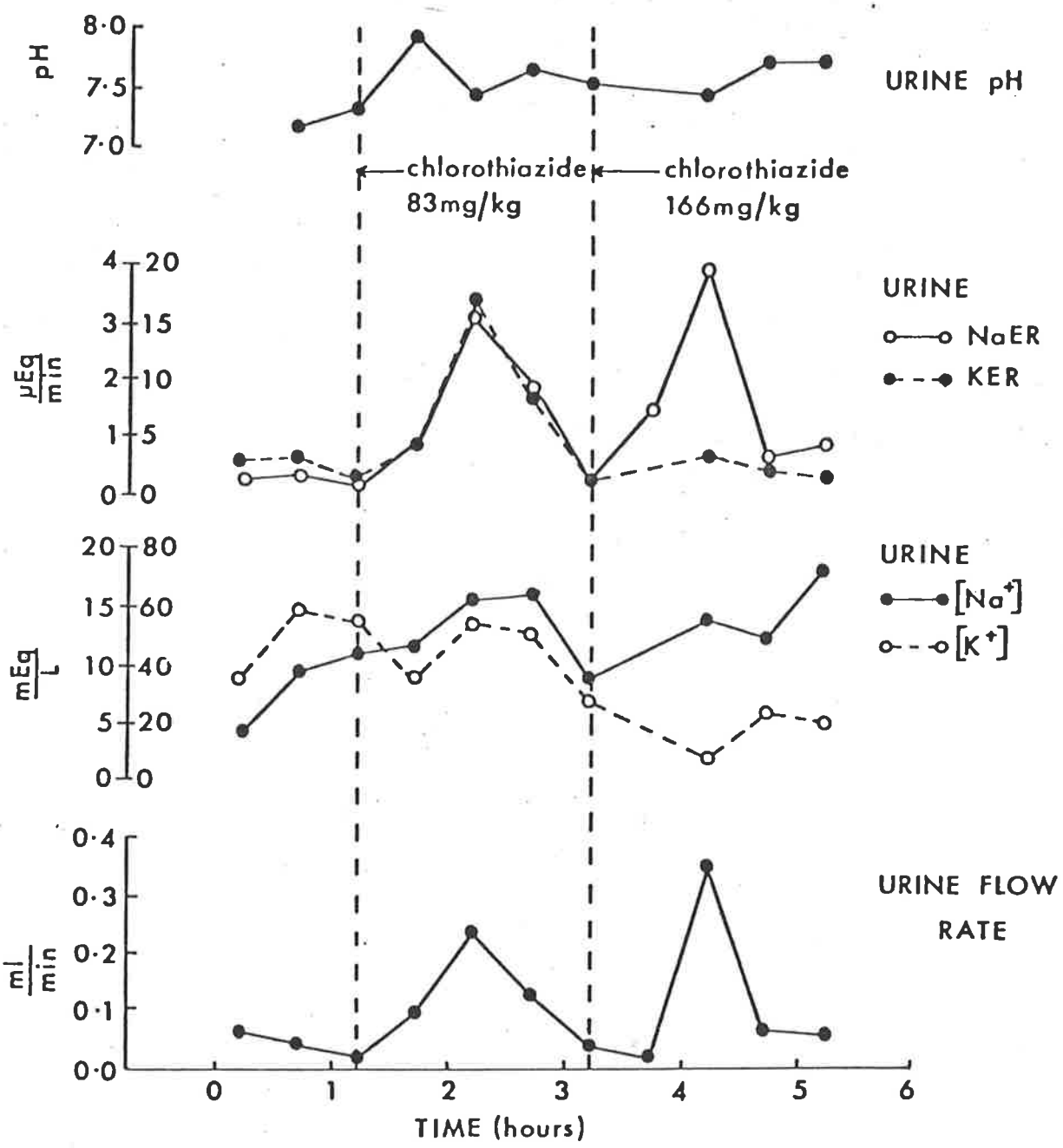

FIGURE 51. Changes in foetal urinary parameters following injections of sodium chlorothiazide into foetus 275 (113 days old).
(See appendix table 59 and text pages 130 and 131)

b. Foetus 257 (129 days) (appendix table 60)

A comparatively low dose of chlorothiazide was administered to this foetus (13mg/kg) but diuresis commenced within fifteen minutes. In one hour the urine flow rate reached a maximum of 0.49ml/min. which was 4.5 times the pre-treatment average. The flow rate remained at that level for about 30 minutes and then, over the following 4½ hours, gradually returned to control levels.

The second chlorothiazide injection was twice the dose of the first (26mg/kg) and although the urine flow rate increased more rapidly, the maximum flow rate was only 3.5 times the pre-treatment average. Urinary $[Na^+]$ was again effected by chlorothiazide treatment. Within 45 minutes of the first injection urinary $[Na^+]$ reached a maximum of 79.0 MEq/L which was 2.6 times the control average. Thereafter the $[Na^+]$ fell slowly and at the time of the second injection the urinary $[Na^+]$ was still 2.2 times the control level. Following this second chlorothiazide treatment there was a further rise in urinary $[Na^+]$ and one hour after treatment the $[Na^+]$ had risen to 74.0 mEq/L (2.5 times the pre-treatment average). For the remaining four hours of the experiment, urinary $[Na^+]$ remained near that level.

As a result of these changes, Na^+ excretion rate increased markedly after each chlorothiazide treatment. One hour after the first treatment the Na^+ excretion rate reached a maximum which was 11.4 times the pre-treatment average. Following the second treatment, Na^+ excretion rate began to rise immediately, but it was not until 3½ hours after the injection that the maximum Na^+ excretion rate was recorded. At that point the excretion rate was 7.1 times the control average.

The remaining urinary solutes, K^+, uric acid and creatinine all showed similar concentration changes. In general, the concentration of these solutes was reduced during periods of diuresis, although there was a small increase in the excretion rate of each solute, with the maximum excretion

rate coinciding with the maximum urine flow. Urine pH was again effected by the chlorothiazide treatment. The pre-treatment average for urine pH was 7.0 but within one hour of the first treatment the pH had reached 8.0. Immediately prior to the second treatment the pH was 7.4 and it reached 7.9 within 30 minutes of that treatment and 8.0 three hours after treatment. (See fig. 52).

c. Foetus 3 (143 days) (appendix table 61)

An initial chlorothiazide dose of 30mg/kg was injected into this foetus. The urine flow rate began to increase within 10 minutes of the injection and a maximum flow rate, which was 5.4 times the pre-treatment average was recorded 40 minutes after treatment. Over the next 80 minutes the urine flow rate decreased gradually, although at the end of that period it was still 1.8 times the control flow rate. At that point a second injection of 15mg/kg of chlorothiazide was given. Again the flow rate increased within 10 minutes and reached a maximum 40 minutes after treatment, but on this occasion the maximum flow rate was 4.1 times the pre-treatment average. As previously, the $[Na^+]$ of foetal urine increased after chlorothiazide treatment. Following the first injection there was no increase in $[Na^+]$ for 50 minutes, but thereafter it increased slowly reaching a maximum of 81.5 mEq/L (1.5 times the pre-treatment average) 1 hour and 40 minutes after treatment. At the time of the second chlorothiazide injection, the urinary $[Na^+]$ was still 1.4 times the pre-treatment average and no further rise was induced. The changes in Na^+ excretion rate following chlorothiazide treatment reflected primarily the changes in urine flow rate. Following each injection the peak in Na^+ excretion rate corresponded with the peak in urine flow rate although the natriuresis persisted longer than the diuresis as a result of the prolonged changes in urinary $[Na^+]$.

As previously, the other urinary solutes, K^+, uric acid and creatinine showed similar concentration changes. Following the first

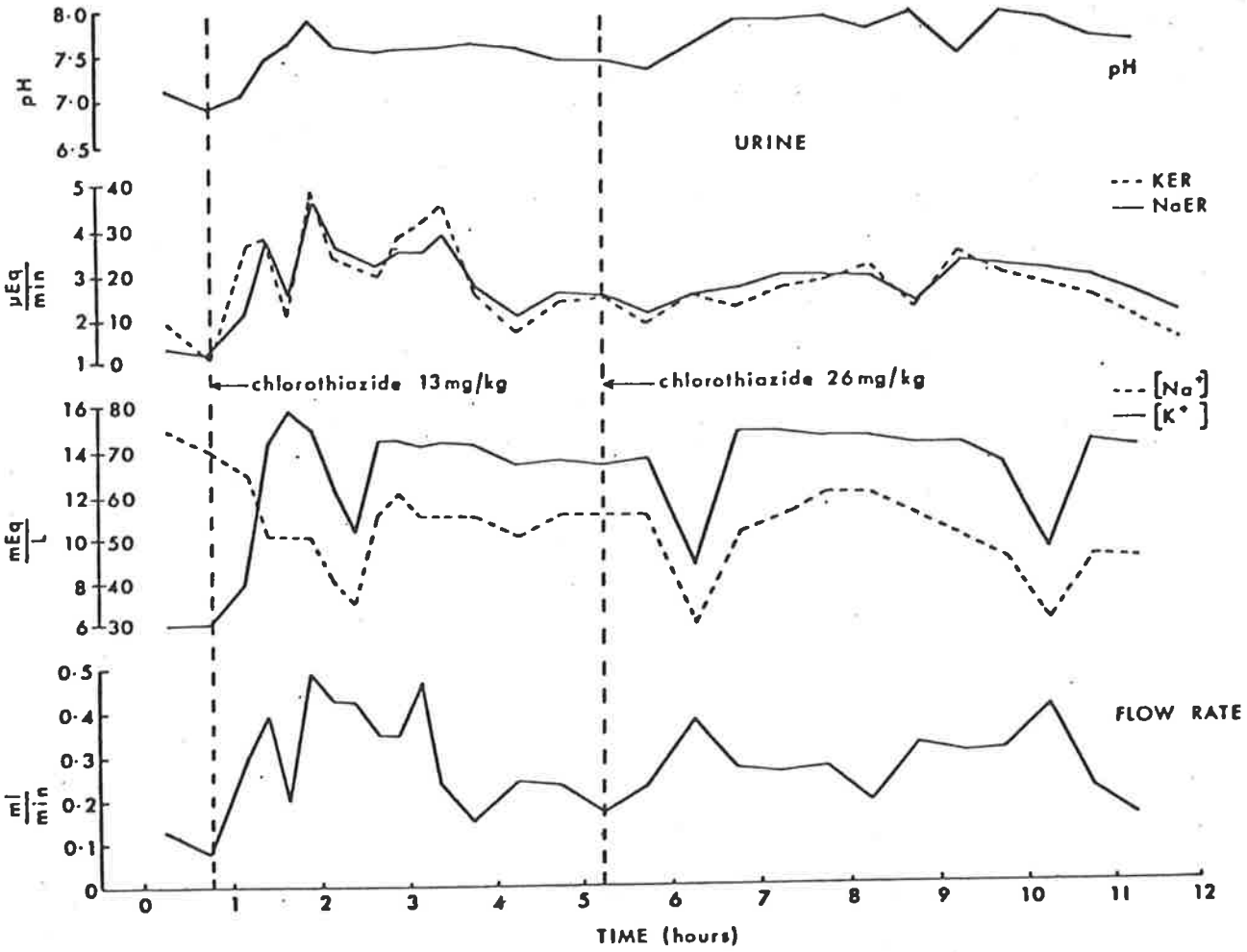

FIGURE 52. Changes in foetal urinary parameters following injections of sodium chlorothiazide into foetus 257 (129 days old).
(See appendix table 60 and text pages 132 and 133)

treatment the concentration of these solutes decreased, beginning at about the time of maximum diuresis and continuing throughout the experiment. Nevertheless the excretion rate of each of these solutes did increase following the chlorothiazide injections. In all cases the maximum excretion rate corresponded with the peak in urine flow rate although the excretion rate changes were not as great as the diuresis.

The pH of foetal urine was once more altered by the chlorothiazide treatment. The average pH before treatment was 7.0 and apart from a small decrease in the 20 minutes immediately after the first injection, the pH increased steadily reaching a maximum of 8.0, two hours after treatment. At that time the second chlorothiazide injection was administered but no additional increase in pH was induced. (See fig. 53).

Only three plasma samples were taken during this experiment and no significant changes in the composition of foetal plasma were observed.

SUMMARY

In the three foetuses tested, chlorothiazide treatment resulted in an increase in the flow rate and $[Na^+]$ of foetal urine. Accordingly increases in Na^+ excretion rate, of between 6.8 and 17.2 times were recorded. The largest diuresis and natriuresis were seen in the youngest foetus, but the dose of chlorothiazide used in that foetus was six times the dose given to the oldest foetus. In all experiments, the foetus was given two different doses of chlorothiazide and overall there was no evidence of a close dose-response relationship. In the youngest foetus, doubling the dose of chlorothiazide did increase the diuresis and natriuresis, but not twofold; while in foetus 129 doubling the dose did not increase the response at all. It is probable that the limited response following the second chlorothiazide injection in these experiments was due to the operation of renal compensatory mechanisms induced by the water and salt losses following the first treatment. These compensatory mechanisms would still have been in operation at the time of the second injection.

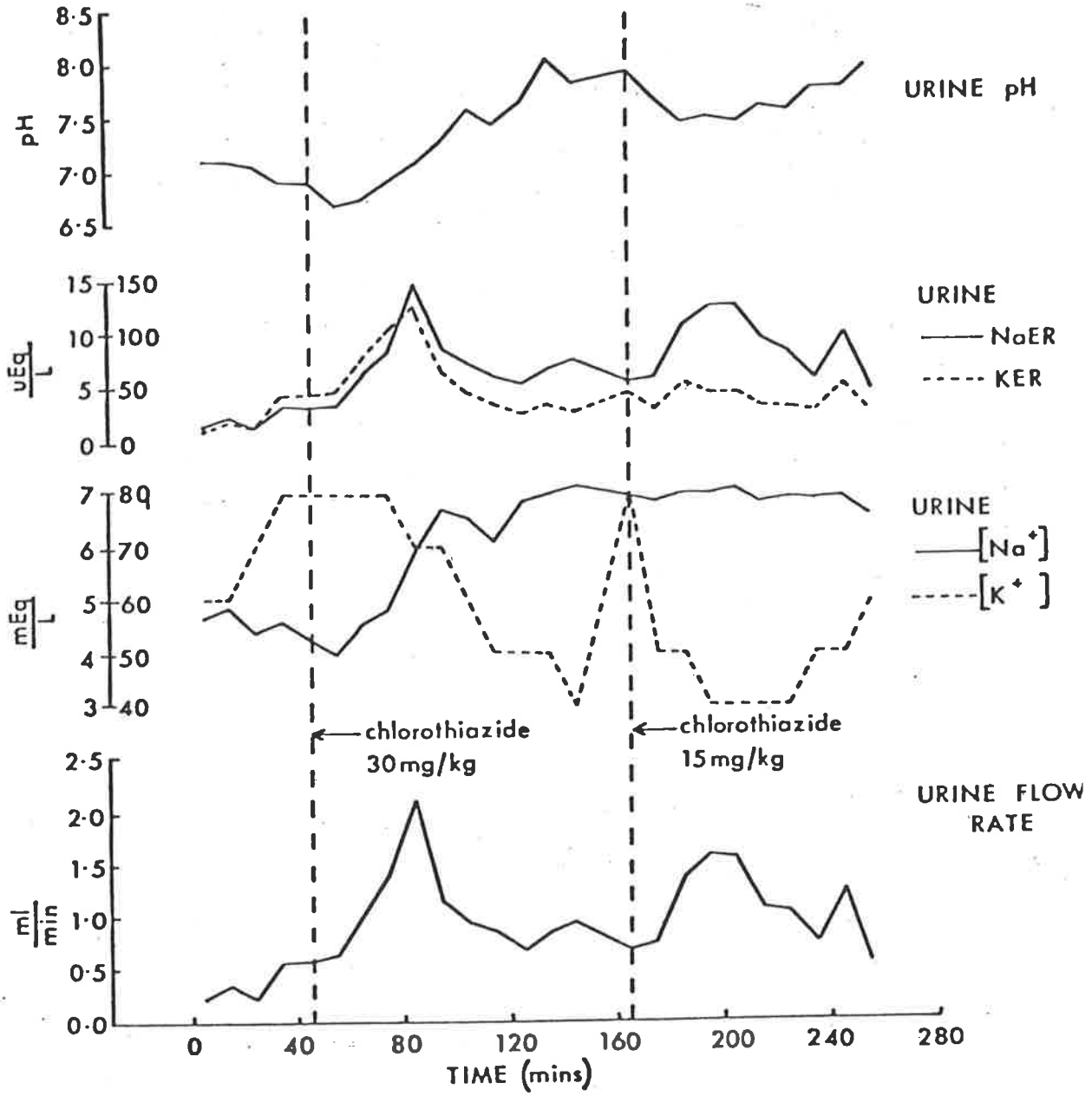

FIGURE 53. Changes in foetal urinary parameters following injections of sodium chlorothiazide into foetus 3 (143 days old). (See appendix table 61 and text pages 133 and 134)

With respect to age-response relationships, the large dose of chlorothiazide given to the 113 day-old foetus precluded it from consideration. Nevertheless, in comparing the effect of comparable doses of chlorothiazide on the remaining foetuses, it would appear that there was no age-response correlation. For example, with a chlorothiazide dose of about 15mg/kg the greatest increase in urine flow occurred with the 129 day-old foetus, but with a dose of about 30mg/kg the 143 day-old foetus produced the largest diuresis. It was noticeable that although the duration of the flow rate responses varied, they were particularly short-lived in the youngest foetus, despite the relatively large doses of chlorothiazide administered. This may indicate a greater responsiveness of older foetuses to chlorothiazide, not in terms of the magnitude of the diuresis induced, but in terms of its duration. Inconsistencies in dose-response and age-response relationships similar to those seen between chlorothiazide treatment and the diuretic response were seen again when the natriuretic responses were considered. As mentioned, chlorothiazide invariably stimulated an increase in urinary $[Na^+]$ and Na^+ excretion rate but little else can be said.

In contrast to the changes in urinary $[Na^+]$ none of the foetuses showed an increase in urinary $[K^+]$ following treatment. The $[K^+]$ either remained unchanged or decreased during the period of increased urine flow. However, a small nett increase in K^+ excretion rate was observed after each chlorothiazide injection. The concentration and excretion rate of uric acid and creatinine followed similar patterns to that of K^+. A more positive response was seen for urinary pH, where in all experiments, the pH increased significantly following chlorothiazide treatment.

5.3.5 Amiloride Hydrochloride (Midamor - Merck Sharp and Dohme)(N - amidino - 3,5 - diamino - 6 - chloropyrazine carboxamide hydrochloride)

Three foetuses of approximately the same age (135 - 139 days) were treated with amiloride hydrochloride. In two of the three experiments the urinary concentrations of uric acid and creatinine were not measured,

however in the experiment where these parameters were measured they were unaffected by amiloride hydrochloride treatment.

a. Foetus 3 (135 days) (appendix table 62)

This foetus received 2.0mg/kg of amiloride hydrochloride, infused over a five minute period. Within a ½ hour of treatment the urine flow rate began to rise and about one hour after treatment it reached a maximum of 1.35 ml/min. This maximum was 1.5 times the average pre-treatment flow rate. After reaching this peak the flow rate returned to control values within 30 minutes and no further increase occurred during the remaining three hours of the experiment. The $[Na^+]$ of foetal urine began to rise immediately after the amiloride hydrochloride treatment and it reached a maximum which was 1.3 times the pre-treatment average for $[Na^+]$. The peak in $[Na^+]$ coincided with the period of maximum diuresis and the nett result was a twofold increase in Na^+ excretion rate. Despite the increase in urinary $[Na^+]$ there was no increase in urinary $[K^+]$ following amiloride hydrochloride treatment. There was, however, a brief kaliuresis corresponding to the rise in urine flow rate. The maximum K^+ excretion rate was 1.5 times the control average. Finally the pH of foetal urine was unaffected by amiloride hydrochloride treatment. (See fig. 54).

b. Foetus 273 (137 days) (appendix table 63)

The dose of amiloride hydrochloride infused into this foetus was again 2.0mg/kg and the maximum flow rate of foetal urine (0.86ml/min.) was recorded within 30 minutes of treatment. This maximum flow rate was 1.9 times the pre-treatment average. Amiloride hydrochloride treatment again induced an increase in the $[Na^+]$ of foetal urine and the $[Na^+]$ rose to a maximum of 43.5 mEq/L, which was 1.7 times the average pre-treatment concentration. This rise occurred over three hours, although near maximum levels were reached within two hours of treatment. The nett result of these changes was a natriuresis which reached a peak within one hour of treatment and during which the Na^+ excretion rate increased 2.9 times. Since the $[Na^+]$ of foetal urine did not return to control levels as quickly as

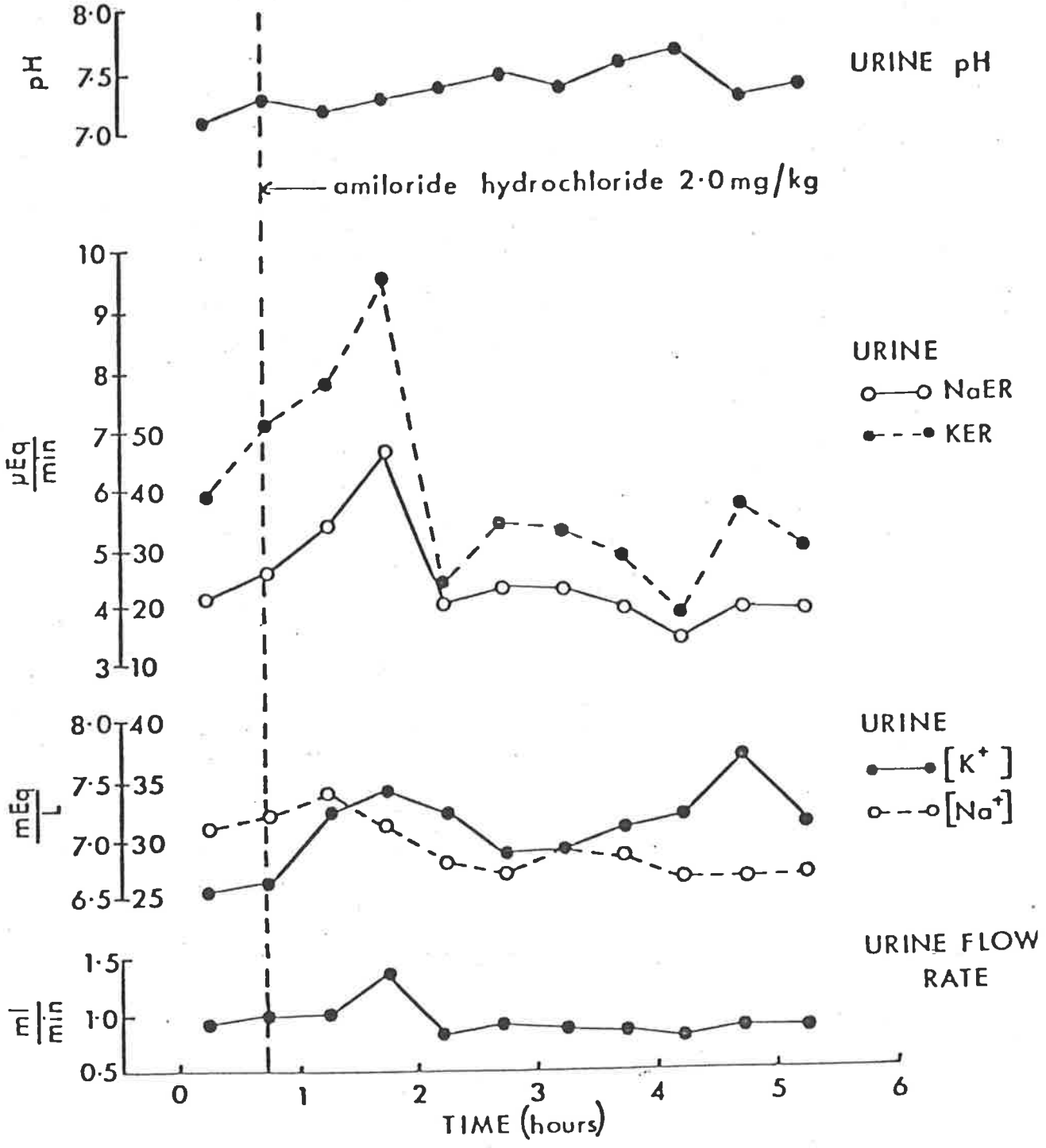

FIGURE 54. Changes in foetal urinary parameters following the injection of amiloride hydrochloride into foetus 3 (135 days old).
(See appendix table 62 and text page 136)

flow rate, the natriuresis persisted for approximately 4 hours. In contrast to the rise in $[Na^+]$; the $[K^+]$ of foetal urine decreased by 53% following the amiloride hydrochloride infusion. The fall in urinary $[K^+]$ did not commence until a ½ hour after treatment and since urine flow rate increased during the first ½ hour after treatment, there was a brief kaliuresis. Following the kaliuresis, K^+ excretion rate fell sharply until at the end of the experiment it was only 18% of the pre-treatment average. During the first two hours of the post-treatment period, the pH of foetal urine increased from a control average of 6.9 to a maximum of 7.2. Thereafter the pH declined toward control levels. (See fig. 55).

With respect to foetal plasma composition, it may be significant that the plasma samples taken after treatment showed a progressive decrease in $[Na^+]$ and a progressive increase in $[K^+]$.

c. Foetus 3 (139 days) (appendix table 64)

In this experiment the foetus received a dose of 3.8mg/kg of amiloride hydrochloride (this foetus was treated with 2.0mg/kg in the first experiment of this series). Following treatment, the output of foetal urine reached a maximum of 0.87ml/min. after one hour and 20 minutes. This maximum was 2.9 times the pre-treatment average and when compared with the increase obtained in the first experiment using this foetus, it suggests that a larger dose of amiloride hydrochloride induces a greater response. However, although the increase in urine flow rate, relative to the pre-treatment average, was greater in this experiment, the maximum flow rate achieved was lower. This dichotomy reflects the fact that the average pre-treatment flow rate in this experiment was about 1/3 of that in the previous experiment involving foetus 3.

With respect to urinary $[Na^+]$, the larger dose of amiloride hydrochloride did not induce a larger rise. The maximum $[Na^+]$ achieved following treatment was only 1.1 times the pre-treatment average and even this small rise was short-lived. Accordingly, natriuresis occurred following treatment, but it appeared to be largely related to the rise in urine flow. There was no significant change in the $[K^+]$ of foetal urine but

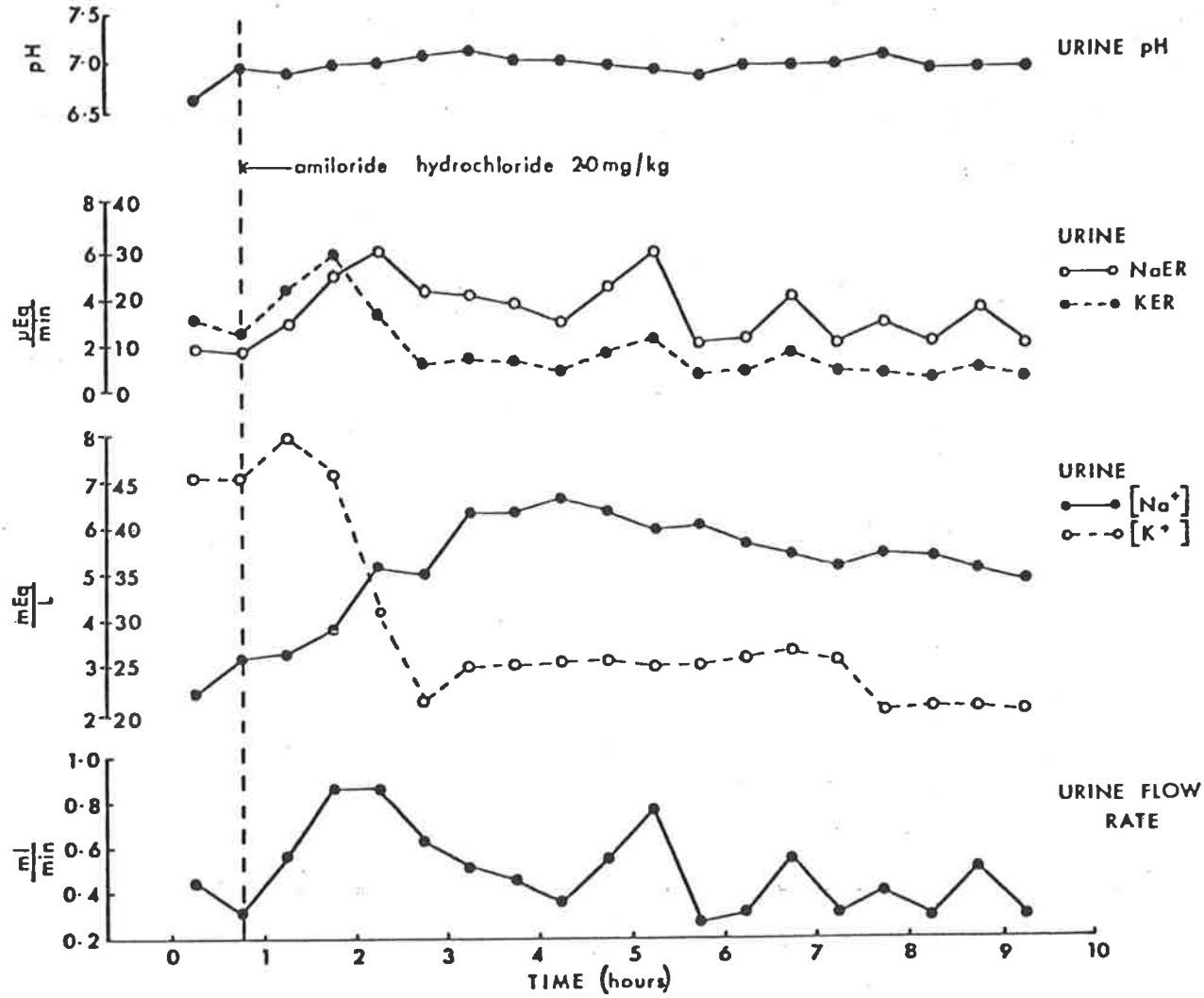

FIGURE 55. Changes in foetal urinary parameters following the injection of amiloride hydrochloride into foetus 273 (137 days old). (See appendix table 63 and text pages 136 and 137)

there was a small transient increase in K^+ excretion rate which paralleled the flow rate change. Finally the pH of foetal urine decreased after the first 20 minutes of the post-treatment period. The pre-treatment average for urinary pH was 7.7, yet 1½ hours after the amiloride hydrochloride infusion the pH was 7.2. (See fig. 56).

SUMMARY

In all three experiments, amiloride hydrochloride treatment induced diuresis, with the urine flow rate increasing between 1.5 and 2.9 times. The speed of onset and the duration of the diuretic responses was variable, despite the similar ages of the foetuses treated. Maximum urine flow rates were recorded between 30 and 90 minutes after treatment and the elevated flow rates persisted for 1 to 2 hours. Amiloride hydrochloride also induced natriuretic responses, although the increases in urinary $[Na^+]$ were not as great as the rises in urine flow rate. With the exception of foetus three, at 139 days of age, the elevated $[Na^+]$ persisted longer than the diuresis and accordingly the natriuretic response was prolonged.

The $[K^+]$ of foetal urine was either unaffected or decreased by amiloride hydrochloride treatment. In the two experiments using foetus three the $[K^+]$ was essentially unchanged but small transient increases in K^+ excretion rate occurred during periods of diuresis. In foetus 273 the urinary $[K^+]$ decreased and apart from a short initial increase, the K^+ excretion rate fell to a fraction of the pre-treatment level. No consistent effect of amiloride hydrochloride on urinary pH was observed.

Since the foetuses used in these experiments were all of a similar age, no inferences can be drawn regarding age-response relationships. However with respect to dose-response relationships, it is apparent that the 75% increase in the dose of amiloride hydrochloride used for the second experiment with foetus 3, did not produce a comparable increase in the diuretic and natriuretic responses induced by the drug.

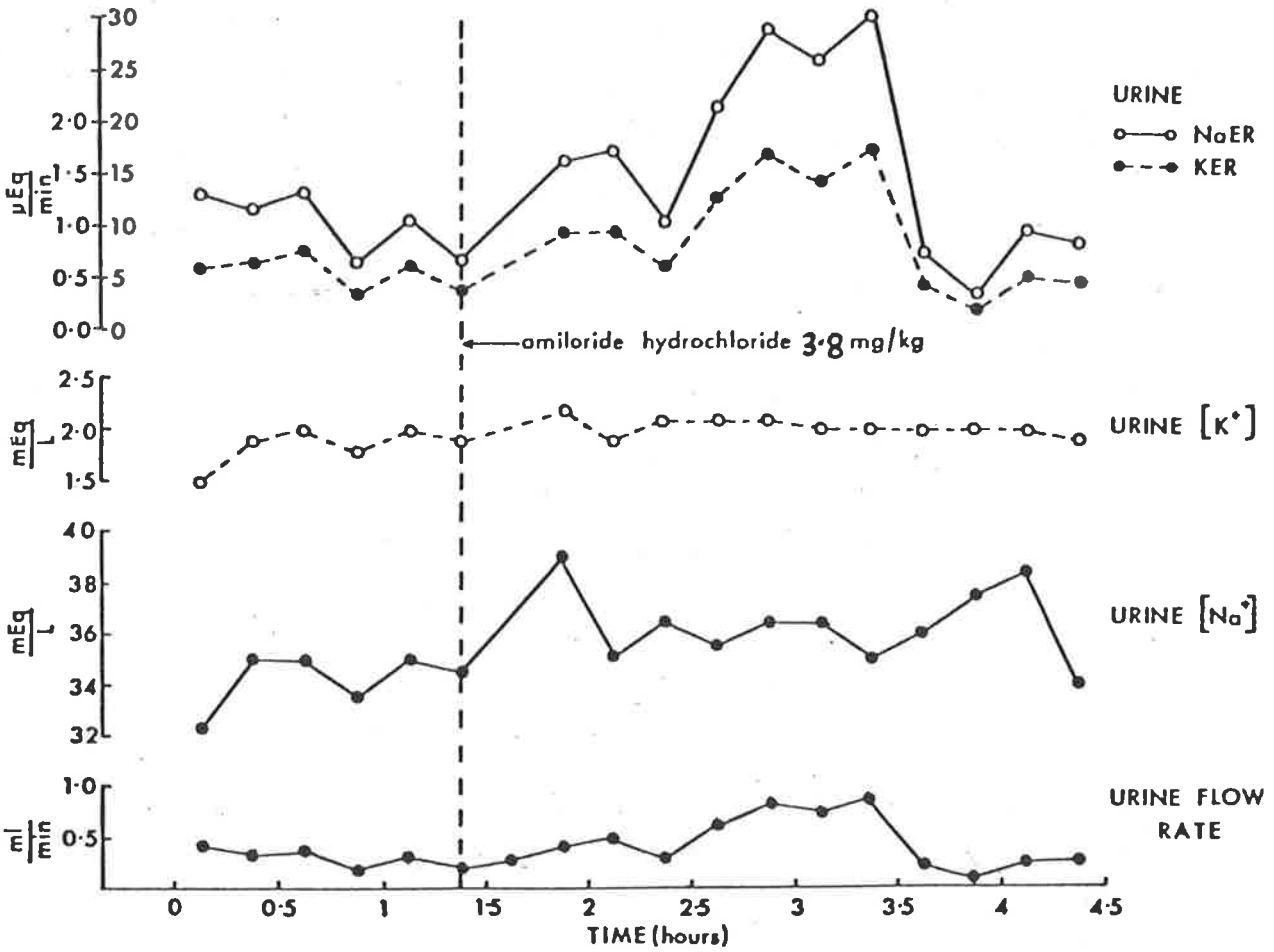

FIGURE 56. Changes in foetal urinary parameters following the injection of amiloride hydrochloride into foetus 3 (139 days old).
(See appendix table 64 and text pages 137 and 138)

5.3.6 Mersalyl (Evans)

Mersalyl is an organic-mercurial diuretic that is known to occasionally produce a fatal reaction following intra-venous administration. Death is thought to be caused by a rapid decrease in blood pressure and cardiac output as a result of ventricular fibrillation. To avoid such reactions it is recommended that the drug be administered slowly. The recommended paediatric dose for mersalyl is 1-5mg/kg. In view of these facts it was decided to test the responsiveness of the foetal kidney to organic-mercurial diuretics by infusing a dose of 100 μg/kg of mersalyl over a 60 minute period. In the first experiment, foetus 254 (125 days old) was infused, but after 35 minutes, foetal urine output ceased and foetal MAP dropped sharply. To this point 105 μg of mersalyl had been injected. An autopsy was performed and foetal death was confirmed; however, no abnormalities were revealed which could have been responsible for the death of the foetus. Accordingly, it was assumed that mersalyl had killed the foetus.

Subsequently a second attempt was made to treat a foetus with mersalyl. In view of the previous experience, an older foetus (foetus 223, 141 days old), was chosen and a dose of 10 μg/kg was to be infused over one hour. However, again the foetus died before the infusion was completed and at the estimated time of death only 27 μg of mersalyl had been infused. As a result of these deaths it was concluded that foetal sheep are particularly susceptible to the toxic effects of organic-mercurial diuretics and no further experiments were carried out using mersalyl.

5.4 Experimental Manipulation of Foetal GFR

In section 4.9 results were presented showing the GFR of normal untreated foetuses at various stages of gestation. The GFR was estimated by calculating endogenous creatinine clearance and by measuring the clearance of inulin ^{14}C. For the experiments described in this section the same techniques were used to estimate GFR before and during

various experimental treatments.

A total of six experiments were carried out using foetuses aged between 128 and 146 days. In two of these experiments drugs were used to alter vascular resistance in the foetal kidney and to assess its effect on foetal GFR. In the third experiment an attempt was made to examine the effect on GFR of reduced hydrostatic pressure in the foetal glomerular capillaries. In the remaining experiments, foetuses were treated with diuretic drugs to examine GFR during drug-induced diuresis. The drugs used were furosemide, sodium ethacrynate and amiloride hydrochloride which were chosen because, as determined in the preceding section, they represent the extremes of diuretic potency in foetal sheep.

5.4.1 Renal Vascular Resistance and GFR

To increase renal vascular resistance, adrenalin tartrate was infused into the foetal sheep. In man it has been established that even low doses of adrenalin, which have little effect on MAP, produce a substantial increase in renal vascular resistance and a corresponding reduction in renal blood flow (Smythe et al 1952; Gombos et al 1962). In the present experiments the adrenalin infusions resulted in increased foetal MAP. These pressure changes were monitored to assess the foetal response to adrenalin and thus to indirectly assess the changes in renal vascular resistance. It is assumed that foetal MAP and renal vascular resistance are correlated in the hypertensive state induced by adrenalin.

a. Foetus 3 (130 days)

Inulin ^{14}C was injected into the femoral vein of this foetus and at regular intervals, beginning 3½ hours after the inulin injection, 30 minute urine fractions were collected and blood samples were taken at the mid-point of each urine collection. The activity of these samples was measured, and an aliquot of each was analysed to determine the concentration of Na^+, K^+, creatinine and uric acid. Thus it was possible to calculate

the clearance rate of inulin ^{14}C plus the clearance rates of endogenous creatinine, uric acid, Na^+ and K^+.

In this experiment, no treatment was applied during the first two collection periods. The MAP of the foetus during these periods, varied between 58.5 and 61mm Hg. Throughout the third and fourth collection periods, adrenalin tartrate was infused (0.04mg/min.) and the MAP was between 79 and 82mm Hg and 81 and 84mm Hg respecitvely. It can be seen in table 11 that the increase in MAP and presumably vascular resistance was associated with a substantial reduction in GFR.

Despite the relatively uniform MAP during the control period, the GFR during the second collection period was twice that of the first collection period and similar differences were seen for the clearance values of all other solutes. Also, during the third and fourth collection periods, where adrenalin was infused, there was a marginally higher MAP in the fourth period but the GFR in that period was higher than during the third collection period. When however, the control and treatment values were averaged and compared, it was seen that a 36% increase in the MAP of the foetus was associated with an 86% reduction in GFR. Also, following this increase in MAP, the clearance rates of endogenous creatinine, uric acid, Na^+ and K^+ were reduced by 83%, 77%, 77% and 85% respectively.

b. Foetus 274 (130 days)

In this experiment three control collections were made after the inulin ^{14}C injection and before any further treatment was applied. However, during the fourth collection period the MAP and renal vascular resistance was increased by infusing adrenalin tartrate (0.05mg/min.). Before the adrenalin infusion, the MAP of the foetus varied between 51.5 and 54.0mm Hg but during the adrenalin infusion the MAP was between 75.0 and 81.0mm Hg. It can be seen in table 12 that again the rise in foetal blood pressure and renal vascular resistance was associated with

TABLE 11: FOETAL RENAL CLEARANCES AND URINE FLOW RATE IN RELATION TO M.A.

FOETUS 3 (130 DAYS)

TIME AFTER INULIN INJECTION (Hours)	MAP (mmHg)	CLEARANCE						TREATMENT
		URINE FLOW (ml/min)	INULIN (^{14}C) (GFR)	Cr	UA	Na^+	K^+	
		(ml/min)						
3½ - 4	58.5-61	0.26	1.09	0.73	0.10	0.1072	0.37	
5½ - 6	58.5-61	0.48	2.76	2.16	0.60	0.1567	0.95	
7 - 7½	79-82	0.04	0.21	0.20	0.07	0.0193	0.06	Adrenalin infusion (0.04 mg/min)
8½ - 9	81-84	0.09	0.32	0.27	0.08	0.0409	0.14	

(See text pages 140 - 141)

a substantial decrease in GFR. This fall in GFR resulted in a decreased urine output and a reduction in the rate of clearance of all the urinary solutes analysed. If, as in the previous experiment, the control clearances are averaged and compared with the clearance rates which occurred during the adrenalin treatment, we find that a 48% increase in MAP was associated with a 92% decrease in GFR. Also, after the increase in MAP and renal vascular resistance, the clearance rates of creatinine, uric acid, Na^+ and K^+ were reduced by 94%, 92%, 96% and 96% respectively. The relationship between foetal blood pressure, GFR and urine flow rate which was revealed in the present experiments, has also been observed in a study by Daniel et al (1975). These workers increased foetal blood pressure by partially occluding the umbilical cord and found that a resultant 29% increase in blood pressure was associated with an 86% reduction in urine flow rate and a 74% decrease in GFR.

c. Foetus 3 (132 days)

In this experiment the aim was to assess the effect on foetal kidney function of reducing the blood pressure in the glomerular capillaries. It was intended to achieve this by reducing systemic blood pressure. Since the bleeding of foetal sheep has been shown to produce only a transient reduction of blood pressure (see section 5.1.2) and since haemorrhage would distort the physiology of the foetus, an attempt was made to reduce foetal blood pressure using aldomet (methyldopate HCl - Merck Sharp and Dohme). Aldomet was chosen because the hypotension it induces does not involve any major reduction of renal blood flow (Goldberg et al 1960; Onesti et al 1962). Accordingly it was intended that the effect of reduced hydrostatic pressure in the glomerular capillaries could be separated from effects caused by changes in renal blood flow. In adult humans, doses of up to 2gm of aldomet are given by intravenous injection to produce a reduction in blood pressure. However, in the present experiment doses of 100mg, 300mg and 600mg were tried over a period of 6

TABLE 12: FOETAL RENAL CLEARANCES AND URINE FLOW RATE IN RELATION TO M.A.I

FOETUS 274 (130 DAYS)

TIME AFTER INULIN INJECTION (Hours)	MAP (mmHg)	URINE FLOW (ml/min)	CLEARANCE					TREATMENT
			INULIN ^{14}C (GFR)	Cr	UA	Na^+	K^+	
			(ml/min)					
3½ - 4	51.5-54	0.33	3.69	3.03	0.34	0.0421	0.53	
5 - 5½	51.5-54	0.27	4.39	3.59	0.24	0.0327	0.49	
7 - 7½	51.5-54	0.18	3.16	2.58	0.18	0.0241	0.43	
8½ - 9	75-81	0.01	0.26	0.18	0.02	0.0013	0.02	Adrenalin infusion (0.05 mg/min)

(See text pages 141 & 142)

hours, but all failed to significantly reduce foetal blood pressure. Throughout the experiment, during both control and treatment periods, the MAP of the foetus remained between 54 and 58 mm Hg. Table 13 shows the results obtained following 300mg and 600mg doses of aldomet, compared with control results. It is difficult to interpret these results since although the MAP was unaffected by the aldomet injections the GFR did change in an erratic manner. Although creatinine clearance followed a similar pattern of change to GFR the changes were not as great, while uric acid clearance, unlike both GFR and creatinine clearance, decreased following the final dose of aldomet. This divergence between uric acid clearance and GFR is not consistent with the other similar experiments. No explanation for the observed changes in GFR can be offered on the basis of the results obtained in this experiment.

5.4.2 Foetal GFR during Diuresis

Three experiments were carried out in this series all using foetus 274.

a. Foetus 274 (139 days)

In this experiment a set of control samples was collected 3½ hours after the inulin injection. Then 0.9mg/kg of sodium ethacrynate was injected into the foetus and a second set of samples was collected during the period of maximum urine flow. At the time of the second urine collection the flow rate was 1.9ml/min. compared with 0.5ml/min. during the control collection. The GFR was also greater during the second collection period although the increase in GFR was only 60% compared with a 309% rise in urine flow rate. This indicates that a rise in GFR did contribute to the diuretic response induced by sodium ethacrynate, but only in part (about 20%). Presumably the effect of sodium ethacrynate on the tubular reabsorption of water, accounts for the remaining increase in urine flow rate.

The clearance rate of all of the urine solutes analysed, increased following the sodium ethacrynate injection (see table 14). The percentage

TABLE 13: FOETAL RENAL CLEARANCES AND URINE FLOW RATE IN RELATION TO M.A.P

FOETUS 3 (132 DAYS)

TIME AFTER INULIN INJECTION (Hours)	MAP (mmHg)	URINE FLOW (ml/min)	CLEARANCE					TREATMENT
			INULIN ^{14}C (GFR)	Cr	UA	Na^+	K^+	
			(ml/min)					
3½ - 4	54-58	0.27	4.25	3.16	0.40	0.0664	0.76	aldomet (100mg)
5½ - 6	54-58	0.34	5.00	3.26	1.18	0.0944	0.75	aldomet (300mg)
7½ - 8	54-58	0.54	3.42	2.52	0.98	0.1876	0.87	aldomet (600mg)
8½ - 10	54-58	1.26	7.14	4.09	0.48	0.5426	1.76	

(see text pages 142 - 143)

increases were as follows; creatinine 53%, uric acid 136%, Na^+ 1663% and K^+ 170%. It is apparent that the rise in GFR would account for the total rise in creatinine clearance (as expected), about ½ of the rise in uric acid clearance, about 1/3 of the rise in K^+ clearance but only a small fraction of the rise in Na^+ clearance. This confirms that sodium ethacrynate has a substantial effect on the exchange of Na^+ in the renal tubules.

b. Foetus 274 (143 days)

On this occasion two sets of control samples were collected and then furosemide (1.8mg/kg) was injected into the foetus. During the resultant diuresis two further sets of samples were obtained; the first when the urine flow rate was 4.3 times the pre-treatment average and the second when it was 1.4 times the pre-treatment average. It can be seen in table 15 that despite some variation between the two control values for GFR there was a substantial increase in GFR in the first collection period following the furosemide treatment. During that period the GFR was 77% greater than the control average, while the urine flow rate was 325% greater than its pre-treatment average. Also the clearance rate of each urine solute was again increased during the diuresis and the percentage increases, compared with the average control clearances, were as follows; creatinine 88%, uric acid 94%, Na^+ 1164% and K^+ 212%.

During the second collection period after treatment, the GFR was 47% greater than the control average while the urine flow rate was 40% above the control level. It is apparent that the readjustment following the diuretic treatment was more rapid for flow rate than for GFR. This implies that the effect of furosemide on the activity of the renal tubules is more transient than its effect on glomerular filtration. Finally, in this fourth collection period, the clearance rates of the urine solutes were, with the exception of uric acid clearance, still above their control averages. The clearance rates of creatinine, Na^+ and K^+ were 28%, 400% and 62% greater than their respective pre-treatment averages and as with flow rate, it is apparent that the solute clearance rates have decreased more rapidly

TABLE 14: THE EFFECT OF DIURETIC TREATMENT ON FOETAL RENAL CLEARANCES

FOETUS 274 (139 DAYS)

TIME AFTER INULIN INJECTION (Hours)	URINE FLOW (ml/min)	CLEARANCE					TREATMENT
		^{14}C INULIN (GFR)	Cr	UA	Na^+	K^+	
3½ - 4	0.46	4.60	4.23	0.53	0.0689	1.01	
							sodium ethacrynate 0.9 mg/kg
6 - 6½	1.88	7.35	6.49	1.25	1.2145	2.73	

(see text pages 143 - 144)

TABLE 15: THE EFFECT OF DIURETIC TREATMENT ON FOETAL RENAL CLEARANCES

FOETUS 274 (143 DAYS)

TIME AFTER INULIN INJECTION (Hours)	URINE FLOW (ml/min)	CLEARANCE					TREATMENT
		INULIN ^{14}C (GFR)	Cr	UA	Na^+	K^+	
		(ml/min)					
3½ - 4	0.97	5.30	4.98	0.25	0.1688	2.15	
6 - 6½	0.77	4.83	3.85	0.38	0.1117	2.03	
							furosemide (1.8 mg/kg)
8 - 8½	3.70	8.99	8.33	0.62	1.7653	6.52	
11 - 11½	1.22	7.46	5.64	0.30	0.7009	3.38	

(See text page 144)

than GFR.

c. Foetus 274 (146 days)

The experimental protocol of the preceding experiment was duplicated in this experiment. Two control collections of foetal plasma and urine were made and then amiloride hydrochloride (4.2mg/kg) was injected into the foetus. However, despite the fact that this dose of amiloride hydrochloride had previously induced a diuretic response, on this occasion there was about a 50% reduction in urine flow rate. The urine flow rate during the control period was particularly high for a foetus of this age (pre-treatment average 1.45ml/min.). In such circumstances it would not have been surprising if amiloride hydrochloride, which has limited potency in foetal sheep, had not produced an additional rise in urine flow. However, it is difficult to understand why amiloride hydrochloride treatment would be followed by a fall in urine flow rate; unless the initial atypical flow rates were in the process of being reduced by other physiological mechanisms that could not be over-ridden by the relatively mild effect of amiloride hydrochloride. Whatever the reason for its occurrence, this abnormal response prevented the fulfillment of the objective of the experiment. Nevertheless it is interesting that coincident with the decrease in urine flow rate was a decrease in GFR of approximately the same magnitude (See table 16).

5.5 Foetal kidney response to hormone administration

A total of 19 experiments were carried out in which a variety of hormones were administered to foetal sheep to assess the effects on kidney function. In all experiments urine was collected continuously, usually in 30 or 15 minute fractions. A limited number of blood samples were obtained in most experiments and close attention was paid to haematocrit changes to avoid excessive bleeding. All urine and plasma samples were analysed for Na^+, K^+, uric acid and creatinine concentration while urinary flow rate and pH were also determined. The excretion rates of the various urine solutes were calculated from the results for each set of samples.

TABLE 16: THE EFFECT OF DIURETIC TREATMENT ON FOETAL RENAL CLEARANCES

FOETUS 274 (146 DAYS)

TIME AFTER INULIN INJECTION (Hours)	URINE FLOW (ml/min)	CLEARANCE					TREATMENT
		INULIN ^{14}C (GFR)	Cr	UA	Na^+	K^+	
		(ml/min)					
3½ - 4	1.46	6.05	5.84	0.55	0.3799	3.26	
5 - 5½	1.44	6.36	5.84	0.32	0.3243	2.48	
							amiloride hydrochloride (4.2 mg/kg)
6 - 6½	0.71	3.43	3.14	0.18	0.2970	1.02	
7½ - 8	1.04	7.97	5.72	1.49	0.3416	1.96	

(see text pages 145)

To simplify the presentation of data, the individual results for those experiments in which the treatment did not induce a response are presented in the appendix and not in the text. Also, in those experiments where a response was induced, only those parameters effected will be shown in graphs or tables.

5.5.1 Cortisol

Four cortisol infusion experiments were carried out on 3 foetuses.

a. Foetus 66-440 (133 days) (appendix table 65)

In this experiment 700 µg of cortisol was infused over 5 hours (52 µg/kg/hr.) following a 2 hour control period. It can be seen in figure 57 that the urinary concentration of Na^+ and K^+ increased during the first 1½ hours of the infusion, reaching levels nearly double the pre-treatment concentrations. Urine flow rate increased slightly during this period but the significance of that change is doubtful as there was some increase in urine flow prior to treatment. Within 2½ hours of commencing the cortisol infusions, the electrolyte concentrations had returned to control levels. Electrolyte excretion rates reflected changes in urinary electrolyte concentration. Of the other urinary solutes, creatinine increased in concentration during the same period as the electrolytes, but uric acid concentration and urinary pH were unaltered.

With respect to the plasma electrolytes, there was some indication that plasma $[Na^+]$ and plasma $[K^+]$ were reduced by the increased excretion of electrolytes. Plasma $[K^+]$ was particularly effected, but control levels were re-established by the end of the treatment period.

b. Foetus 66-440 (137 days) (appendix table 66)

In this experiment 240 µg of cortisol was infused over 4 hours (24 µg/kg/hr). Again, the urinary concentration of Na^+ and K^+ was affected. In the first 1½ hours of the infusion period $[K^+]$ increased by about 70% compared with its pre-treatment average, while $[Na^+]$ showed a similar

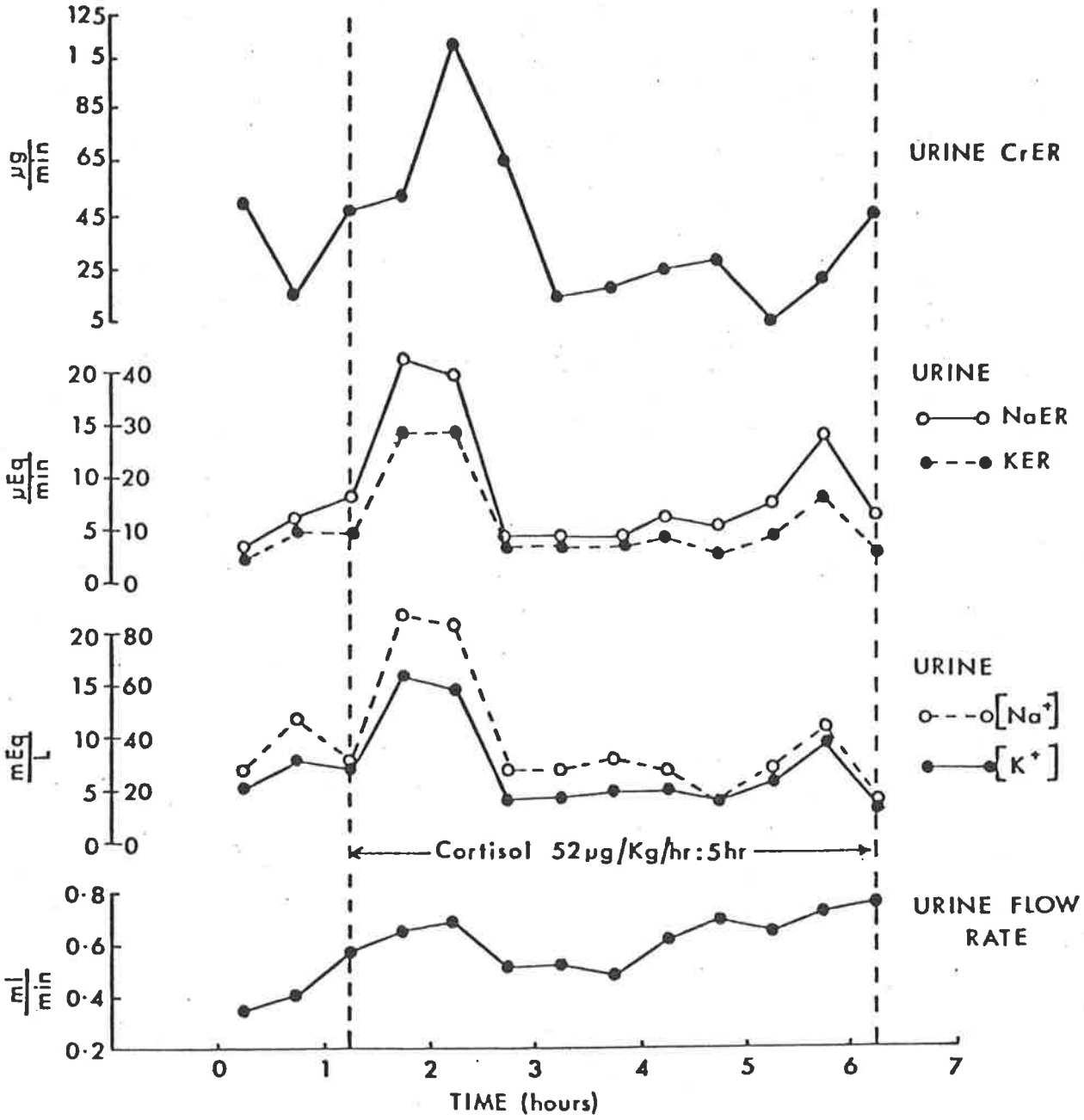

FIGURE 57. Changes in foetal urinary parameters following the infusion of cortisol into foetus 66-440 (133 days old).

(See appendix table 65 and text page 146)

increase in the first three hours of infusion. The $[K^+]$ declined during the remainder of the experiment as did $[Na^+]$, but the $[Na^+]$ was still elevated at the end of the experiment. The uric acid and creatinine concentrations of foetal urine also increased during the first 2 hours of infusion, while urinary pH showed changed that closely paralleled the changes in uric acid concentration. Urine flow rate, although erratic, did show an overall increase during the treatment period and changes in electrolyte excretion rate, generally paralleled the changes in urinary electrolyte concentration. (See fig. 58).

Plasma $[Na^+]$ fell slightly during the cortisol infusion possibly due to the increased loss of Na^+ in the urine.

c. Foetus 220 (145 days) (appendix table 67)

This foetus received the same dose of cortisol as the preceding foetus (240 µg in 4 hours; 20 µg/kg/hr.) and despite the difference in age, the changes in the $[Na^+]$ and $[K^+]$ of urine were similar. Again $[K^+]$ rose steadily, reaching a maximum concentration twice that of the pre-treatment average after about 3½ hours of cortisol infusion. The $[Na^+]$ of foetal urine also increased and continued to increase until the end of the experiment, by which time it was more than double the pre-treatment average. Urine flow rate decreased during the cortisol treatment and consequently the electrolyte excretion rates, which had begun to rise early in the treatment period, also declined. The remaining urinary solutes; creatinine and uric acid, showed irregular increases in concentration during treatment and both reached concentrations which were about twice their pre-treatment averages. (See fig. 59).

In this experiment the changes in the concentration of the plasma solutes were not consistent with those in the preceding experiments. Both $[Na^+]$ and $[K^+]$ increased slightly following cortisol treatment, while in contrast, creatinine concentration decreased.

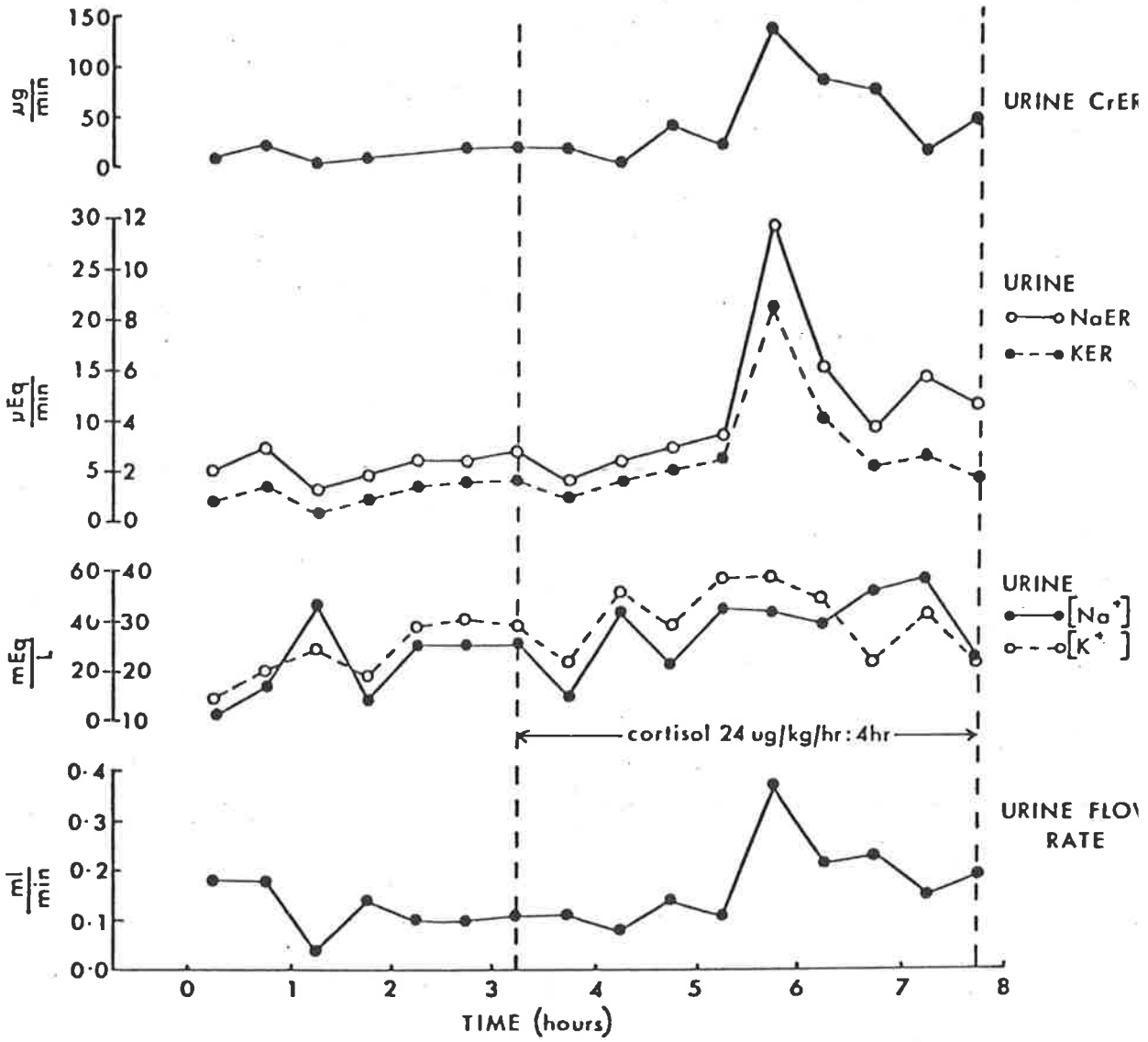

FIGURE 58. Changes in foetal urinary parameters following the infusion of cortisol into foetus 66-440 (137 days old).
(See appendix table 66 and text pages 146 and 147)

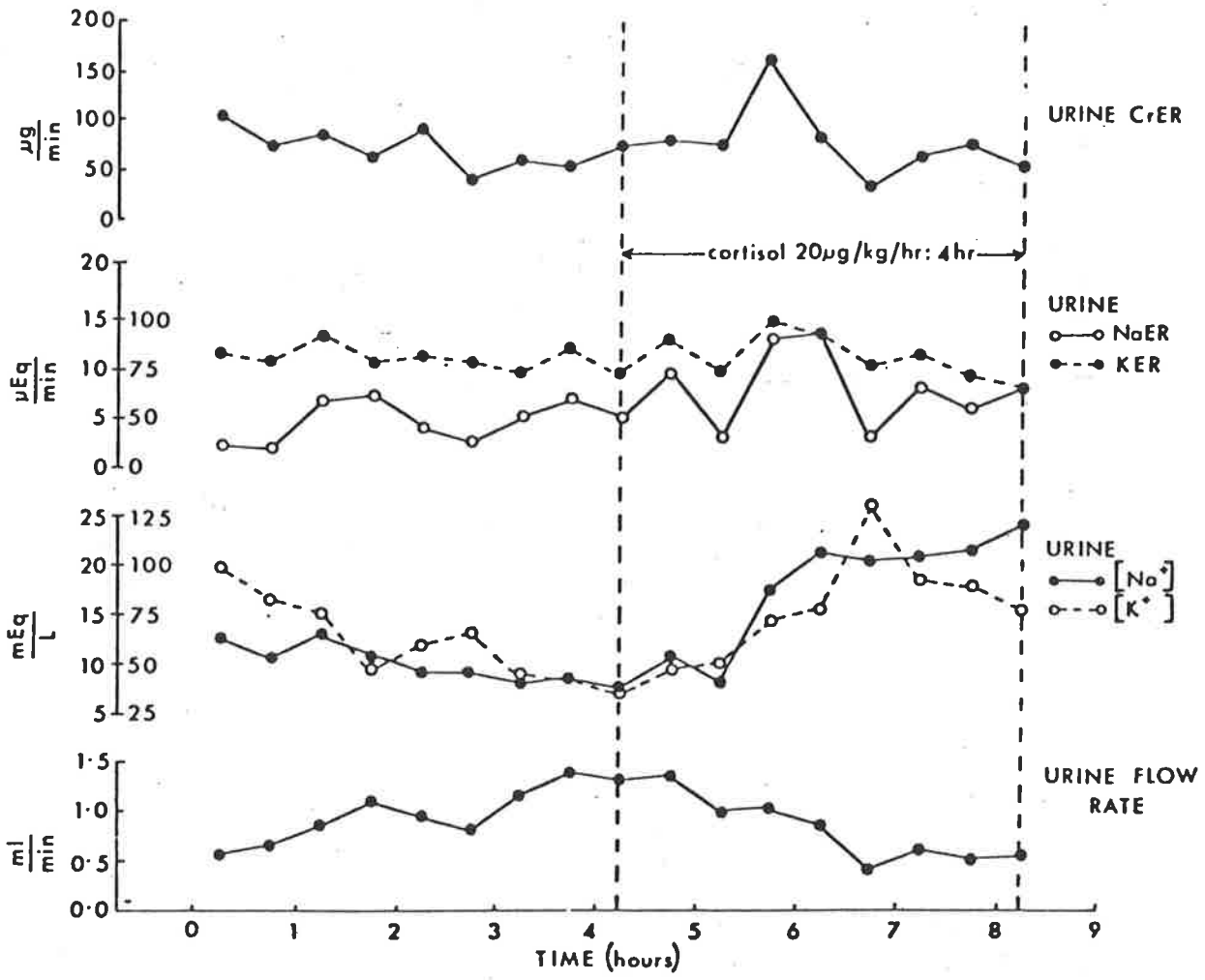

FIGURE 59. Changes in foetal urinary parameters during the infusion of cortisol into foetus 220 (145 days old).
(See appendix table 67 and text page 147)

d. Foetus 157 (148 days) (appendix table 68)

This foetus was near term and its urinary [Na^+], before treatment, was exceptionally high. This may have contributed to the unique response of the foetus. It can be seen in figure 60 that a sharp decrease in urinary [Na^+] and a lesser decrease in urinary [K^+] occurred within 1 hour of commencing treatment (20 µg/kg/hr. for 6 hours). However, about 3 hours after the infusion began, the concentration of both electrolytes increased, with [K^+] stabilising at values slightly above the pre-treatment average and [Na^+] still below its pre-treatment average. Generally, after the decline in urinary electrolyte concentration during the first hour of treatment, the changes in [Na^+] and [K^+] were similar to those observed in foetus 220. The concentration of uric acid and creatinine in foetal urine showed patterns of change similar to those of [K^+].

In the plasma, there was a general increase in [Na^+] and [K^+] as the experiment proceeded. But the fact that this trend was apparent in 2 successive control samples suggests that it was independent of the cortisol treatment.

In summary, it is evident that in 3 of the 4 experiments carried out, most of the urinary solutes analysed increased in concentration following cortisol treatment. This was especially true of urinary Na^+ and K^+ and of these, the most prolonged effect was on urinary [Na^+]. These changes usually began within an hour of commencing treatment and in the case of [K^+] reached a peak 3 to 4 hours after beginning treatment. Sodium concentration took longer to reach a peak and declined more slowly. The magnitude of the electrolyte increases compared with the control levels, were 70 - 100% for both Na^+ and K^+. There was no reliable indication of age or dose-dependence in the responses observed.

The changes in plasma electrolyte concentration were even less consistent than those for urine and no generalisations could be made. In the final experiment of this series, using a near-term foetus (157), the

urinary solutes increased in concentration only after an initial decrease during the first ½ hour of treatment. This unique response may have been related to the relatively high concentration of urinary solutes present during the control period.

5.5.2 Aldosterone

Two aldosterone experiments were carried out.

a. Foetus 223 (127 days) (appendix table 69)

In this experiment 625 µg of aldosterone was infused over 2½ hours (130 µg/kg/hr.) (see fig. 61). The most important result was the decrease in urinary [Na^+] (50%) which occurred during the first 2 hours of the aldosterone infusion. This was followed by a sudden return to pre-treatment Na^+ levels in the last ½ hour of the infusion and these levels were maintained for the rest of the experiment. Erratic changes in urine flow rate obscured trends in Na^+ excretion rate. From about 2 hours after the commencement of the infusion, there was a gradual increase in urinary [K^+] and K^+ excretion rate.

The concentration of the other urinary solutes, creatinine and uric acid, fell during the infusion period to levels which were about 50% of their pre-treatment levels. However, the control levels were re-established 2 hours after completion of the aldosterone infusion. Urine flow rate was variable, as usual, and it was impossible to infer any hormonal influence on urine flow rate or on the excretion rates derived from it. Similarly, there were no significant changes in the concentration of the plasma solutes.

b. Foetus 220 (128 days) (appendix table 70)

In this experiment the infusion rate was 460 µg/kg/hr. for 3½ hours (see fig. 62). Urine flow rate, which was surprisingly uniform before treatment, declined during the infusion period and continued to fall after the infusion was completed. Urinary [Na^+] and Na^+ excretion

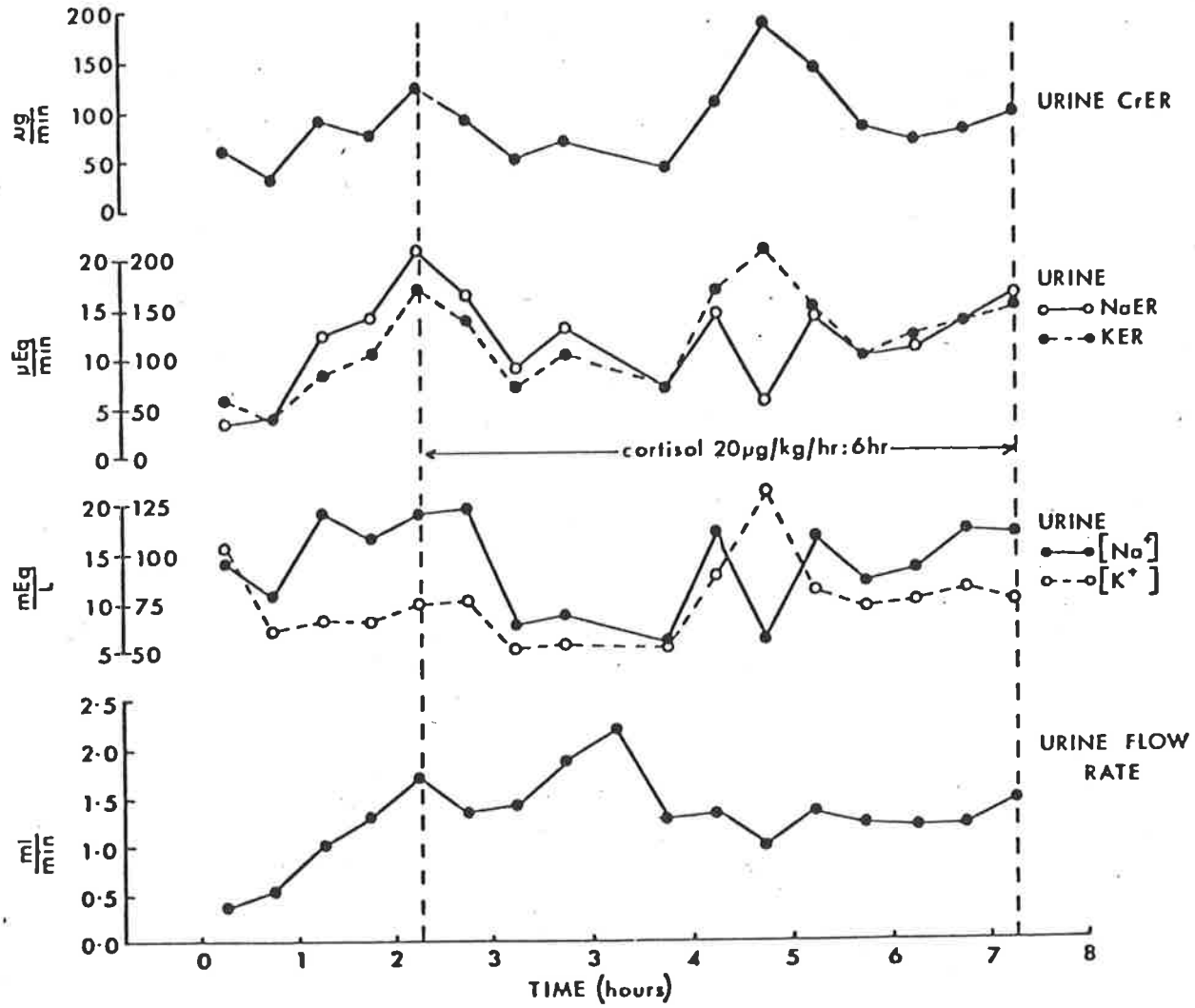

FIGURE 60. Changes in foetal urinary parameters during the infusion of cortisol into foetus 157 (148 days old).
(See appendix table 68 and text pages 148 and 149)

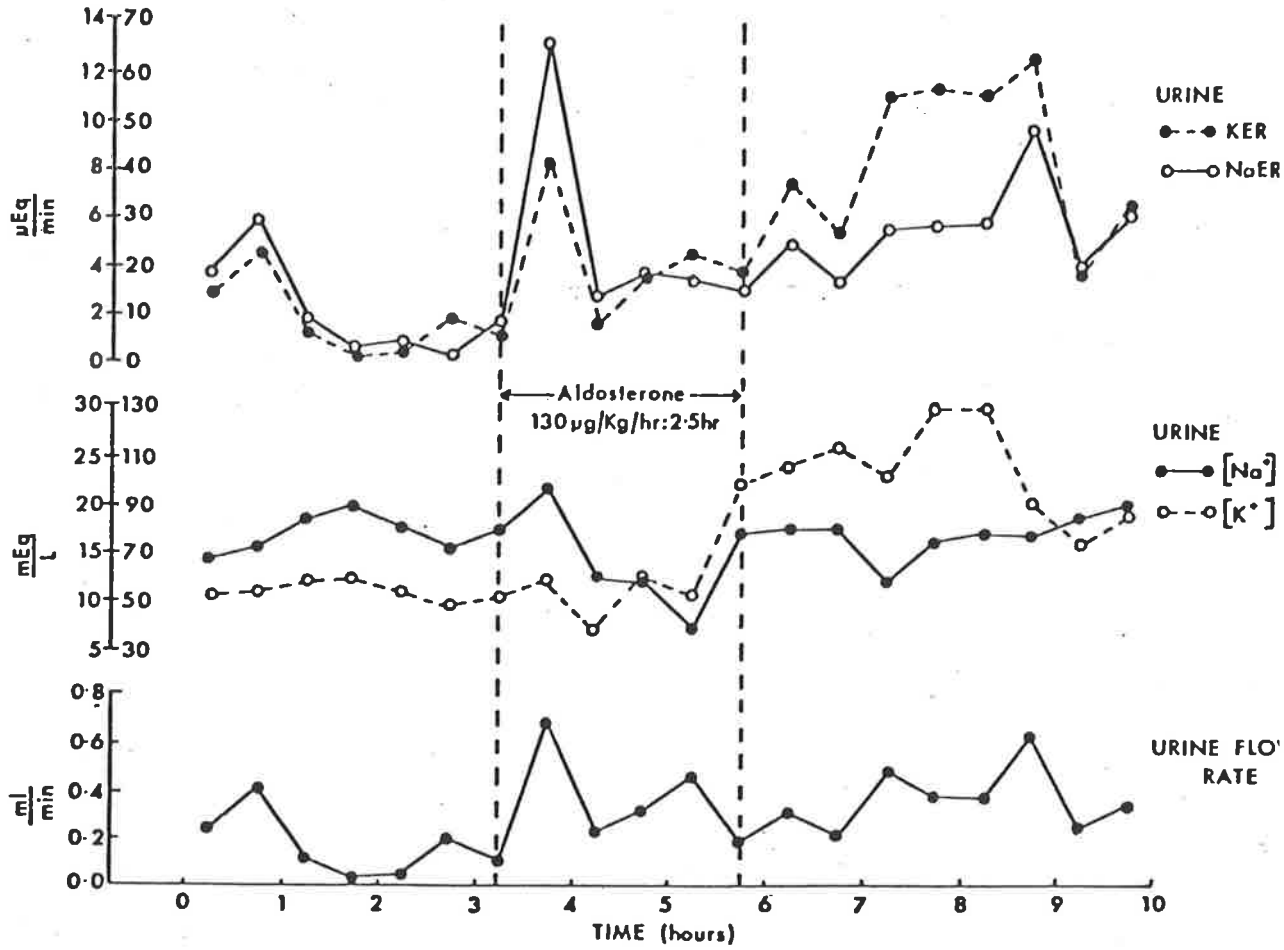

FIGURE 61. Changes in foetal urinary parameters during and after the infusion of aldosterone into foetus 223 (127 days old). (See appendix table 69 and text page 149)

rate also decreased but not until about 2 hours after the infusion commenced and the former returned to pre-treatment levels within 1 hour of completing the infusion. Potassium concentration began to rise when $[Na^+]$ fell and the rise in $[K^+]$ and K^+ excretion rate continued until the last hour of the experiment. The significance of the changes in $[Na^+]$ and Na^+ excretion rate is clouded by the fact that there was a downward trend in urinary $[Na^+]$ during the control period and this was reflected in the Na^+ excretion rate changes prior to treatment. In fact, however, after the first 30 minutes of the control period the pre-treatment Na^+ levels were relatively stable. In the case of urinary $[K^+]$ and K^+ excretion rate no such complication exists, for it appears that the hormone treatment has reversed the pre-treatment trends displayed by these parameters. In view of the probable existence of a Na^+ - K^+ exchange mechanism in the foetal nephron, the K^+ changes render the observed Na^+ changes more significant.

The concentrations of the remaining urinary solutes showed no significant changes, however urine pH decreased during treatment.

Four plasma samples were taken during this experiment and the $[Na^+]$ in the 2 collected after commencing the aldosterone treatment was lower than in the control plasmas. No reliable trends were evident for the concentrations of the remaining plasma solutes.

The results from these 2 experiments give some indication of an aldosterone influence on urine formation. In the first experiment the $[Na^+]$ results indicate that aldosterone caused Na^+ retention. The return to pre-treatment levels during the latter part of the infusion may reflect the intervention of other mechanisms which compensate for the continued administration of aldosterone. In the second experiment there was a decrease in both the $[Na^+]$ of foetal urine and Na^+ excretion rate. The increase in urinary $[K^+]$ in both experiments was possibly due to Na^+ - K^+ exchange in the renal tubules. Finally the urinary pH changes in both experiments, but particularly in the second, were consistent with increased Na^+ - H^+ exchange in the nephron.

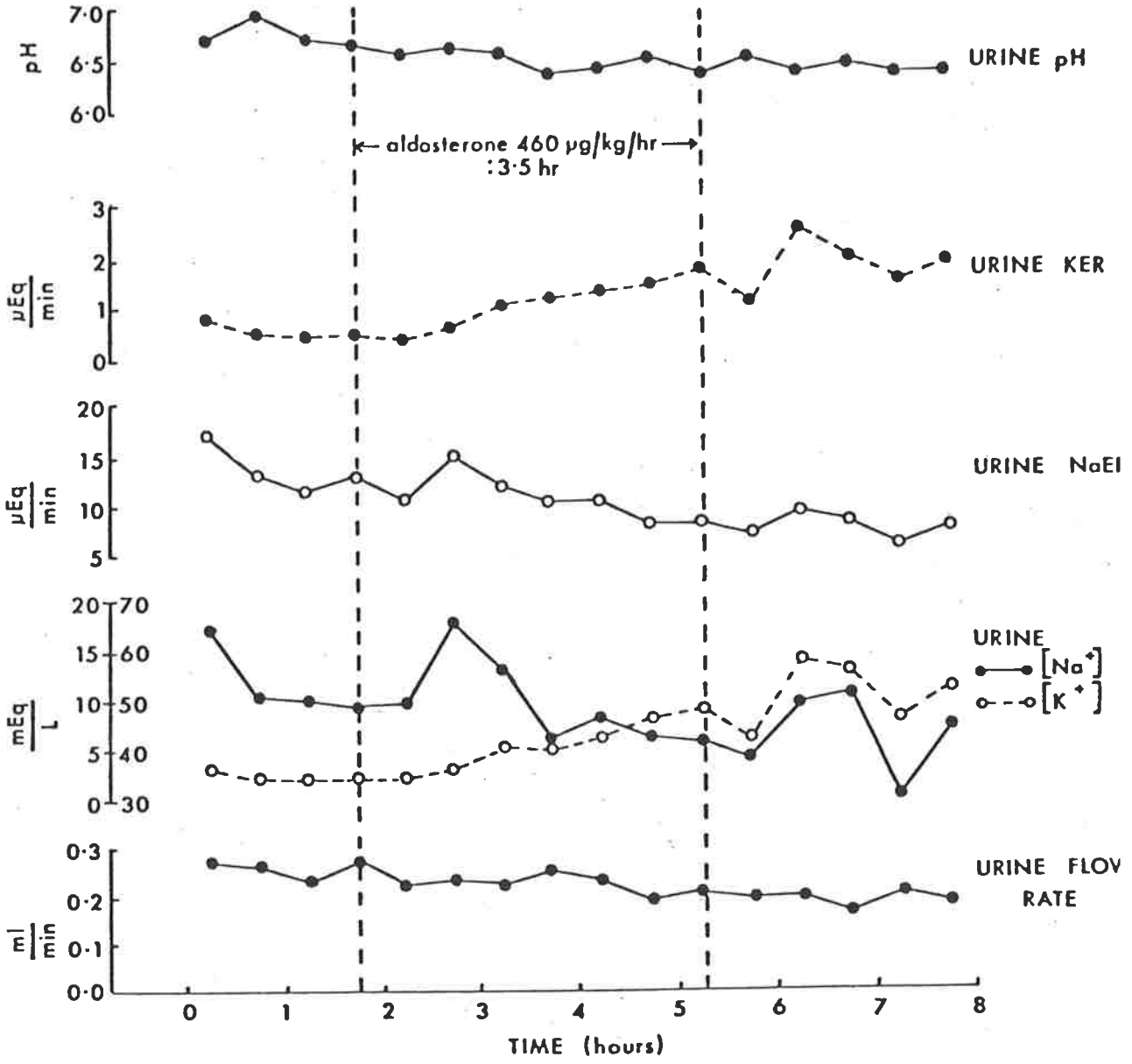

FIGURE 62. Changes in foetal urinary parameters during and after the infusion of aldosterone into foetus 220 (128 days old). (See appendix table 70 and text pages 149 and 150)

5.5.3 Progestins

a. Foetus 275 (111 days) (appendix table 71)

Because of the difficulty of solubilising progesterone, this hormone was not infused. Instead, 5mg of progesterone dissolved in 0.1ml of ethanol was injected every ½ hour and flushed in with 0.5ml of saline. In the control period 0.1ml of plain ethanol was injected every ½ hour and washed in with 0.5ml of saline.

During the progesterone treatment, the plasma progesterone concentration of the foetus increased from a pre-treament average of 4.3ng/ml (n = 3) to a maximum of 8.0ng/ml. Similarly the concentration of 17 α HP increased from a control average of 0.7ng/ml (n = 3) to a maximum of 1.9ng/ml. Despite these increases in the level of plasma progestins there was no evidence of any changes in urine composition that could be related to the hormone treatment. Urine flow rate and $[K^+]$ did decrease during the treatment period but these changes began before the progesterone was administered.

b. Foetus 275 (116 days) (appendix table 72)

In this experiment 4mg of 17 α HP was injected (as above) every ½ hour for 5 hours following a 3½ hour control period (total dose 40mg). During treatment the plasma concentration of 17 α HP increased from 0.3 to 0.5ng/ml although progesterone concentration did not increase. The plasma level of 20 α HP was variable but did show an overall increase.

Neither the individual injections of 17 α HP, nor the cumulative effect of the total 40mg of hormone produced a significant alteration of the concentration of any urinary or plasma solute.

In summary, although the number of experiments carried out using progestins is small, it does appear that at the doses used, progestins have no direct effect on foetal kidney function in foetuses aged between 110 and 115 days.

5.5.4 Dexamethasone Sodium Phosphate (Decasone - Ciba)

a. Foetus 220 (133 days) (appendix table 73)

Dexamethasone is a synthetic adreno-cortical steroid which in adult animals has a gluco-corticoid effect and a low mineralo-corticoid effect. In this experiment, 5mg of dexamethasone was infused over 2½ hours (0.4mg/kg/hr.) (see fig. 63). It should be noted that the dose of dexamethasone used in this and the following experiment was very large, since each represents a glucocorticoid potency that is approximately 1000 times that of the cortisol used in the preceding experiments. Although such doses are unphysiological in the foetal lamb, they were chosen in an attempt to ensure that ACTH secretion by the foetal pituitary would be inhibited and the secretion of endogenous mineralo-corticoids minimised.

During the infusion period, there was a small increase in K^+ excretion rate, but a more significant increase occurred in the 1½ hours after the infusion ceased. In humans, the duration of dexamethasone activity has been shown to be at least 4 hours so it is likely that dexamethasone was responsible for the observed increase in K^+ excretion. Urinary $[Na^+]$ showed a similar pattern to K^+ excretion rate since little change occurred during treatment but $[Na^+]$ increased 1½ times in the 90 minutes immediately after the infusion. Over the next hour control levels were re-established. Sodium excretion rate began to increase toward the end of the infusion period and continued to rise until 1½ hours after the infusion, at which point it was 4 times the pre-treatment average. The flow rate of foetal urine increased during the last hour of the infusion period reaching a maximum which was about 3 times the average pre-treatment flow rate. Immediately after the infusion period, flow rate began to fall and control values were re-established by the end of the experiment. The concentration of the urinary solutes, other than electrolytes, showed no reliable trends.

Despite the observed changes in K^+ excretion rate; the plasma concentration of K^+ (and Na^+) increased throughout the experiment. In contrast, the concentrations of plasma creatinine and uric acid fell during most of the experiment.

b. Foetus 242 (121 days) (appendix table 74)

On this occasion, a priming dose of 5mg (3mg/kg) of dexamethasone was injected and an additional 5mg was infused over 2 hours (0.8mg/kg/hr.). The response obtained was similar to that observed in the preceding experiment (see fig. 64). Again there was an increase in urine flow rate, particularly during the last 90 minutes of the infusion period. Hence the response occurred in a similar manner to that seen in the preceding experiment although the magnitude and duration of the flow rate increase was greater on this occasion, possibly due to the priming dose. The $[Na^+]$ and the $[K^+]$ of foetal urine increased following the infusion period and substantial increases in the rate of excretion of these electrolytes was again observed. In fact the sixfold increase in urine flow rate contributed to increases in the excretion rate of all the urinary solutes analysed.

The changes in plasma composition in this experiment did not correspond as closely with the preceding experiment as did the urinary changes. The $[Na^+]$ of plasma increased, but $[K^+]$, uric acid concentration and creatinine concentration declined overall. These changes in plasma solute concentration may have been related to the large increase in excretion rates. Also the rise in $[Na^+]$ may have been due to a maternal contribution of Na^+ to the foetus in response to the initial decline in foetal plasma $[Na^+]$.

5.5.5 Metyrapone (Metyrapone Ditartrate - Ciba)

a. Foetus 188 (124 days) (appendix table 75)

Metyrapone is an adreno-corticostatic agent that inhibits the synthesis of cortisol, corticosterone and aldosterone by blocking 11β--hydroxylase activity in the adrenal cortex of adults. The effect

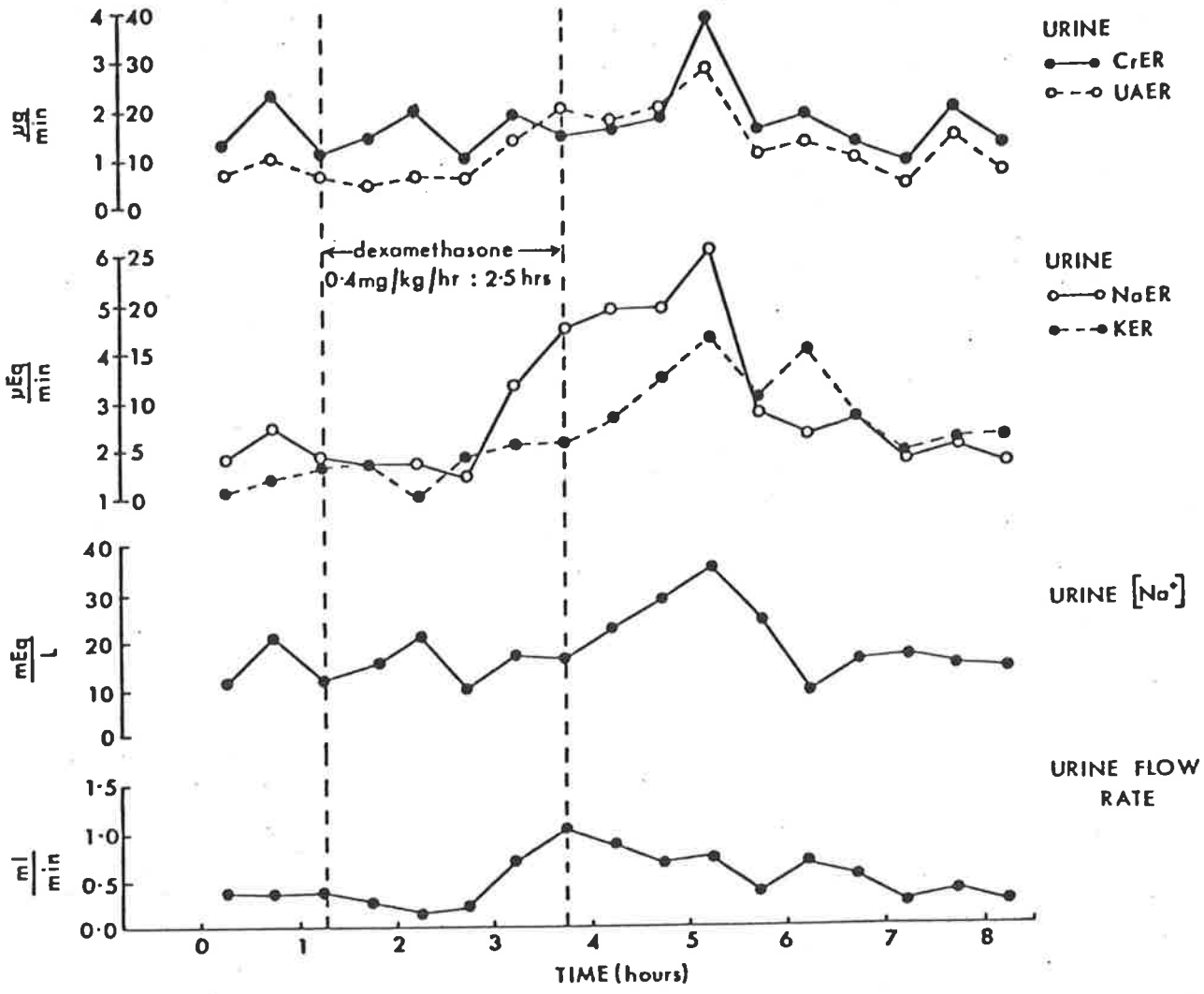

FIGURE 63. Changes in foetal urinary parameters during and after the infusion of dexamethasone sodium phosphate into foetus 220 (133 days old).

(See appendix table 73 and text pages 152 and 153)

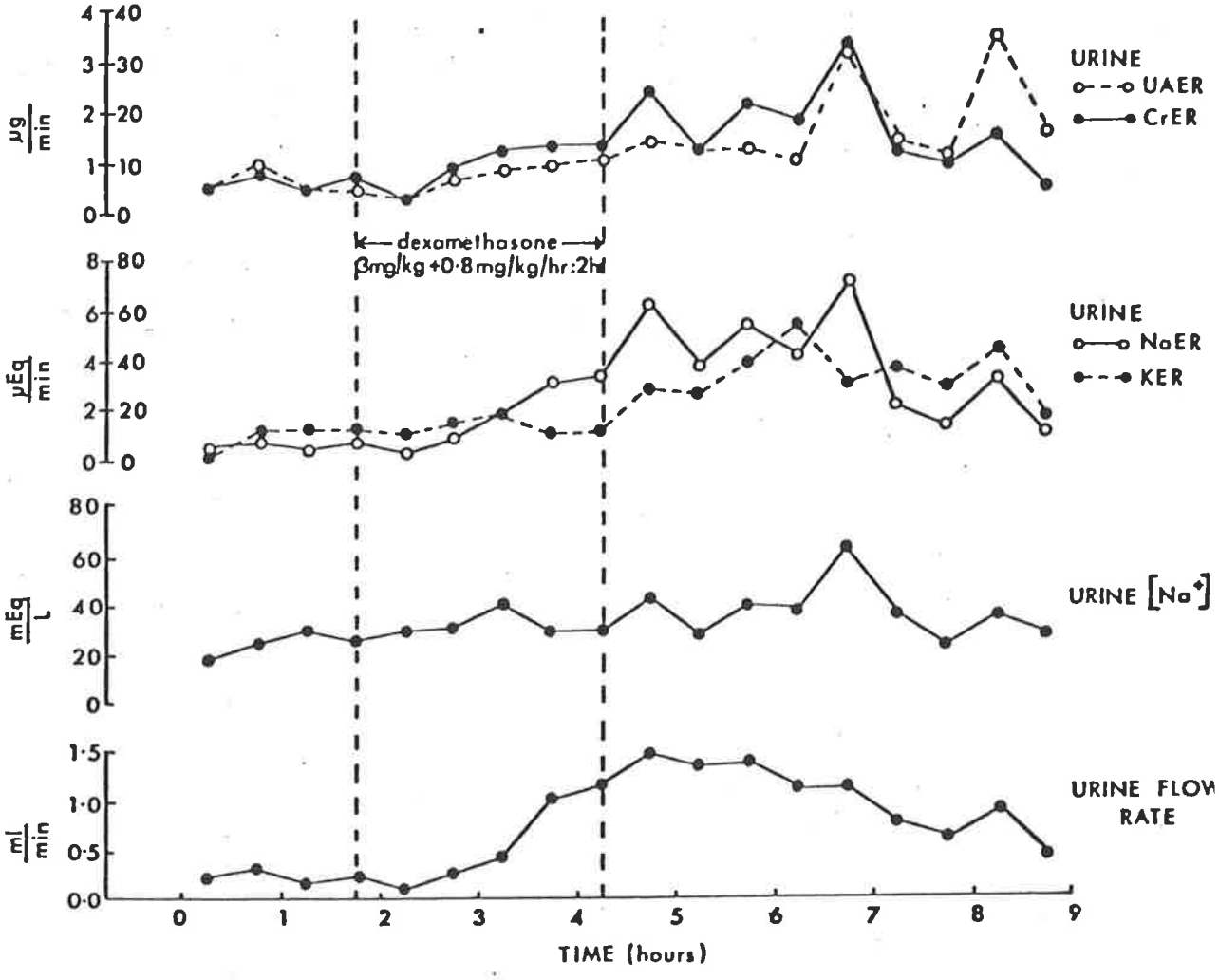

FIGURE 64. Changes in foetal urinary parameters during and after the infusion of dexamethasone sodium phosphate into foetus 242 (121 days old).

(See appendix table 74 and text page 153)

of this hormone in foetuses is of particular interest because of the conflicting opinions concerning the activity of 17α and 11β hydroxylases in foetal adrenals. It has been demonstrated in humans, that 1.0 to 2.0gm of metyrapone ditartrate infused over 4 hours produces its maximum effect within 8 hours of commencing the infusion. So in this experiment 44mg of metyrapone (24mg/kg) was injected intravenously as a priming dose then an additional 352mg infused over the following 8 hours (24mg/kg/hr.). One gm. of metrapone ditartrate administered intravenously corresponds to 0.44gm of metyrapone base and the doses quoted for this and the next experiment are in terms of metyrapone base.

The results of this experiment are shown in figure 65. It can be seen that although urine flow rate and urine [Na^+] showed some variation during the control period, there was a definite decline in both of these parameters during the metyrapone infusion. For the 5 urine samples collected immediately prior to commencing the infusion, the average urine flow rate was 1.69ml/min., while for the last 5 samples of the infusion period the average was 0.71ml/min. The corresponding averages for [Na^+] in these sets of samples were 50.2 and 31.9 mEq/L. There was no reliable trend in urinary [K^+] nor in the concentrations of creatinine and uric acid. Sodium excretion rate fell by about 80% during the infusion period due to the decline in flow rate and urinary [Na^+], while potassium excretion rate fell to a lesser extent due mainly to the flow rate changes. Urine pH increased from the beginning of the experiment, reaching a peak midway through the infusion period and declining thereafter. Since the rise in pH began before treatment, it is unlikely that it was due to the metyrapone treatment.

In the plasma there was an increase in [Na^+] during the metyrapone treatment while [K^+] and uric acid concentration decreased.

b. Foetus 250 (115 days) (appendix table 76)

In this experiment a priming dose of 44mg (34mg/kg) of metyrapone was again administered and the infusion rate was 34mg/kg/hr. for 20 hours.

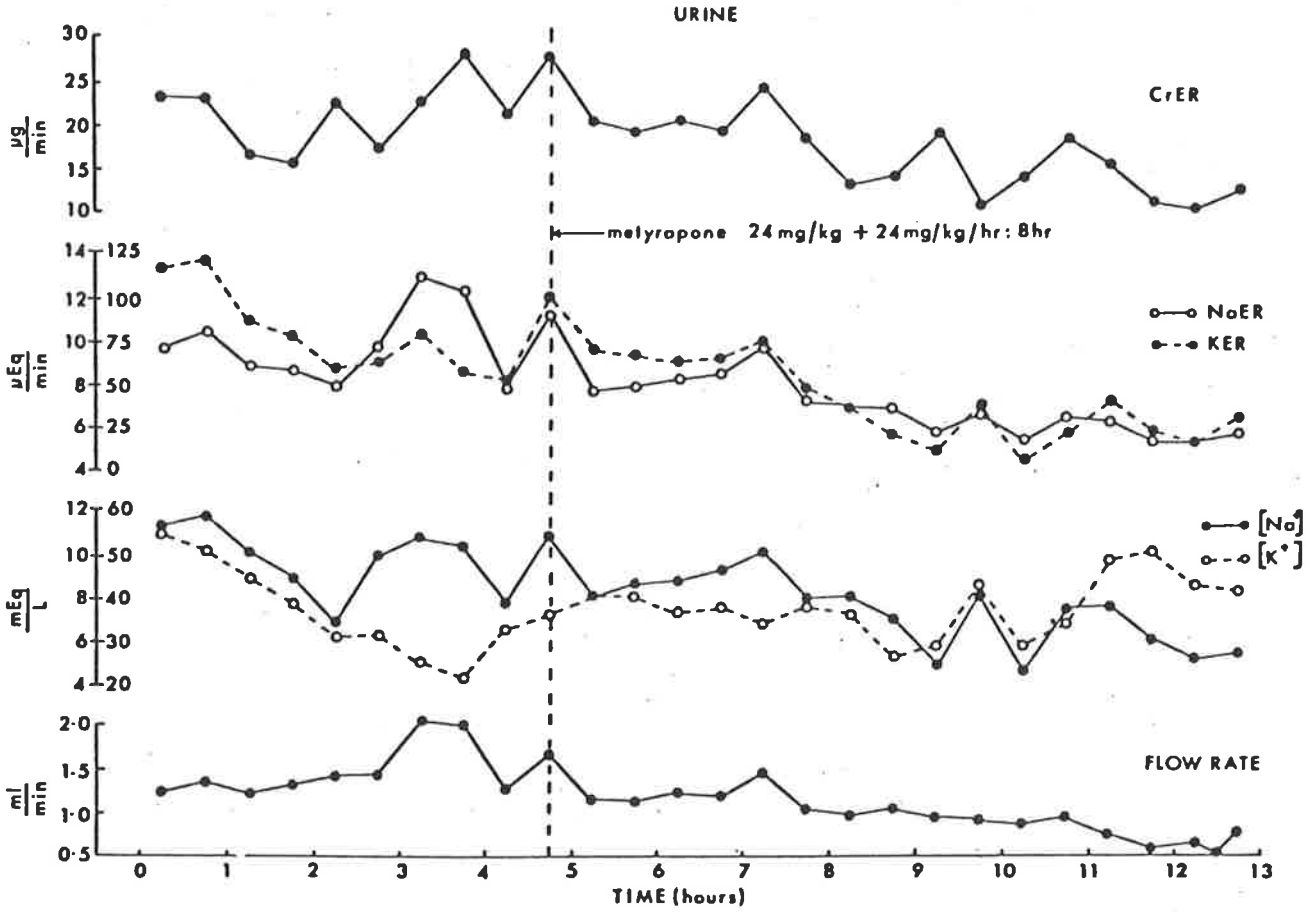

FIGURE 65. Changes in foetal urinary parameters during and after the infusion of metyrapone into foetus 188 (124 days old).
(See appendix table 75 and text pages 153 and 154)

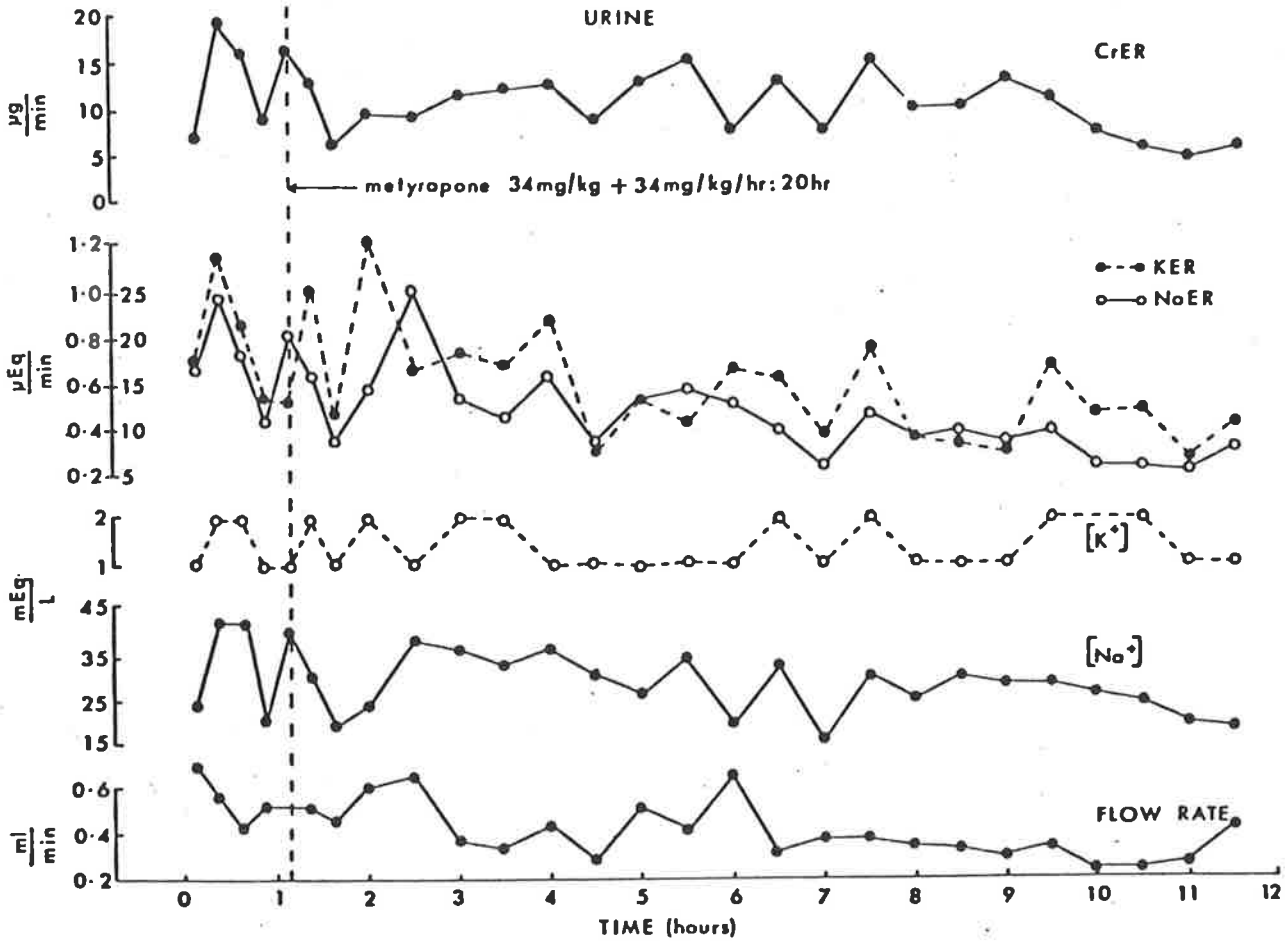

FIGURE 66. Changes in foetal urinary parameters during and after the infusion of metyrapone into foetus 250 (115 days old).
(See appendix table 76 and text pages 154 and 155)

No blood samples were taken from this foetus. It can be seen in figure 66 that again there was a decrease in urine flow rate and urine [Na^+]. The average flow rate during the control period was 0.55ml/min. and the average [Na^+] was 43.0 mEq/L. In comparison, during the final 3 hours of the experiment the averages for flow rate and [Na^+] were 0.30ml/min. and 24.17 mEq/L respectively. The [K^+] showed no regular trend although K^+ excretion rate fell during the infusion period. Similarly, Na^+ excretion rate fell by about 75% due to the parallel decreases in [Na^+] and flow rate. The remaining urinary parameters, creatinine concentration, uric acid concentration and pH showed no trends that could be related to the metyrapone treatment.

c. Foetus 69-825 (123 days) (appendix table 77)

In mature animals it has been demonstrated that the simultaneous administration of metyrapone plus a gluco-corticoid such as decasone can inhibit ACTH production and thereby inhibit the synthesis of 11-desoxy-corticoids. This in turn would result in diuresis and natriuresis. An experiment was conducted to see whether such a response could be induced in the foetus.

Priming doses of 44mg (26mg/kg) of metyrapone and 0.2mg of decasone (0.12mg/kg) were injected and over the next 8 hours the foetus was infused with metyrapone and decasone at the rates of 26mg/kg/hr. and 0.12mg/kg/hr. respectively. The [Na^+] of foetal urine was variable during the control period, however during treatment there was a definite increase in [Na^+] which reached a peak about 4 hours after beginning the infusion. The [Na^+] remained elevated for the next 2 hours and then returned to control levels. Urinary [K^+] showed a pattern of change which closely paralleled that of Na^+. At its peak [Na^+] was about 60% greater than the pre-treatment average, while the peak [K^+] was approximately 3 times the control average. The urine flow rate recorded during the first hour of the infusion period was lower than the control flow rate but thereafter flow rate increased to a maximum of 2.23ml/min. which was 93% greater than

the pre-treatment average. This increase in urine flow rate occurred more rapidly than the changes in urinary electrolyte concentration but the effect was not as prolonged. As a result of the overlapping increases in urine flow rate and $[Na^+]$ and $[K^+]$, the excretion rates of these electrolytes were elevated during the first ½ of the infusion period. Of the remaining urinary parameters, creatinine and uric acid concentrations do not appear to be effected by the hormone treatment, while pH showed a pattern of change which was independent of the treatment (see fig. 67).

No changes in plasma composition occurred that could be related to the hormone treatment.

In summary, experiments in which decasone and metyrapone were infused, either alone or in combination, yielded consistent results, particularly with respect to water and electrolyte excretion. Decasone not only induced diuresis, natriuresis and kaliuresis, but also the pattern of response was very similar despite differences in the age of the foetuses used and the dose of hormone infused although the hormone doses were very large in all cases; Metyrapone produced the reverse effect; it promoted Na^+ and water retention but it had no definite effect on urinary $[K^+]$

When these hormones were administered simultaneously, the expected natriuresis and diuresis was observed along with a lesser kaliuresis. However, none of these responses persisted beyond the middle of the 8 hour infusion period. In fact, the responses obtained resembled very closely those obtained when decasone was administered alone. The highest Na^+ and K^+ concentrations occurred about 4 hours after treatment began which corresponds with the responses obtained using decasone alone. One difference in the response of the foetus treated with both hormones was that the overall increase in urine flow was less than in the foetus which received only decasone. This may have been due to the high pre-treatment flow rates in foetus 69-825.

5.5.6 ACTH (Synacthen - Ciba) (appendix tables 78, 79 and 80)

Initially two experiments were conducted in which ACTH was

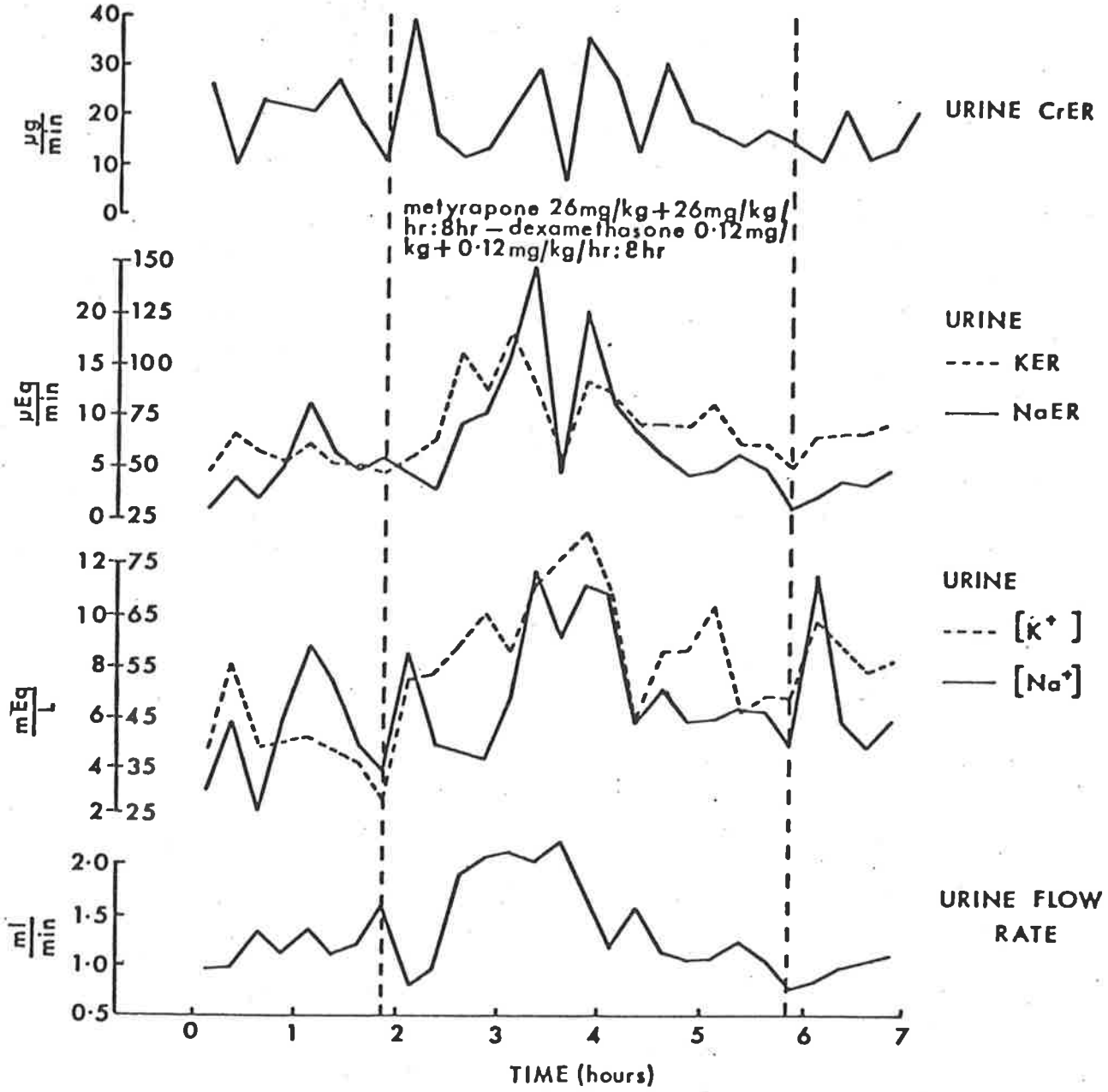

FIGURE 67. Changes in foetal urinary parameters during the simultaneous infusion of dexamethasone and metyrapone into foetus 69-825 (123 days old).

(See appendix table 77 and text pages 155 and 156)

infused. In the first experiment 130 µg of synacthen was infused over 8 hours (17 µg/kg/hr.) into a 121 day-old foetus (foetus 223) (see fig. 68). In the second experiment 154 µg was infused over 10 hours (10 µg/kg/hr.) into a 113 day-old foetus (foetus 219) (see fig. 69). Neither experiment yielded results which indicate that synacthen influences the concentration of Na^+, K^+, uric acid or creatinine in foetal urine. In fact, despite similar foetal ages and similar synacthen infusion rates, there was little consistency in the responses produced. This inconsistency was particularly obvious when the flow rates in the 2 experiments were compared. In the first experiment there was a 75% increase in flow rate between the control period and the final sample of the infusion period. In the second experiment there was an 84% decrease in flow rate over the same period. The two foetuses also showed converse trends in urine pH. With foetus 223 there was a small increase in urine pH during the experiment, while with foetus 219 the urine pH fluctuated in the early part of theinfusion period and then decreased. There does appear to be some relationship between increased creatinine and uric acid concentration and decreased urine pH. Finally in both experiments, the concentration of the plasma solutes were not significantly effected by the exogenous ACTH.

In view of these variable results a third experiment was carried out on a slightly older foetus (foetus 233, 124 days). This foetus was infused with 170 µg of ACTH over 12 hours (10 µg/kg/hr.). By the third hour of infusion both urine flow rate and urinary $[Na^+]$ had begun to rise. Flow rate continued to increase, in an irregular manner, until the final hour of treatment; however, $[Na^+]$ reached a peak after 9 hours of infusion. Sodium excretion rate exhibited a pattern of change similar to that of $[Na^+]$ while urine pH followed a trend which paralleled that of flow rate. The concentration and excretion rates of the remaining urinary solutes showed changes that paralleled the changes in flow rate (see fig. 70).

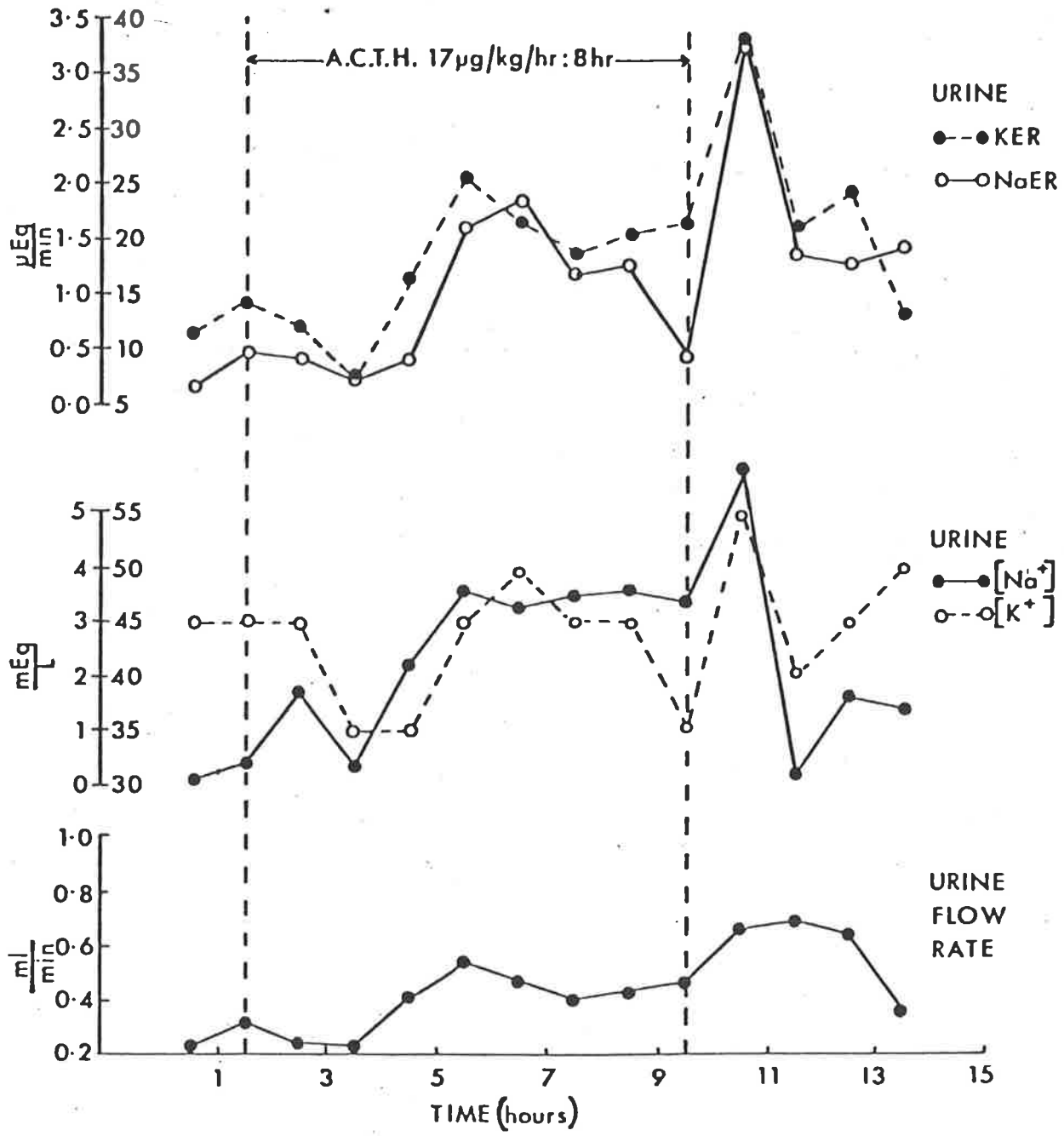

FIGURE 68 Changes in foetal urinary parameters during and after the infusion of ACTH into foetus 223 (121 days old).
(See appendix table 78 and text page 157)

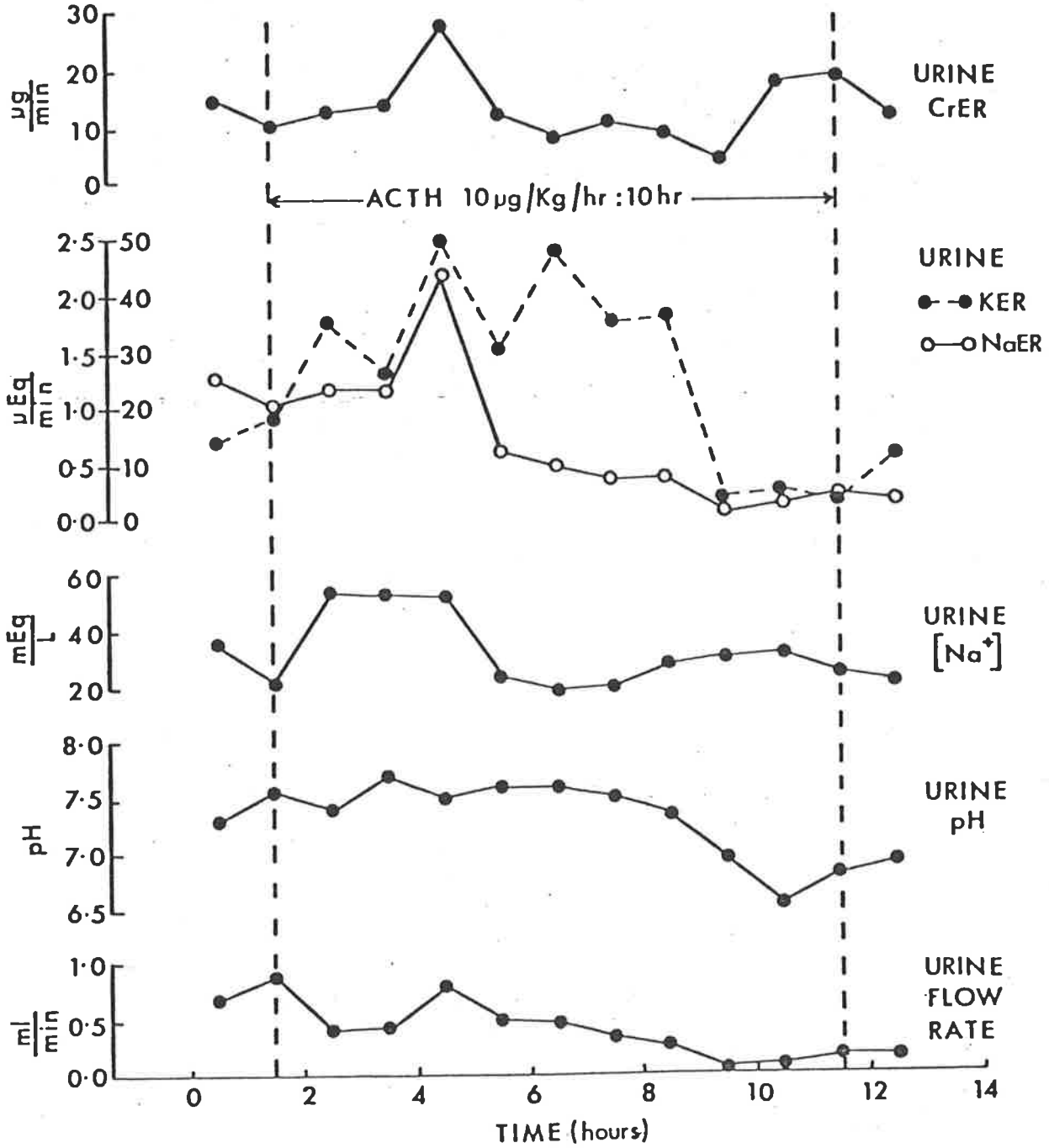

FIGURE 69. Changes in foetal urinary parameters during the infusion of ACTH into foetus 219 (113 days old).
(See appendix table 79 and text page 157)

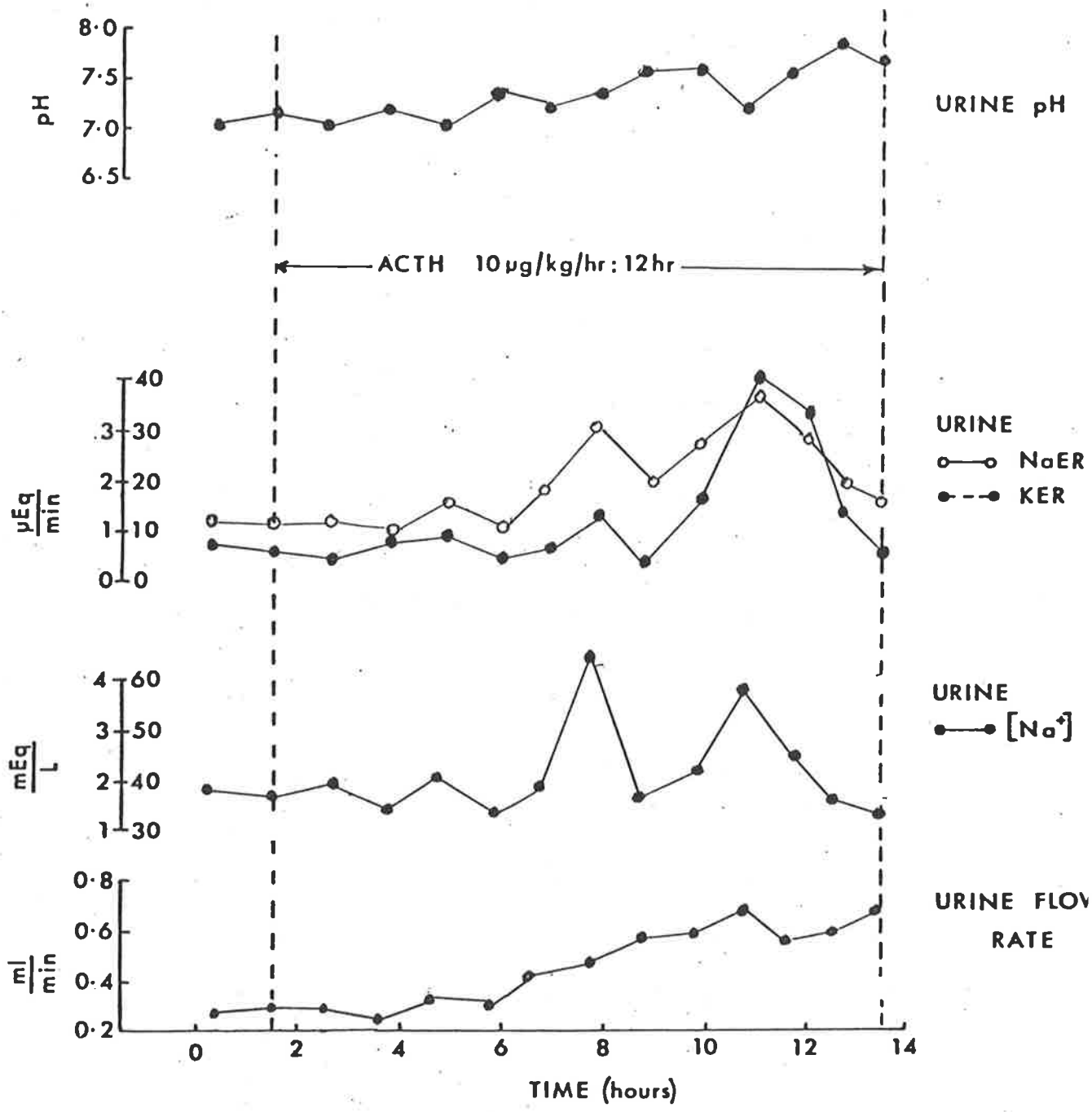

FIGURE 70. Changes in foetal urinary parameters during the infusion of ACTH into foetus 233 (124 days old).
(See appendix table 80 and text page 157)

5.5.7 ADH (Pitressin - Parke Davis) (appendix tables 81 and 82)

To test the responsiveness of the foetal kidney to ADH it was infused into two foetuses aged 114 (69-444) and 124 days (230). The infusion rates were 96 mU/kg/hr. for 2 hours and 110 mU/kg/hr. for 5½ hours respectively. On both occasions ADH produced immediate and substantial increases in the mean arterial blood pressure (MAP) of the foetus. In foetus 69-444, the MAP immediately before treatment was 50mm Hg and it increased to 62mm Hg within five minutes of commencing the ADH infusion. The maximum MAP during theinfusion period was 69mm Hg. In foetus 230 the blood pressure response was similar. From an average of 41.5mm Hg in the control period, the MAP increased to 49mm Hg within five minutes of commencing the ADH infusion and fluctuated between 48.5 and 51mm Hg during the rest of the infusion period. Thus the ability of foetal sheep to respond to the pressor activity of ADH, as early as the 114th day of gestation, was established. However, as will be seen, ADH had no consistent effect on foetal urine volume or urine composition.

In the experiment carried out on foetus 69-444 (114 days) the pre-treatment urine flow rates were high but variable (see fig. 71). The average pre-treatment flow rate was 1.36ml/min., yet within one hour of commencing the ADH infusion the flow rate had fallen to 0.37ml/min. Urine flow rate remained low throughout the infusion period and although it rose slightly when the treatment was completed, it did not return to control levels. The $[Na^+]$ of foetal urine increased by about 25% during the ADH treatment probably because of the reduced urine volume rather than additional Na^+ excretion. This also appears to be true of urinary K^+ and creatinine, as both solutes showed rises in concentration that were inversely related to urine flow rate.

The plasma samples collected during the period of reduced urine flow all showed reduced concentrations of Na^+, K^+ and uric acid. This may have been due to water retention, although there was no comparable change in haematocrit.

Although the first experiment did suggest that the foetal kidney was responsive to ADH, this was not confirmed in the second experiment. In the second experiment the pre-treatment flow rates were relatively low (average 0.48ml/min.). On commencing treatment, there was a decrease in flow rate during the first hour, but thereafter it increased steadily to a maximum of 1.02ml/min. recorded between 2½ and 3 hours after the ADH infusion began. Over the next hour the flow rate returned to control levels. The $[Na^+]$ and $[K^+]$ of foetal urine increased within two hours of beginning treatment, and reached levels 4 and 6 times the respective pre-treatment averages. Both then decreased erratically toward control levels. The concentrations of creatinine and uric acid showed similar patterns of change, while urine pH increased from an average of 6.6 before treatment to 7.5 midway through the treatment period and then fell toward control levels. (See fig. 72).

The $[Na^+]$ of foetal plasma showed an overall decline similar to that observed in the preceding experiment, but no reliable trends in concentration were noted for the other plasma solutes analysed.

In summary, foetus 69-444 appeared to show an anti-diuretic response following ADH administration; yet a foetus which was 10 days older gave almost the converse response. Although the second foetus received a higher total dose of ADH, the infusion rates in terms of foetal body weight were similar and accordingly, the dichotomy in the results is more difficult to explain. On the basis of these experiments, no conclusions can be drawn regarding the effect of ADH on perinatal kidney function, despite the presence of a definite pressor response.

5.5.8 Angiotensin II (Hypertensin - Ciba) (appendix tables 83, 84 and 85).

Initially two foetuses were treated with angiotensin II. The first, foetus 219 (116 days), was infused at the rate of 22 µg/kg/hr. for five hours, while the second, foetus 69-495 (140 days) received 14 µg/kg/hr. for four hours. Both foetuses showed immediate rises in MAP.

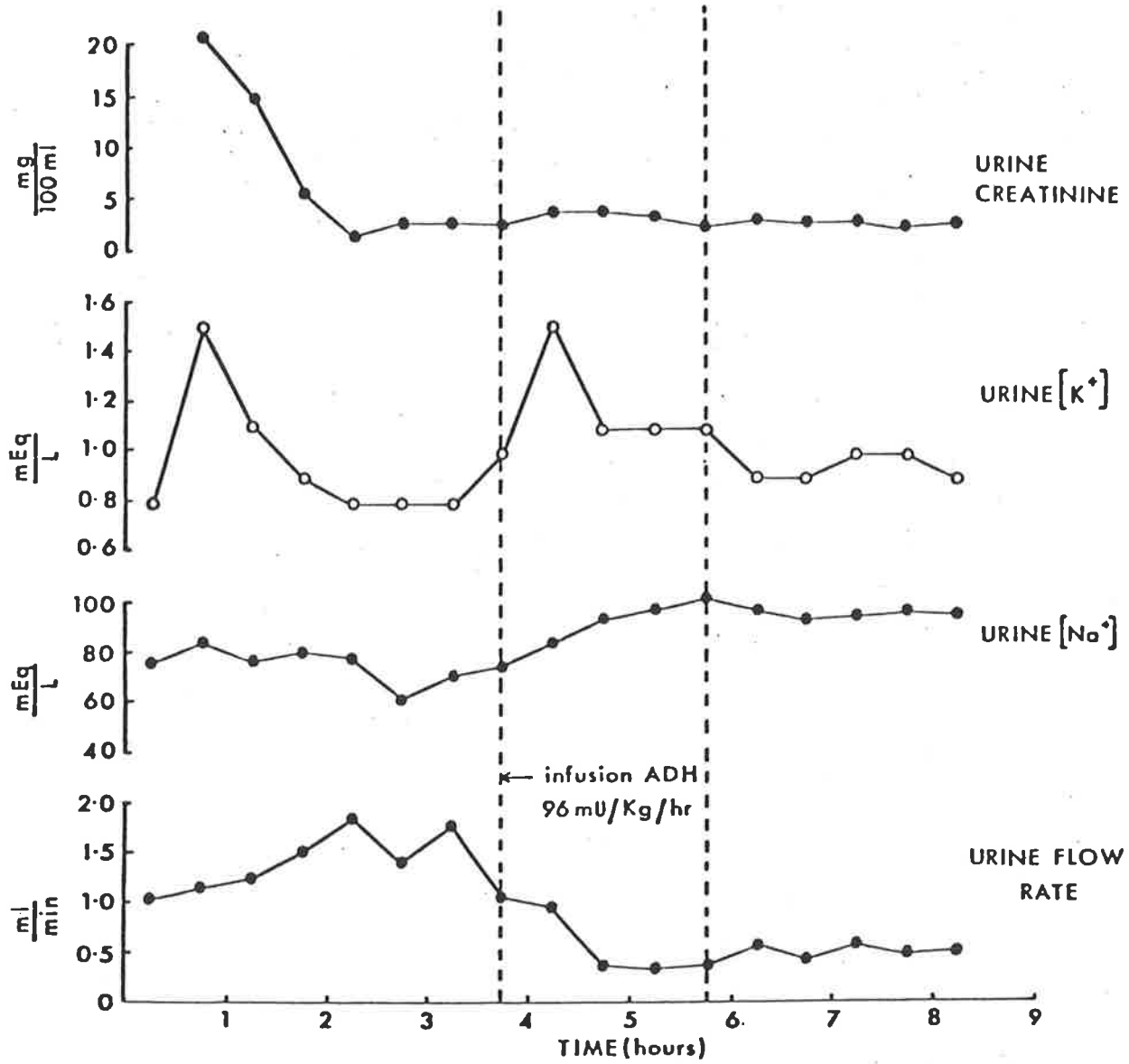

FIGURE 71. Changes in foetal urinary parameters during and after the infusion of ADH (vasopressin) into foetus 69-444 (114 days old). (See appendix table 81 and text pages 158 and 159)

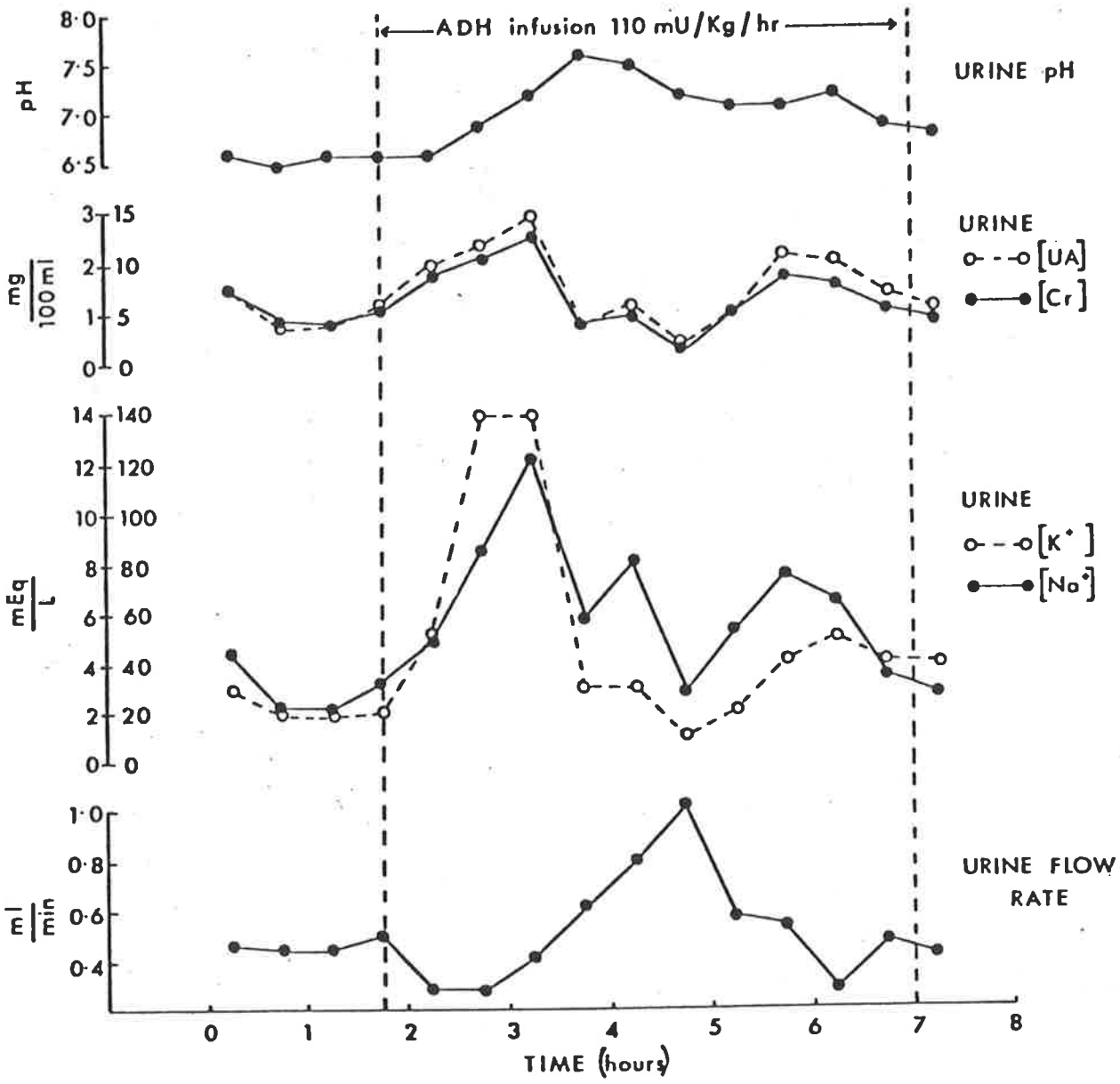

FIGURE 72. Changes in foetal urinary parameters during the infusion of ADH (vasopressin) into foetus 230 (124 days old).
(See appendix table 82 and text pages 158 and 159)

In foetus 219, the MAP rose from 32 to 41mm Hg within five minutes of commencing the infusion and remained near that pressure throughout the infusion period. The MAP fell to 34 mm Hg within five minutes of stopping the angiotensin treatment. Foetus 69-495 showed a similar response, with the MAP rising from 33.5 to 43.5mm Hg in the first five minutes of treatment and falling from 46.5 to 33.0mm Hg in the five minutes following the end of the infusion.

Despite the obvious sensitivity of foetal sheep to the pressor activity of angiotensin II, no consistent effect on water or electrolyte excretion was observed. (See figs. 73 and 74).

In both experiments there was a decrease in urine flow rate during the first hour of the angiotensin infusion, after which it returned to control levels or above. It is possible that the initial decrease in flow rate was due to constriction of the afferent arterioles and that subsequent re-adjustment within the kidney enabled the flow rate to recover. Alternatively, the initial drop in flow rate may have been due to water retention following an aldosterone induced increase in Na^+ reabsorption. The latter possibility is unlikely since the fall in urine flow rate occurred within 30 minutes in both foetuses and, as seen, aldosterone activity has a latent period of approximately one hour.

Following these parallel changes, the trends in urine flow rate, $[Na^+]$ and Na^+ excretion rate in the two foetuses were quite different. In foetus 219, urinary $[Na^+]$ fluctuated widely during the infusion period as did the electrolyte excretion rates. In contrast, foetus 69-445 maintained a relatively constant urinary Na^+ level, but Na^+ and K^+ excretion rates increased as urine flow rate increased. The remaining urinary solutes, in both foetuses, showed patterns of change, which appeared to be due to fluctuations in water excretion, since the concentrations of uric acid and creatinine increased when urine flow decreased and vice versa. Finally, neither urine pH, nor the concentration of any of the plasma solutes, showed changes that could reliably be related to the angiotensin treatment.

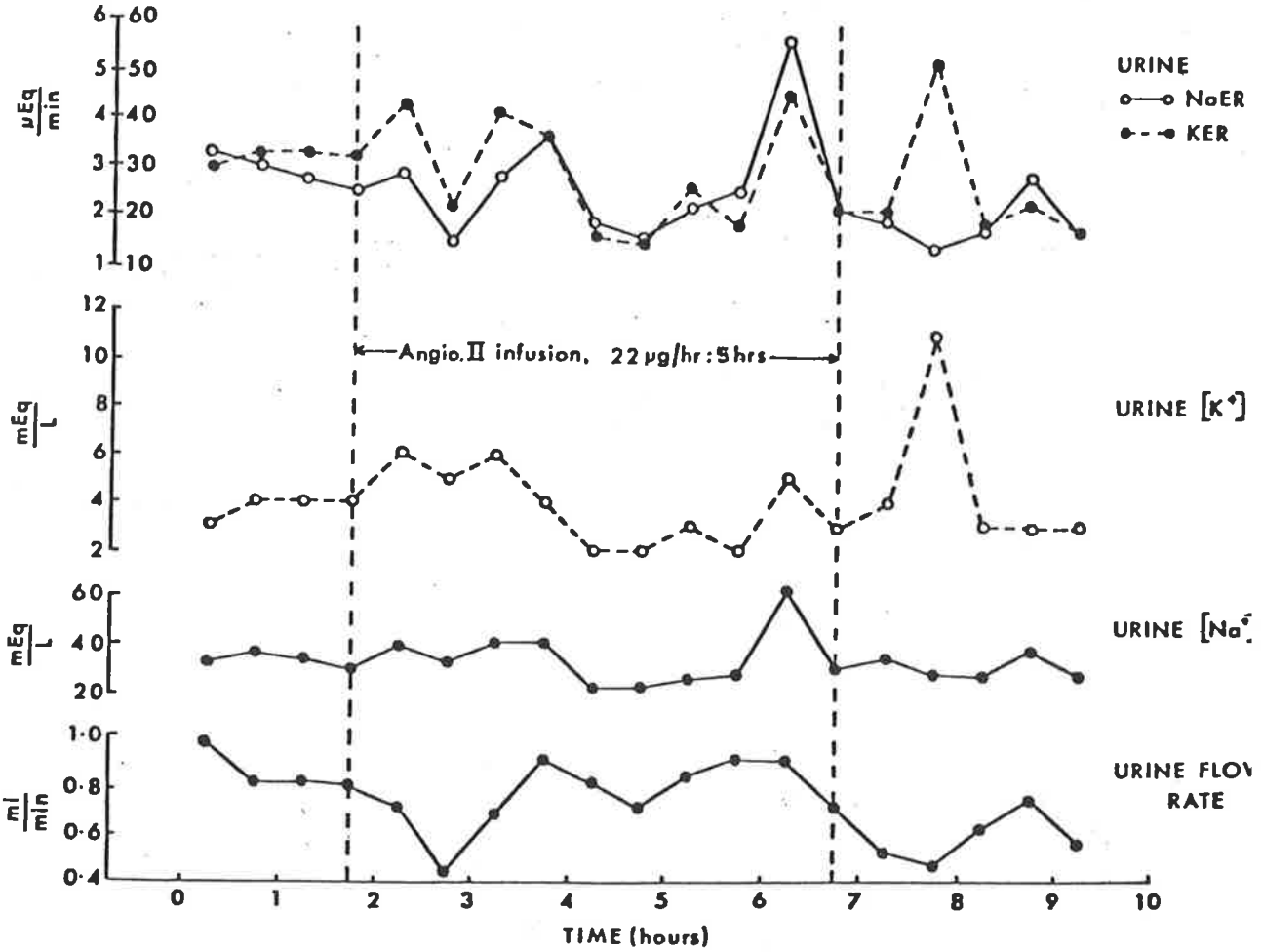

FIGURE 73. Changes in foetal urinary parameters during and after the infusion of angiotensin II into foetus 219 (116 days old). (See appendix table 83 and text pages 159 and 160)

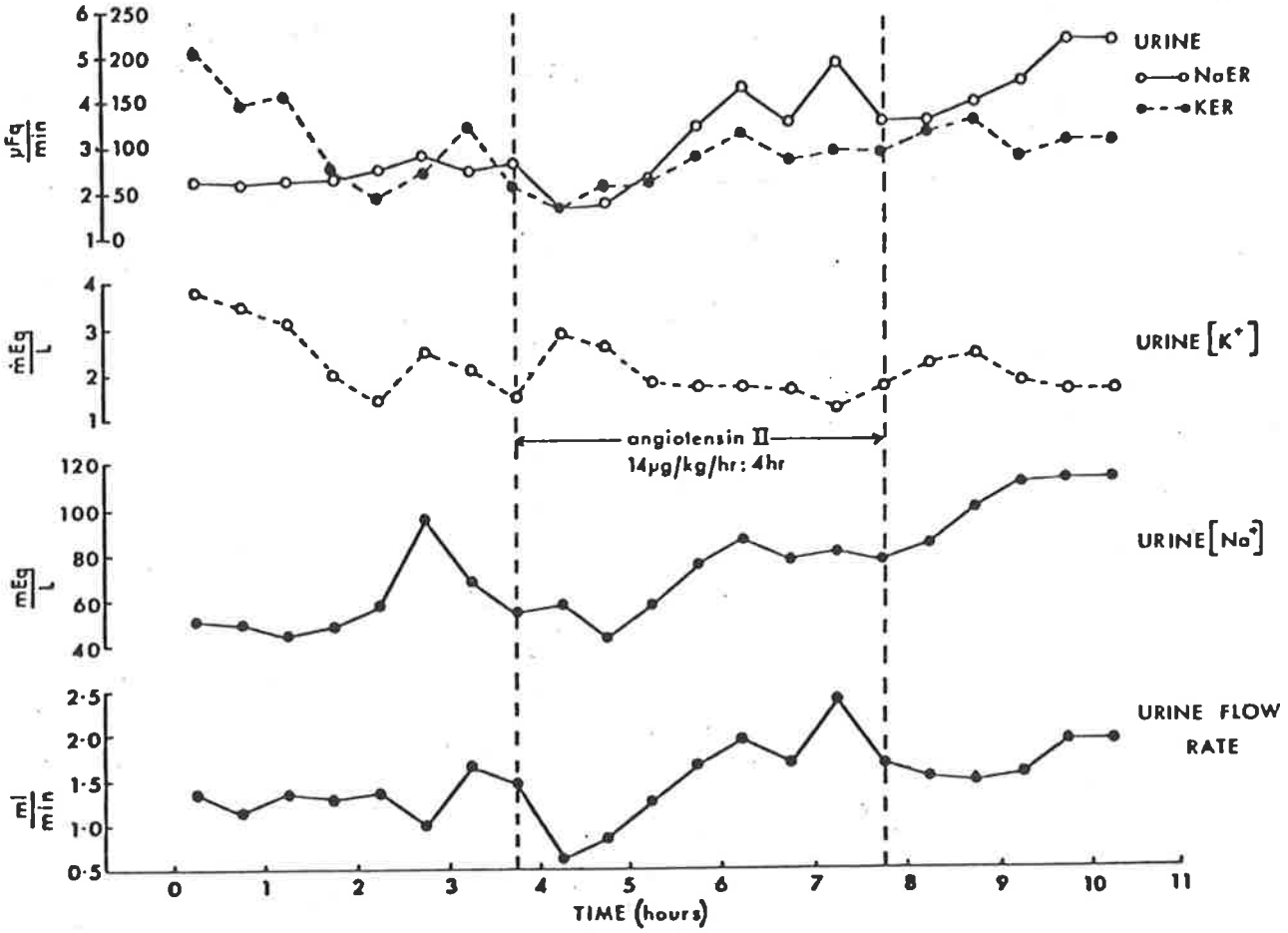

FIGURE 74. Changes in foetal urinary parameters during and after the infusion of angiotensin II into foetus 69-495 (140 days old). (See appendix table 84 and text pages 159 and 160)

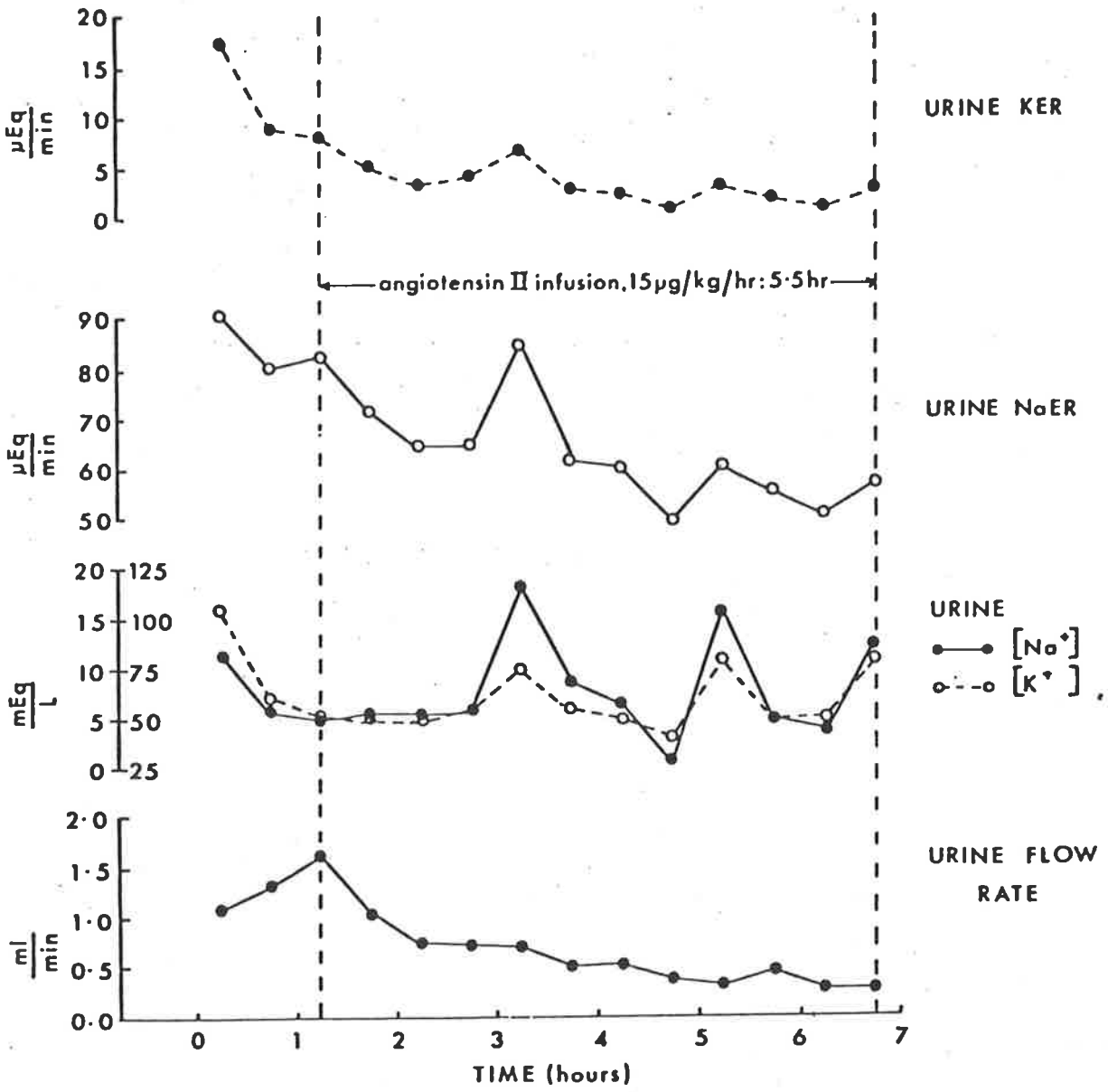

FIGURE 75. Changes in foetal urinary parameters during the infusion of angiotensin II into foetus 223 (129 days old).
(See appendix table 85 and text page 160)

In summary, both foetuses showed some fluctuation in urine output and composition that could be interpreted as being due to the angiotensin treatment. Since this is particularly true of the older foetus, sensitivity to angiotensin may be related to foetal maturity. However, the variation of all parameters during the control period of the experiment carried out on foetus 69-495, plus the lack of consistency between the results of the two experiments precludes the formulation of reliable conclusions.

In view of these difficulties a third experiment was carried out on a 129 day-old foetus (foetus 223) which was infused with angiotensin at the rate of 15 µg/kg/hr. for 5½ hours. Again there was an initial decline in urine flow rate, but on this occasion the decrease in flow rate continued throughout the infusion period. The $[Na^+]$ and $[K^+]$ of foetal urine varied considerably during infusion, but the changes could not be related to the angiotensin treatment. However, the electrolyte excretion rates fell progressivley during treatment because of the decrease in urine flow rate. (See fig. 75). Therefore the response to angiotensin II infusion by this foetus was different to that exhibited by the other foetuses and overall the confusion was compounded rather than resolved by this experiment.

6. *DISCUSSION AND CONCLUSIONS*

6.1 *Glomerular filtration*

It has been established in the present work, that GFR in foetal lambs increases during the last third of gestation. In exploring the reasons for this rise, factors which are known to influence GFR in adults have been considered. These factors can be summarised algerbraically as follows:

$$GFR = Kp \quad ([Pb - Pc] \quad - \quad \pi b)$$

where Pb is the hydrostatic pressure within the glomerular capillaries, Pc the hydrostatic pressure in the glomerular capsule, πb the osmotic pressure attributable to the plasma proteins and Kp a filtration coefficient incorporating factors such as glomerular surface area and glomerular permeability. The gross anatomy and fine structure of the foetal kidneys would obviously be involved in Kp and the first consideration was to assess the relationship between GFR and kidney size. However only a small fraction of the GFR - foetalage correlation, was lost when the values for GFR were standardised for kidney weight. Accordingly GFR was assumed to be largely independent of kidney weight and other anatomical factors were considered. Glomerular size did not vary significantly in foetuses aged between 119 and 145 days, but the functional characteristics of the glomerulii, such as internal surface area and permeability, may have been less uniform. No quantitative information concerning these possibilities can be advanced, but qualitative observations of glomerular ultra-structure give no indication that these factors are relevant. Glomerular permeability would be a function of the permeability of the endothelium of the glomerular capillaries, the permeability of the capsular epithelium and the permeability of the basement membrane which is interposed between these

barriers. Endothelial permeability would be related to the relative amount of thin endothelium and the number of fenestrae it contains. The diameter of the endothelial fenestrae was found to be constant during the last 30 days of pregnancy and there appeared to be no significant change in the number of fenestrae per unit length of thin endothelium nor in the proportion of thick to thin endothelium. Secondly the permeability of the epithelial membrane would, in part, be a function of the number of epithelial cell foot-processes and the width of the filtration slits between these processes. There was however no evidence that these factors change during the final month of gestation. Thirdly there was no apparent change in the width or density of the basement membrane.

From a functional point of view, it was found that high molecular-weight proteins such as albumin and haemoglobin, which are present in foetal plasma were not present in significant amounts in foetal urine. According to Pappenheimer et al (1951) this indicates that pores approximately 100Å in diameter exist in the filtration barrier and that these pores do not increase in diameter as gestation proceeds. This proposal is consistent with the ultra - structural observations of foetal glomerulii. However, functional studies in human infants, led Arturson et al (1971) to conclude that there is an increase in the diameter of the glomerular pores as the infant matures. Since this post-natal enlargement would presumably be a continuation of enlargement during foetal life, these results conflict with those of the present work.

Although the anatomical features of foetal glomerulii appear to be constant during the last 30 days of pregnancy, the total intra-renal surface area, available for filtration, would increase if there was an increase in the number of glomerulii per unit volume of cortical tissue.

To assess this possibility estimates were made of the number of glomerulii per μ^2 of cortical tissue (as seen in section) and of the proliferation of cortical tissue in relation to overall kidney growth. There was in fact a small reduction in the number of glomerulii per μ^2 of cortical tissue as gestation proceeded, indicating growth of the tubular elements of kidney tissue. However this reduction was more than compensated for by the growth of the renal cortex, so there would have been a greater number of functioning glomerulii in the older foetuses. This increase would be responsible for that fraction of the GFR - foetal age correlation that was found to be associated with kidney size. Nevertheless there remains a substantial fraction of the correlation that cannot be accounted for by the anatomical observations.

Obviously these anatomical studies yielded limited information. Glomerular permeability would depend not only on the structural characteristics of the filtration barriers, but also on the physiological and biochemical processes occurring within the membranes and cellular elements that constitute these barriers. Such mechanisms and their involvement in molecular transport are poorly understood and whether or not, they develop progressively during foetal or neonatal life is unknown. Secondly, GFR would rise if there was a progressive increase in the area of the renal capillary bed that was exposed for filtration. There is evidence in lower vertebrates that glomerular filtration is regulated by varying the proportion of glomerulii involved in filtration (Foster, 1938; Richards and Schmidt 1924). However in mammals, the evidence suggests that under normal circumstances all glomerulii are continually active. In the present work the glomerular capillaries of all foetuses contained erythrocytes irrespective of foetal age which suggests that all glomerulii were

functional. Nevertheless it cannot be concluded that the rate of filtration by each glomerulus was uniform since haemodynamic factors such as vascular resistance can vary from glomerulus to glomerulus.

In adults a major factor in determining GFR is intracapillary hydrostatic pressure which is directly related to arterial blood pressure and inversely related to the resistance offered by the afferent arterioles. In newborn dogs a good relationship has been found between blood pressure and the maturation of GFR. In the present work the MAP of foetal lambs increased with increasing foetal age and MAP and GFR were highly correlated. However from the 128th day of gestation until term, there was a 61% rise in GFR and only a 28% rise in MAP; yet together with an estimated 25% increase in the number of functioning glomerulii these changes could account for the rise in GFR. The influence of MAP on the pressure within the glomerular capillaries would be amplified if there was a fall in renal vascular resistance. In the present study no evidence was obtained that vascular resistance in the foetal kidney decreased during gestation but in piglets, renal maturation has been found to involve a fall in vascular resistance (Gruskin et al 1970). On the other hand it has been reported that renal vascular resistance is elevated in newborn guinea-pigs (Spitzer and Edelmann 1971) and dogs (Jose et al 1974) and this has been implicated in the low GFR of these neonatal animals. In general the role of vascular resistance in the maturation of GFR is ill defined.

A series of experiments was described in section 5.4 in which vascular resistance was manipulated. It was found, as anticipated, that renal vascular resistance and GFR were inversely related. Secondly, since vascular resistance and presumably therefore the degree of constriction of the afferent arterioles was affected by a sympathomimetic drug, renal vascular resistance is probably regulated by the autonomic nervous system.

The probable existence of such neural controls over renal vascular resistance in foetuses as young as 130 days, suggests that foetal sheep have the ability to regulate RBF and thus to regulate GFR. This ability would be of significance in the homeostatic activity of the foetal kidney.

The remaining factors that are potentially involved in the maturation of GFR are; the osmotic effect of plasma proteins and intra-capsular hydrostatic pressure. Since no regular increase in the total protein concentration of arterial plasma was detected in foetal lambs, it is unlikely that colloid OP contributes significantly to the observed rise in GFR. No information was obtained concerning the influence of intra-capsular pressure on the maturation of foetal GFR. The general opinion concerning adult kidneys, is that variation of the intra-capsular pressure contributes little to the regulation of GFR under normal physiological circumstances. Whether or not this is true of the maturing foetus, where the structure of the nephron and the overall anatomy of the urinary tract is changing, is uncertain. However in the last 35 days of gestation, during which time GFR rises 61%, the metanephric kidney is well developed and the urethra is patent, so variations in intra-capsular pressure would be slight.

In summary, the observed increase in foetal GFR during the last third of pregnancy is probably due, in part, to an overall increase in the number of glomerulii in the growing kidney and to a rise in filtration pressure reflecting a rise in MAP. Changes in renal vascular resistance are possibly involved in the maturation of GFR although this was unassessed. However it does appear that mature foetuses have the ability to vary vascular resistance and hence control RBF and GFR. Many subtle factors such as membrane transport have not been studied in this or any other investigation of foetal glomerular function nor have they been well studied in adult kidneys. Accordingly their involvement in the maturation of GFR is unknown.

6.2 *Tubule Function*

In the present work, assessment of the activity of the renal tubules has relied upon inferences drawn from data concerning renal output in normal and experimental conditions. Apart from the use of micropuncture techniques, which would be particularly difficult to apply in foetal studies, this is the only method available. It has been possible to estimate the fractional reabsorption of water and electrolytes at various stages of gestation and to study the results of inhibiting tubular reabsorption using pharmacological agents. However the secretory activity of the renal tubules has not been studied at all, even though uric acid, which was analysed in all studies, is probably secreted by the tubules as well as being cleared at the glomerulii. Nevertheless uric acid was included in the analyses because the level of uric acid in foetal urine is an index of purine metabolism and because uric acid excretion rate provides an indication of the specificity of the drugs used to inhibit water and electrolyte excretion. For example in section 5.4.2 it was found that sodium ethacrynate treatment of a 139 day-old foetus (foetus 274) resulted in an increase in creatinine excretion rate that was comparable with and presumably due to, the rise in GFR induced by the drug. In contrast, the rise in uric acid excretion rate was twice that which could be accounted for by the increase in GFR, which suggests that ethacrynate not only inhibits water and electrolyte reabsorption but also inhibits uric acid reabsorption. Similar results were obtained when an older foetus (foetus 274, 148 days) was treated with furosemide. These findings are consistent with evidence advanced by Burg and Green (1973) that high doses of ethacrynate and furosemide promote the excretion of uric acid.

With respect to water and electrolyte excretion, the experiments mentioned above indicate that during ethacrynate and furosemide treatment,

increased GFR accounts for 20% of the flow rate increase, 40-45% of the rise in K^+ excretion rate, but only 4-6% of the rise in Na^+ excretion rate. The rest of these increases were obviously due to the inhibition of normal tubular reabsorption. The magnitude of the reabsorptive capacity of the foetal tubules was confirmed in a study of 26 untreated foetuses; where it was found that over the last 35 days of gestation the fractional reabsorption of water increased from 75% to 86%, the reabsorption of K^+ from 45% to 56% and the reabsorption of Na^+ from 90% to 98%.

These studies indicate how active the reabsorptive mechanisms of the renal tubules are, particularly those concerned with water and Na+ reabsorption, but they give little indication of the nature or specific sites of the reabsorptive mechanisms. Accordingly the diuretic studies were expanded and foetal sheep of various ages were treated with a range of diuretic drugs.

Since a considerable amount of information is available concerning the pharmacology of these drugs in adults it was hoped that a comparison of adult and foetal responses would permit inferences to be drawn regarding the site and mode of action of these drugs in foetal kidneys. This in turn, would possibly indicate the nature and degree of maturity, of the reabsorptive mechanisms in foetal kidneys.

Of the diuretic drugs used, sodium ethacrynate had the greatest effect on foetal kidney output. In adults, sodium ethacrynate prevents the reabsorption of up to 23% of the filtered Na^+ load. When administered intra-venously ethacrynate begins to act within 15-30 minutes and has a peak effect after about 45 minutes. The overall effect lasts about 3 hours. Under normal circumstances, the movement of Na^+ from the tubular cells across the peritubular membrane and into the peritubular fluid is thought to be accomplished by an active ionic pump. Further, it has been postulated that sodium-potassium activated, adenosine triphosphatase (Na^+ - K^+ ATPase),

may be the carrier molecule for the pump. (Martinez-Maldonado et al 1974) and that ethacrynate produces its diuretic effect by inhibiting Na^+ - K^+ ATPase (Inagaki et al 1973). Other aspects of the function of tubule cells, including the activity of sulfhydryl-containing enzymes, may be inhibited by the binding of sodium ethacrynate to the -SH groups, thereby impairing Na^+ reabsorption (Komorn and Cafruny 1965). Sodium ethacrynate is thought to exert maximal inhibition of Na^+ reabsorption along the whole length of the ascending limb of the loop of Henle. However other sites have not been excluded and although the evidence is controversial, some workers suggest that ethacrynate inhibits Na^+ reabsorption in the proximal tubule. It is also believed that because ethacrynate inhibits Na^+ reabsorption in the loop of Henle there is an increased Na^+ load presented to the Na^+ - K^+ and Na^+ - H^+ exchange site in the distal tubule. This results in increased Na^+ reabsorption in exchange for K^+ and H^+ ions. Finally it is generally thought that ethacrynate has little effect on the GFR of adults, except that with excessive diuresis the reduced blood volume may lead to a fall in GFR. In contrast Vorburger (1964) and Hook et al (1966) claim that both ethacrynate and furosemide increase RBF and GFR in man.

In adult sheep sodium ethacrynate produces a large diuresis and natriuresis, plus a smaller rise in K^+ excretion rate. Doses of ethacrynate of up to 10 mg increase urine flow rate to as much as 10 ml per minute and Na^+ excretion rate to between 1000 and 1400 µEq per minute. These rates were achieved within 20-40 minutes of treatment (Peter 1969). There is also a fall in urinary pH from around 7.4 to as low as 4.9. No precise dose - response relationship has been determined in adult sheep and increasing the ethacrynate dose beyond 10 mg does not increase the magnitude of the diuresis but does extend its duration.

When this information is compared with the data obtained from foetal sheep, the similarity of the responses is apparent. The speed of onset and the duration of the foetal responses, were similar to those described for adults and the percentage inhibition of filtered Na^+ was comparable. In foetuses as in adults, there appeared to be a maximum diuretic and natriuretic response beyond which higher ethacrynate doses merely increased the duration rather than the magnitude of the response. The maximum levels of urine flow and Na^+ excretion in foetal sheep following ethacrynate treatment were not comparable, in absolute terms, with those achieved in adults. However the percentage increases were comparable, with the exception of K^+ excretion rate where the foetal rises were comparatively low. No information is available concerning the effect of ethacrynate on the excretion of uric acid by adult sheep, but there was an increase in uric acid excretion by the foetuses which is comparable with the response obtained in adult dogs and humans treated with high doses of ethacrynic acid.

Apart from the comparatively limited rise in K^+ excretion rate in foetal sheep, the most obvious difference between the adult and foetal responses to ethacrynate treatment, was the effect on urine pH. In adults urine pH decreases after treatment but in all foetuses it increased. The fall in urine pH in adults is presumably due to increased Na^+ - H^+ exchange in the distal tubules. Obviously the distal tubules of the foetuses would receive increased Na^+ loads following ethacrynate treatment and accordingly increased H^+ secretion would be anticipated. It appears significant that this was not the case, particularly since the increase in K^+ excretion rate that occurred in most foetuses was probably due to increased Na^+ - K^+ exchange in the distal tubules. Finally sodium ethacrynate treatment induced an 80% rise in foetal GFR which is consistent with some observations

in adult humans (Vorburger 1964, Hook et al 1966) but contrary to others (Gussin and Cafruny 1966).

Overall, it can be concluded that within the context of relative kidney size, foetal sheep exhibit a response to sodium ethacrynate similar to that known to occur in adult mammals, including adult sheep. Accordingly it seems that the mechanisms for Na^+ and water reabsorption are similar in adults and foetuses and that apart from obligatory Na^+ reabsorption in the proximal tubules, the ascending limb of the loop of Henle is the major site of Na^+ reabsorption in the foetal tubules. Secondly a Na^+ - K^+ exchange mechanism appears to operate in the foetal nephrons, probably in the distal convoluted tubules. However since the percentage increase in K^+ excretion rate following ethacrynate treatment is less in foetuses than adults, the capacity for foetal Na^+ - K^+ exchange may be limited. Finally the rise in the pH of foetal urine following ethacrynate treatment suggests that the Na^+ - H^+ exchange mechanism thought to be active in the distal tubules of adults is either absent in foetal sheep, or its activity is limited.

With respect to the biochemical basis of these exchange mechanisms, the close similarity between the inhibitory effect of ethacrynate on Na+ exchange in foetal and adult kidneys suggests not only that the sites of inhibition but also the mechanisms of inhibition are similar. Therefore it can be argued that Na^+ reabsorption in the foetal nephron is an active process mediated by Na^+ - K^+ ATPase. However it must be remembered that although there is considerable evidence for such a mechanism in adults, it has not been conclusively demonstrated. With respect to water reabsorption it is clear that in foetuses, as in adults, this is a passive process resulting from the development of an osmotic gradient following active Na^+ reabsorption. It has been clearly

demonstrated that the inhibition of Na^+ reabsorption in the foetal kidney results in a large diuresis. Accordingly the tubular membranes of the foetus must be freely permeable to water, at least in the proximal tubule. The ascending limb of the loop of Henle is also a site of active Na^+ reabsorption but it is not permeable to water in adults and there is no evidence, in the present work, to suggest that it is permeable in the foetus.

The second diuretic agent used in foetal sheep was furosemide. The pharmacological properties of furosemide have been less extensively studied than those of sodium ethacrynate. However they appear to be very similar to those of sodium ethacrynate. In adults furosemide prevents reabsorption of 15-30% of the filtered Na^+ and it has been established that the inhibition occurs in the ascending limb of the loop of Henle (Suki et al 1965, Morrin 1966). In addition proximal sites of inhibition appear to be involved (Brenner et al 1969) and partial inhibition of distal Na^+ reabsorption is also possible (Fraser et al 1967). When administered intra-venously to adults, furosemide begins to act within 5 minutes and the response reaches a peak after 30 minutes while residual activity persists for about 2 hours. The biochemical action of furosemide has not been as widely studied as that of sodium ethacrynate, but furosemide is generally thought to inhibit Na^+ - K^+ ATPase. The diuretic action of furosemide supplements the action of most diuretics but not ethacrynate, which supports the proposal that both drugs act in a similar manner.

In adult sheep, furosemide induces a large diuresis and natriuresis similar to that produced by ethacrynate (Peter 1969), although the maximum urine flow achieved with furosemide is slightly higher (12 ml per minute). Peter (1969) observed a rough dose - response pattern for furosemide, but points out that intra-venous doses, in excess of 5 mg, increase the duration of the response rather than its magnitude. Furosemide,

like ethacrynate, stimulates an increase in H^+ excretion by adult sheep resulting in a fall in pH from over 7.0 to as low as 5.5.

It can be seen in section 5.3.1 that the foetal responses to furosemide were comparable with the adult responses, particularly with respect to Na^+ and water excretion. However the effects on foetal K^+ and H^+ excretion were not the same as in adults. The relative increases in Na^+ and water excretion induced by furosemide are of a similar order to those obtained in adult sheep, but the onset of action in the foetus is slower and the duration of action longer. The prolonged response may be due to the lower capacity of the foetus to excrete the drug. Furosemide did not act as quickly in the foetus as ethacrynate which may reflect the high affinity of furosemide for plasma proteins (Gayer 1965). The natriuretic and diuretic effects of furosemide in foetal sheep were not dose related, although there did appear to be a maximum dose beyond which no additional response could be induced, which is consistent with the proposed pharmacological action of the drug. The excretion rate of K^+ by the foetuses was usually affected by furosemide, but not to the extent of Na^+ excretion. Finally there was no consistent effect of furosemide on the pH of foetal urine.

These results confirm the existence of a mechanism for active Na^+ reabsorption in the foetal nephron and indicate that it is located in the proximal tubule and the ascending limb of the loop of Henle. As with ethacrynate there is evidence in adults that the inhibition of these mechanisms by furosemide is due to inhibition of Na^+ - K^+ ATPase activity in the cells of the renal tubules. Since it is likely that furosemide exerts its effects on the foetal kidney in the same way, Na^+ reabsorption in the foetal nephron is probably mediated by Na^+ - K^+ ATPase (Frazier and Yager 1973).

The effect of furosemide on foetal K+ excretion was limited, and again there was no fall in urine pH; instead the pH of foetal urine increased. These findings are consistent with those obtained in the ethacrynate experiments and support the proposition that distal Na^+ - K^+ and Na^+ - H^+ exchange mechanisms are either absent in the foetal sheep or poorly developed. If these mechanisms normally involve membrane bound Na^+ - K^+ ATPase, it may be that this enzyme is absent from the distal tubules of foetuses or relatively inactive at that site. However it is known that Na^+ - K^+ ATPase actively is high in the distal tubules of neonatal rabbits (Davis and Dixon 1971) and if that is the case in newborn lambs, renal development during the transition from foetal to neonatal life in sheep may include a rise in Na^+ - K^+ ATPase activity in the distal tubules.

With respect to GFR, furosemide like ethacrynate induced about an 80% rise in foetal GFR. In mature animals, furosemide is known to produce mild renal - vasodilation and in adult sheep 1 mg of furosemide causes a rapid increase in GFR from 30 mls per minute to about 100 mls per minute and this is associated with a three fold rise in RBF (Peter 1969). Presumably a similar change in renal vascular resistance and RBF following treatment accounted for the increase in foetal GFR. There are no reports available on the effect of sodium ethacrynate on renal vascular resistance in adults or foetuses; but it is likely that the rise in foetal GFR produced by that drug is also due to reduced vascular resistance and increased RBF.

The third diuretic administered to foetal sheep was acetazolamide which is a carbonic anhydrase inhibitor. Within the cells of the renal tubules, carbonic anhydrase catalyses the hydration of CO_2 to form carbonic acid which then dissociates providing H^+ ions to be secreted in exchange for Na^+ ions. The secretion of H^+ ions also facilitates the reabsorption of filtered bicarbonate and the excretion of titratable acid. Following the administration of acetazolamide, less H^+ ions are available for secretion so Na^+ and HCO_3^- ions remain in the filtrate and an alkaline diuresis

results. Also, because of the lack of H^+ ions for competitive cation exchange with Na^+ ions, the urinary excretion of K^+ increases. Thus in adults acetazolamide treatment causes a rise in urine volume, an increase in urine pH and an increase in the excretion of Na^+ and K^+.

Although the general pharmacology of acetazolamide is established, many details remain unknown including the exact location of the enzyme within cells and the nature of the exchange mechanism (Maren 1967). With respect to the site of action, originally only the distal tubule was implicated but it now appears that the entire nephron is involved (Clapp et al 1963).

In adult sheep, acetazolamide administered intra-venously causes urine pH to rise from 7.0 to 8.4 and increases the excretion of water, Na^+ and K^+. Doses of acetazolamide of between 10 mg and 300 mg cause urine flow rate to rise between 10% and 120% while Na^+ and K^+ excretion rates increase between 15% and 100% (Peter 1969). In foetal sheep, the changes in urine flow rate resulting from acetazolamide treatment were of a similar magnitude to those observed in adults, despite obvious differences in the absolute values. Also the speed of onset and the duration of the diuretic responses were similar in adults and foetuses. However the relative increases in foetal Na^+ and K^+ excretion rates exceeded the adult rises. This was particularly true of the youngest foetus, where acetazolamide treatment caused the Na^+ excretion rate to increase 9.5 times and K^+ excretion rate 6.4 times. However the foetal acetazolamide doses standardised on a body weight basis are about 5 times the maximum adult dose. This may account for the disparity in electrolyte excretion rates although flow rate and pH changes were comparable in adults and foetuses. In both mature sheep and foetal sheep, there appears to be a threshold beyond which increased doses of acetazolamide produce no further rise in pH and presumably at those doses (about 50 mg/kg in foetal sheep) maximum carbonic anhydrase inhibition has been achieved.

By comparing the foetal response to the adult model, it can be concluded that foetal tubule cells, under normal circumstances exhibit

substantial carbonic anhydrase activity. This is not consistent with a report that in newborn humans, renal carbonic anhydrase activity is low (Maren 1967) which again indicates the comparative maturity of the lamb kidney during the last third of gestation. Further this cellular activity apparently facilitates Na^+ reabsorption, H^+ secretion (via Na^+ - H^+ exchange) and HCO_3^- reabsorption. Finally, the fact that inhibition of this enzyme produces an increase in K^+ excretion, confirms the existence of a Na^+ - K^+ exchange mechanism in the tubules of the foetal kidney.

The next substance tested in foetal sheep was chlorothiazide, a representative of the thiazide group of diuretics. All of the thiazides produce an increase in the excretion of water, Na^+ and K^+ and during maximum diuresis they prevent reabsorption of 5-7% of the filtered Na^+ load. In addition the thiazides increase the excretion of HCO_3^- ions which was originally thought to be due to carbonic anhydrase inhibition. However some of the drugs in this group have been found to have virtually no ability to inhibit carbonic anhydrase. It is now thought that increased Na^+ - K^+ exchange results in less Na^+ - H^+ exchange and therefore fewer H^+ ions in the tubular fluid. This prevents the formation of carbonic acid and since the HCO_3^- ions cannot be reabsorbed they are excreted in the urine. The specific nature of the chemical interaction of the thiazides with renal receptors is unknown. The oxidation of non-esterified fatty acids (NEFA) may contribute to the energy required for Na^+ transport within the renal cortex. Chlorothiazide, in adults, depresses the nett renal uptake of NEFA and may exert part of its action by producing alterations in energy metabolism (Barac-Nieto, and Cohen 1968). Apart from this, no critical enzymatic factors involving thiazides have been identified. In preparations from mammalian kidneys that were treated with chlorothiazide, Na^+ - K^+ ATPase was not depressed and presumably inhibition of that enzyme is not involved in the biochemical effect of chlorothiazide.

Fernandez and Puschett (1973) have recently shown that chlorothiazi causes inhibition of Na^+ reabsorption in the proximal tubules of mature dogs, but they concluded that this did not contribute to the final diuresis.

Other workers (Anderton and Kincaid-Smith, 1971, Steinmuller and Puschett, 1972) have concluded that the thiazides exert their major inhibitory effect on Na^+ reabsorption in the outer medullary segment of the ascending limb of the loop of Henle. Because of reduced Na^+ reabsorption at that site, a higher concentration of Na^+ reaches the late distal tubule resulting in enhanced Na^+ - K^+ exchange and increased K^+ excretion. Finally hyperuricemia is not uncommon in adults treated with thiazides, presumably because the thiazides (like furosemide and ethacrynic acid) compete with uric acid for secretion via the organic-acid transport system in the proximal tubule.

In adult sheep cyclothiazide produces only minor changes in urinary excretion. Peter (1969) reported small increases in urinary water, K^+ and Na^+ losses which were partly independent of the thiazide doses administered. Large doses (10 mg) of cyclothiazide extended the duration rather than the size of the response. Urinary pH was unaffected by cyclothiazide treatment (Peter 1969). Similar findings were made by Cross and Thornton (1966) using related thiazide compounds; in fact two of the compounds they tested were almost completely ineffective. They concluded that thiazide diuretics either do not affect electrolyte movement in the renal tubules of the sheep, as they do in other species, or the mechanisms they do affect are relatively unimportant in that species.

The findings in adult sheep are not consistent with the results obtained in foetal sheep where chlorothiazide rapidly induced diuretic and natriuretic responses with flow rate increasing 4-6 times and Na+ excretion rate increasing 6-17 times. In contrast the $[K^+]$ of foetal urine decreased after chlorothiazide administration but because of the increases in urine flow rate there was usually a small transient rise in K^+ excretion rate. Creatinine and uric acid excretion rates were generally unaffected by chlorothiazide but in all experiments the pH of foetal urine increased significantly.

The doses of chlorothiazide used in foetal sheep were much greater in relation to body weight, than those used in adult sheep. Nevertheless doses of up to 30 mg/kg do not appear to have achieved maximum inhibition

in the foetal nephron. Therefore it is likely that the poor response in adult sheep is due to the limited doses of thiazide used rather than any real difference in the responsiveness of adult and foetal sheep. In fact, on a body weight basis, the thiazide doses used in adult sheep were considerably less than the optimally effective doses in humans. Thus there appears to be no grounds for the conclusion that the mechanisms inhibited by thiazide diuretics are of little significance in adult sheep. It is certainly not the case in foetal sheep where substantial increases in Na^+ excretion rate are induced and where the total response, including decreased H^+ excretion, is remarkably similar to that described for humans and rats.

On the basis of the foetal experiments using chlorothiazide, it can be concluded that the kidney of the foetal sheep has a mechanism for active Na^+ reabsorption that does not involve Na^+ - K^+ ATPase. This mechanism is possibly located in the outer medullary segment of the ascending limb of the loop of Henle. It may be that the energy for this mechanism is provided by the oxidation of NEFA. An important feature of the foetal response to chlorothiazide was the lack of a significant change in K^+ excretion. A rise in K^+ excretion usually occurs in adults and is thought to be due to a rise in distal Na^+ - K^+ exchange following an increase in the Na^+ load reaching the late distal tubule. The lack of a comparable result in foetal sheep again suggests that the Na^+ - K^+ exchange mechanism in the distal tubules is immature. Finally Vesin and associates (1962) have stated that a thiazide is only effective if the GFR is greater than 20-25 ml per minute and that since the GFR is low in infants, the thiazides would be ineffective in inducing substantial diuresis. This is not the case in foetal sheep, where it is unlikely that in any experiment the GFR exceeded 10 ml per minute, yet chlorothiazide treatment induced substantial increases in water and Na^+ excretion.

The final diuretic agent used in foetal sheep was amiloride hydrochloride which in adults increases the renal excretion of Na^+ without a significant change in GFR. This drug may also produce a moderate increase in urine pH due to reduced H^+ secretion. An important characteristic of the

adult response to amiloride hydrochloride is that under all conditions the natriuresis is associated with only a small increase in K^+ excretion rate or alternatively an absolute decrease in K^+ excretion. The experimental evidence indicates that amiloride hydrochloride inhibits K^+ secretion in the late distal tubule which is also where its limited natriuretic action is thought to originate. It is postulated that amiloride hydrochloride inhibits active Na^+ reabsorption in the late distal tubule and that as a result the electrochemical gradients that would normally favour the secretion of K^+ are not established. The slight action of amiloride hydrochloride on H^+ transport remains unexplained but it is not due to carbonic anhydrase inhibition.

No specific information is available concerning the effect of amiloride hydrochloride on renal function in adult sheep. However in the present work amiloride consistently induced a rise in urine flow rate and Na^+ excretion rate in foetal sheep, but the effect on K^+ excretion was more ambiguous. In one of the foetuses tested there was an increase in K^+ excretion rate that was comparable with the rise in Na^+ excretion (1.5 times) in another K^+ excretion was unaffected while in a third it was reduced. The influence of amiloride hydrochloride treatment on H^+ excretion was no less variable. On the basis of these experiments it cannot be concluded that the pharmacological activity of amiloride hydrochloride in the foetus is as described for adults. There is certainly inhibition of active Na^+ reabsorption in the foetal nephron and a resultant decrease in the passive reabsorption of water. However whether or not K^+ secretion in the foetal kidney is affected by amiloride hydrochloride, remains undetermined. Since a relationship between Na^+ inhibition and K^+ secretion has not been established it cannot be concluded that the natriuretic response obtained in foetal sheep is due to inhibition in the late distal tubule. The natriuresis may be the result of inhibition at other sites of Na^+ reabsorption. Unfortunately these results do not provide specific information on the presence or absence, or the state of maturity of $Na^+ - K^+$ exchange mechanisms in the late distal tubules of foetal sheep.

In summary the responses obtained when foetal sheep, in the last third of gestation, were treated with various diuretic agents have provided considerable information on the function of renal tubules. The sodium ethacrynate and furosemide experiments indicate that foetal GFR can increase up to 80% given the appropriate stimulii. The probable mechanism for such a rise in GFR is reduced vascular resistance and a resultant increase in RBF. Secondly these drugs have confirmed the existence of a mechanism for active Na^+ reabsorption in the foetal nephron that may involve Na^+ - K^+ ATPase as a carrier molecule. Since inhibition of active Na^+ reabsorption resulted in a diuretic response it can be concluded that under normal circumstances active Na^+ reabsorption is accompanied by passive water reabsorption. Accordingly the distal segments of the foetal nephron must be permeable to water.

Acetazolamide treatment revealed the existence of carbonic anhydrase activity within the cells of the foetal tubules and confirmed the involvement of that enzyme in the Na^+ - H^+ exchange accomplished by these cells. Also the fact that kaliuresis occurred following acetazolamide treatment confirmed the existence of a Na^+ - K^+ exchange mechanism in the foetal nephron, but the location and capacity of that mechanism is ill-defined. In mature kidneys, the late distal tubule is a site of Na^+ - K^+ and Na^+ - H^+ exchange and invariably following treatment with high-ceiling diuretics like ethacrynate and furosemide, the increase in distal Na^+ load results in a kaliuresis comparable in magnitude with the natriuresis. However this was not usually the case in foetal sheep. Generally the results imply that distal Na^+ - K^+ exchange occurs in foetal sheep but only to a limited extent. The same can be said about distal Na^+ - H^+ exchange. Chlorothiazide treatment indicated the probable existence of Na^+ reabsorption in the outer medullary segment of the ascending limb of the loop of Henle. It is thought that the energy for this process may be

provided by the oxidation of NEFA.

In mature kidneys the proximal tubule is a very active site of reabsorption. In man it is estimated that 75% of filtered solute and 75% of filtered water is reabsorbed in the proximal tubule. The current work provides no direct information concerning the reabsorptive capacity of the proximal tubule in foetal sheep. Nevertheless it is highly probable, in view of the large fractional reabsorption of water (76% to 86%) and Na+ (90% to 98%), that it is an active site of Na^+ and water reabsorption. However it is likely that it is obligatory reabsorption and has no involvement in regulating salt and water excretion. Such regulation, is probably the role of the various mechanisms that have been revealed in the loops of Henle and distal convoluted tubules.

With respect to the maturity of these mechanisms it is apparent that during what appeared to be maximum inhibition, the relative increases in urine flow rate and Na^+ excretion rate by the foetal sheep were comparable with those achieved using optimally effective doses in adult sheep. The differences in absolute values reflect the relative sizes and GFRs of foetal and adult kidneys. Thus with the probable exceptions of $Na^+ - K^+$ and $Na^+ - H^+$ exchange in the distal tubules the reabsorptive capacities of the foetal tubular mechanisms are comparable with the adult mechanisms. Presumably therefore, where enzymes are involved in reabsorption, the enzyme activity per unit mass of renal tissue is comparable in foetuses and adults.

Despite the general equality of adult and foetal responses, there was some indication of progressive maturation of the foetal nephrons during the last third of gestation. However this evidence is not conclusive.

In the experiments involving furosemide, the youngest foetus (118 days) showed some increase in Na^+ excretion rate after treatment. However in the remaining cases the most mature foetuses exhibited the

greatest responses, despite receiving the lowest doses of furosemide. If the results for foetus 69-559 are ignored because of the particularly high diuresis which may have been due to the low pre-treatment flow rates; then for the remaining 6 furosemide experiments the correlation between foetal age and the percentage increase in Na^+ excretion rate following treatment is 0.686 ($0.05 > p > 0.01$ $n = 6$) (see table 10). Thus it may be that the mechanism for Na^+ reabsorption in the ascending limb of the loop of Henle matures progressively, in terms of its reabsorptive capacity, during the last 30 days of gestation. The diuretic and natriuretic responses induced by sodium ethacrynate also appear to be positively age-related (% increase NaER against age: $r = 0.867$, $0.01 > p > 0.001$, $n = 3$) and since there is considerable evidence indicating that the pharmacological mechanism and site of action of ethacrynate is similar to that of furosemide, this supports the contention that the capacity for Na^+ reabsorption increases in late gestation. It may be that such developments involve increasing Na^+ - K^+ ATPase activity in the cells of the renal tubules.

The chlorothiazide experiments did not indicate any age-response relationship with respect to the magnitude of the response, but older foetuses did show a more prolonged response. This is possibly because the biochemical activity involved in NEFA oxidation becomes increasingly sensitive to chlorothiazide suppression. Alternatively if the diuretic activity of chlorothiazide involves binding with a carrier molecule or with an enzyme that facilitates the carrier mechanism then the prolonged response to chlorothiazide in older foetuses may be due to a greater affinity between the carrier molecule and chlorothiazide. In such circumstances, the reabsorptive capacity of the outer medullary portion of the ascending limb of the loop of Henle may well be greater in older foetuses, but because of the larger doses of chlorothiazide used in the young foetuses the affinity differences and therefore the differences in reabsorptive ability were masked.

Generally the diuretic experiments indicate that the capacity for Na^+ reabsorption by the ascending limb of the loop of Henle is greater in more mature foetuses. Other less conclusive evidence suggests that there is a relationship between foetal age and the ability of the outer medullary section of the ascending limb of the loop of Henle to reabsorb Na^+ and finally it appears that Na^+ - K^+ and Na^+ - H^+ exchange in the distal tubules of foetal sheep is limited.

The immaturity of the distal exchange mechanisms may be a factor in the limited concentrating capacity of the foetal kidney. However the Na^+ reabsorption thought to occur in the ascending limb of the loop of Henle would make a more significant contribution of Na^+ to the medullary interstitial fluid than distal Na^+ exchange. Therefore if as the diuretic experiments suggest, Na^+ reabsorption from the loop of Henle increases during the last 20-30 days of gestation (see table 10), this may facilitate the development of an increasingly steep osmotic gradient and promote the reabsorption of water from the distal tubules and collecting ducts of the foetus. As will be seen this may occur in association with the accumulation of urea in the interstitial fluid of the foetal kidney. Together these factors would reduce the hypotonicity of the foetal urine during the last 30 days of pregnancy. Alexander et al (1958a) and Mellor and Slater (1972b) have reported that this does occur, they found that the osmolality of foetal sheep urine increases in the last 20-25 days of gestation.

There are difficulties associated with drawing conclusions concerning the maturity of tubule mechanisms in foetal sheep on the basis of these diuretic experiments. It must be remembered that seemingly age-related differences in the responses induced by the drugs may be due to variations in factors such as the capacity of proteins in the foetal plasma to combine with the drugs and variations in the ability of the foetus to metabolise and excrete the drugs. In human infants thiazide diuretics are eliminated primarily by glomerular filtration and by active secretion in the proximal tubule, while both furosemide and ethacrynate are eliminated in the urine

and faeces (Berger et al 1965). In the urine ethacrynate is found in equal parts as the parent compound and as an unstable metabolite. Furosemide is bound to plasma proteins after absorption and only a fraction is metabolised prior to excretion.

No information is available concerning the ability of the foetal lamb to eliminate chlorothiazide by secretion in the proximal tubule nor on the binding of furosemide and ethacrynate to plasma proteins. Similarly no specific information is available concerning the capacity of foetal lambs of various ages to metabolise and excrete these diuretics. However we can reasonably assume that the ability of the foetal liver to metabolise drugs increases with foetal age (Dawes and Mott, 1964). Also foetal GFR has been shown to increase during the last 1/3 of gestation thus it is likely that the rate of metabolism and excretion of the diuretic drugs increases with foetal age. In the present work the conclusions based on the diuretic experiments are dependent upon the fact that greater responses were observed in older foetuses. Obviously therefore the probable increase in the foetal capacity to metabolise and excrete these drugs does not negate these conclusions but in fact reinforces them.

6.3 Hormone Involvement in Foetal Kidney Function

6.3.1 The influence of exogenous hormones

As described in section 5.5 a variety of hormones were infused into foetal sheep. The objective of these experiments was to assess the sensitivity of the foetal kidney to hormonal influences and to enable gestational changes in foetal kidney function to be related to changes in the endocrinological milieu of the foetus. It is not sufficient to examine morphological and biochemical changes within the foetal kidney if its functional development and homeostatic capacity is to be understood. These

phenomena must be considered in terms of the development of the pituitary-adrenal axis of the foetus.

One hormone considered in the endocrinological survey was ADH (vasopressin) a pepitide hormone from the posterior pituitary which normally has the effect of reducing high urine flow rates by facilitating the reabsorption of water in the renal tubules. In the absence of ADH the epithelium of the distal tubule and collecting duct is relatively impermeable to water. However an increased level of ADH results in greater permeability of these tubules to water, allowing osmotic equilibration between the tubular fluid and the interstitial fluid of the renal medulla. This results in the excretion of hypertonic urine. Apart from this, ADH has an unpredictable effect on electrolyte excretion that appears to vary between species and even within species. How ADH influences electrolyte excretion is not known but Ginetzinsky (1961) claims that the increased permeability of the epithelium of the distal tubules and collecting ducts, is due to greater hyaluronidase activity in the presence of ADH. Generally the findings of other workers have not supported this theory. More recently Orloff and Handler (1964) using amphibian bladder tissue have shown that cyclic adenosine - 3', 5' - monophosphate (cyclic - AMP) causes changes in permeability similar to those caused by ADH. Accordingly they proposed that accumulation of cyclic - AMP is an intermediary step in the action of ADH on mammalian kidney tubules. However the exact effect of ADH on nephron permeability is not known.

Adult sheep show several patterns of response to ADH. Doses of pitressin or arginine vasopressin (ADH) as small as 1 mU by injection or 12 mU/hour by infusion, produce a transient increase in GFR plus rises in Na^+ and K^+ excretion rates. These doses are diuretic when the initial urine flow rate is between 1.0 and 1.5 ml per minute but are anti-diuretic when the initial flow rate is greater than 1.5 to 2 ml per minute,

(Kinne et al 1961, Gross et al 1965, Gans 1964). However Stacy and Brook (1964, 1965) reject the proposal that under certain conditions ADH may increase electrolyte and water excretion. In their experiments they obtained only anti-diuretic responses with the production of hypertonic urine and there was no alteration of total electrolyte excretion.

Only two experiments were completed in which foetal sheep were infused with ADH and the results are inconsistent. Therefore no firm conclusions can be drawn from the present work and obviously further experiments are required. Nevertheless we can speculate on some aspects of the results obtained. The youngest foetus treated with ADH showed a typical anti-diuretic response with an 80% fall in urine flow rate during the first hour of treatment. It was noticeable that on this occasion the initial urine flow rate was 1.36 ml per minute and when it is considered that the GFR in foetal sheep is only about 20% of the adult GFR, such a flow rate represents a high rate of water output. In comparison, the second foetus treated with ADH had a low pre-treatment flow rate (0.48 ml per minute) and about one hour after treatment the flow rate increased.

Therefore it may be that in foetal sheep, as in the adults, ADH induces a response that is related to the initial flow rate and that with high initial flow rates it is anti-diuretic and with low initial flow rates it is diuretic. In such circumstances the response is presumably determined by the state of hydration of the foetus. Alternatively, ADH may simply be anti-diuretic as indicated by the first experiment, for if the low initial flow rate in the second experiment was due to near maximum nephron permeability, exogenous vasopressin could not produce a further reduction in flow rate.

Further the increase in flow rate in that experiment may have been due to a natural resurgence following a phase of restricted urine output. Since the maximum flow rate recorded in the second experiment occurred three hours after the completion of the vasopressin infusion the second

explanation seems more suitable.

If it is accepted that the response in the first experiment was due to the anti-diuretic effect of ADH involving an increase in the permeability of the distal tubules and collecting ducts, an intra-renal osmotic gradient must exist. Despite the proposal that Na^+ transport from the distal tubule and loop of Henle is limited in young foetuses, there must be sufficient transport of Na^+ or other osmotically active substances to have established at least a small intra-renal solute gradient.

The next hormone considered was aldosterone. In humans, intravenous administration of aldosterone is followed, after a delay of about one hour by a decrease in the rate of renal Na^+ excretion and an increase in K^+ and H^+ excretion. Generally no change in water output is observed during aldosterone administration (Simpson and Tait 1955). It is thought that aldosterone acts on the distal tubules to promote the exchange of Na^+ for K^+ or H^+. With long term administration this leads to hypokalemia and alkalosis while Na^+ retention continues and the extra-cellular fluid volume increases. Eventually a compensatory mechanism is initiated, the details of which are unknown, but which results in the inhibition of Na^+ reabsorption in the proximal tubule so that Na^+ excretion rises despite continued aldosterone action. As a result Na^+ balance is re-established but the abnormal excretion of K^+ and H^+ continues.

The mechanism of aldosterone action has not been clearly established. Edelman et al (1963) have suggested that the increase in Na^+ transport is brought about by an increase in the rate of synthesis of the enzymes involved in Na^+ transport. The long latent period in the action of aldosterone on the mammalian kidney is consistent with this hypothesis, but not consistent with a direct action of the hormone on the cell membrane, in which case practically no delay in the onset of action would be anticipated.

In adult sheep, aldosterone causes Na^+ retention (Blair - West et al 1963) but it does not appear to promote K^+ and H^+ loss as it does in other species (Kinne et al 1961, MacFarlane 1963). These findings have been confirmed by Peter (1969) who infused aldosterone into sheep at rates ranging from 50 to 1000 µg per hour and found a clear decrease in urinary $[Na^+]$ and Na^+ excretion rate plus a small reduction in urine flow rate. However there was no effect on K^+ excretion rate.

In foetal sheep, the doses of aldosterone used were comparable on a body weight basis with the doses used in adult sheep.

However the responses induced in foetal sheep although consistent with those observed in adult mammals in general, were not consistent with those displayed by adult sheep in particular. Aldosterone promoted Na^+ retention and K^+ loss by foetuses. In addition there was some increase in H^+ excretion particularly when high doses of aldosterone were injected. In all cases there was a delay of one to two hours before the response began which again indicates that aldosterone does not act directly on tubule cell membranes so the mechanism of aldosterone action in foetuses may be as proposed by Edelman et al (1963).

If the adult model is used to explain the foetal responses, it can be infered that the rises in K^+ excretion rate which followed aldosterone treatment were due to a rise in K^+ secretion by the distal tubules following increased Na^+ reabsorption. On the basis of the diuretic experiments it has been argued that distal Na^+ - K^+ exchange is limited in young foetuses but increases with foetal age. Presumably therefore there would be some Na^+ - K^+ exchange in the distal tubules of foetuses aged about 130 days which is the age of the foetuses used in the aldosterone experiments. Therefore superficially there is no dichotomy between the conclusions derived from the diuretic experiments and those derived from the hormone experiments. However when it is considered that in some experiments K^+ excretion rate

increased as much as five times, the suggestion that this was achieved by poorly developed mechanism seems improbable. Secondly the aldosterone induced kaliuresis had a latent period of over an hour compared with the immediate kaliuresis induced by the diuretic agents. Therefore it is likely that the mechanism of the aldosterone induced kaliuresis is different from the mechanism responsible for the increase in K^+ excretion following diuretic treatment.

The next hormone considered was cortisol which is basically a gluco-corticoid, but which produces variable effects on the renal excretion of water and electrolytes. These alterations depend on the initial patterns of electrolyte excretion and flow rate (Lipsett et al 1961). Cortisol can induce sodium retention and potassium excretion in adult mammals but much less effectively than aldosterone. The increased reabsorption of Na^+ is a result of a direct action of cortisol on the kidney, as in the case with aldosterone. However the increased excretion of K^+ may be largely the result of mobilization of K^+ from tissues rather than a direct action on the kidney. Under certain circumstances cortisol can increase Na^+ excretion and this is almost certainly due to the ability of cortisol to increase GFR. Aldosterone and desoxycorticosterone are ineffective in this regard (Ahmed et al 1967, Share and Travis 1968).

There are few reports available on the renal effects of cortisol in adult sheep, but Peter (1969) has found that cortisol infused at rates of between 60 and 100 µg/hour has no effect on renal function. Cortisol had no consistent effect on urine flow rate or electrolyte excretion rate in adult sheep. In three of the four experiments in which foetal sheep were infused with cortisol the $[Na^+]$ and $[K^+]$ of foetal urine increased following a latent period of 1 to 1½ hours and the electrolyte concentrations reached

were 70-100% greater than the pre-treatment averages. The available evidence suggests that these changes were due to increases in foetal GFR rather than to changes in the tubular handling of Na^+ and K^+. Apart from electrolyte excretion the excretion rates of all urinary solutes increased during cortisol treatment and there was a rise in endogenous creatinine clearance. Thus it seems likely that in foetal sheep as in some adult mammals, cortisol has the ability to increase GFR. The mechanism of this effect is not clear. In adults it is thought to be due to an ill-defined influence on the cardio-vascular system. For instance it is known, that in the absence of corticosteroids there is a reduction in cardiac output. Similarly Brown and Remington (1955) and Lefer and Sutfin (1964) have demonstrated that corticosteroids influence the contractile force of the heart. Irrespective of the mechanism, the possibility that cortisol influences foetal GFR is of significance in explaining the maturation of GFR, since the concentration of cortisol in foetal plasma increases with foetal age.

In conclusion it should be noted that in the cortisol experiment carried out on the oldest foetus, the urinary $[Na^+]$ before treatment was exceptionally high but shortly after treatment began the $[Na^+]$ decreased. Thereafter the concentrations of Na^+, K^+ and the other urinary solutes increased and finally stabilised at levels close to pre-treatment levels. So after the initial fall in urinary $[Na^+]$ the response in this experiment was comparable to the responses obtained in the other three cortisol experiments. However the initial effect on urinary $[Na^+]$ suggests that cortisol may act as a salt retaining hormone in foetal sheep and whether or not it does so may depend on the initial rate of Na^+ excretion.

To this point it has been concluded that during the last third of gestation exogenous aldosterone promotes Na^+ retention in foetal sheep

and that cortisol has a mild natriuretic and diuretic effect. Further, it is likely that the cortisol response is the result of an increase in GFR rather than changes in tubular reabsorption. One observation suggests that when Na^+ excretion rate is high, cortisol may have a salt-retaining effect. All of this information indicates the involvement of the foetal adrenal in regulating kidney function.

The next step in examining the influence of the foetal adrenal was to inject metyrapone into two foetuses aged 115 and 124 days. In adults metyrapone inhibits the biosynthesis of cortisol, corticosterone and aldosterone in the adrenal cortex by blocking 11β hydroxylase activity. Studies in foetal sheep by Wintour et al (1975) have shown that 11β and 17α hydroxylases are present from about the 60th day of gestation. They also claim that the adrenal glands of 40 day-old foetuses are capable of producing more cortisol and aldosterone per gm of body weight, than term adrenals. Nevertheless the plasma concentration of these hormones rises with increasing foetal age. Aldosterone rises regularly over the last 60 days of pregnancy while cortisol rises dramatically during the last 10 days. In foetuses of the same age as those used in the present experiments, the plasma concentration of cortisol is about 20 ng/ml (Liggins et al 1973) and the plasma concentration of aldosterone is about 0.03 ng/ml (Wintour et al 1975). In fact the rate of secretion of cortisol by foetal sheep 20 days before delivery, is similar to that observed in more mature foetuses; presumably therefore, the changes in concentration reflect changes in the metabolic clearance of cortisol (Liggins et al 1973).

All of this information confirms the existence of considerable 11β and 17α hydroxylase activity in the adrenal glands of foetal sheep, much earlier than the 115th day of gestation. Therefore it was anticipated that metyrapone would have the same action in foetuses as in adults and block 11β hydroxylase activity causing aldosterone secretion to be reduced and

Na^+ excretion increased. Alternatively, if the foetal adrenal can respond to ACTH and there is substantial evidence that it can (Wintour et al 1975, Liggins et al 1973), the rise in ACTH secretion that usually occurs during metyrapone treatment would stimulate additional secretion of 11-desoxycorticoi which would reduce Na^+ excretion.

In fact, on both occasions that metyrapone was infused into foetal sheep, there was a significant decrease in the concentration of Na^+ in foetal urine and a reduction in urine flow rate. The nett result in each case was about an 80% decrease in Na^+ excretion rate and with the exception of K^+, no other urinary solute was affected by metyrapone treatment. In one experiment K^+ excretion rate was unaffected, while in the other there was a reduction in K^+ excretion rate that obviously paralleled the change in urine flow rate. Thus the only consistent and significant result following metyrapone infusion was Na^+ retention. If it is accepted that this response was the result of ACTH release and a rise in the secretion of 11-desoxycorticoids then it is assumed that pituitary - adrenal feedback mechanisms are in operation in the foetus and that the foetal kidney is sensitive to the salt-retaining properties of the 11-desoxycorticoids. These assumptions are supported by a variety of evidence including results obtained in the present work.

In adults if metyrapone is administered simultaneously with a gluco-corticoid such as dexamethasone,it is possible to inhibit the rise in ACTH output and hence inhibit the increased secretion of 11-desoxycorticoids. Therefore such combined treatment often results in natriuresis and diuresis. An experiment of this type was carried out using a 123 day-old foetus and the anticipated increases in urine flow rate and Na^+ excretion rate were observed. These rises began after about one hour of treatment. The maximum Na^+ excretion rate recorded was 60% greater than the pre-treatment

average while the maximum flow rate was 93% greater than the pre-treatment average. A rise in GFR may have contributed to these changes, since K^+ excretion rate also increased and there was a 15% rise in endogenous creatinine clearance. However this would only account for part of the natriuresis and diuresis and the bulk of the response was presumably due to a decrease in Na^+ reabsorption by the foetal nephron.

In two further experiments, dexamethasone was administered alone. On both occasions the dexamethasone treatment produced a rise in urine output plus a comparable increase in Na^+ excretion rate and a smaller rise in K^+ excretion rate. Since dexamethasone is a synthetic adreno-cortical steroid, which in adults exhibits basic gluco-corticoid properties similar to cortisol and no Na^+ retaining ability, it may be capable of increasing GFR. The changes in endogenous creatinine clearance during these experiments were compatible with rises in GFR. However the GFR rises indicated by creatinine clearance would not have been sufficient to account for the changes in Na^+ excretion rate which were as much as 400%. In the first experiment, endogenous creatinine clearance increased by 25% and in the second experiment, it increased 20%. Nevertheless, since the metyrapone experiments have indicated the presence of normal pituitary-adrenal relationships, the administration of dexamethasone would presumably inhibit ACTH release. This would limit the secretion of endogenous mineralo-corticoids and since dexamethasone itself has no salt-retaining effect, the Na^+ retaining stimulus on the foetal kidney would be reduced. Consequently active Na^+ reabsorption and passive water reabsorption would be reduced resulting in the observed increases in urine flow rate and Na^+ excretion.

In general, the experiments carried out with dexamethasone and metyrapone indicate the existence of 11β hydroxylase activity in the foetal adrenals. Secondly it appears that when that enzyme is inhibited by metyrapone, the resultant alteration in corticosteroid output results in greater Na^+ and water retention. Since the effect of metyrapone blockage is to increase 11-desoxycorticoid secretion it seems that the foetal nephron is sensitive to the Na^+ retaining properties of those hormones. This is consistent with the observed sensitivity of the foetal kidney to aldosterone. Also the experiments using dexamethasone alone, and in combination with metyrapone, indicate the existence of normal pituitary-adrenal interactions in the foetus and suggest that the administration of synthetic gluco-corticoids suppresses the secretion of endogenous cortico-steroids. Further it appears that dexamethasone, like cortisol, has the ability to increase foetal GFR, although the synthetic compound has a more limited effect on GFR.

In view of the urinary responses displayed by foetal sheep following treatment with exogenous gluco-corticoids, further experiments were conducted in which ACTH was infused in an attempt to increase the secretion of endogenous cortico-steroids. In adult mammals ACTH stimulates the adrenal cortex to secrete cortisol, corticosterone, aldosterone and a number of other hormones. If this pituitary stimulus is absent the secretion of most of these hormones is markedly reduced and in fact the adrenal cortex atrophies. However, aldosterone secretion rate is relatively independent of the pituitary stimulus and the zona glomerulosa of the adrenal cortex, which is mainly responsible for the production of aldosterone, shows least atrophy in the absence of ACTH.

With respect to foetal sheep, the effect of ACTH on cortisol secretion has been studied by Liggins et al (1973) who infused 0.1 mg of

ACTH over 24 hours (4.2 ug/kg/hr) into two foetuses aged 115 days. They found that plasma cortisol concentration increased approximately four fold on both occasions with the peak values occurring within 12 hours. Nevertheless the maximum concentrations (18 and 27 ng/ml) were still approximately one third of those found 24 to 48 hours before birth. In other in vitro and in vivo experiments, Wintour et al (1975) demonstrated the ability of foetuses of a similar age to those used in the present work, to respond to ACTH by increasing cortisol and aldosterone secretion. With a 118 day-old foetus, they found that infusion of ACTH at the rate of 1.25 U/hr increased the secretion of aldosterone from 0.05 µg/hr to 0.5 µg/hr and the secretion of cortisol from 0.9 to 3 µg/hr. In the present work 3 foetuses aged between 113 and 121 days were infused with ACTH at rates of 10 to 17 µg/kg/hr for periods of 8 to 12 hours. On the basis of the work described above, it can be assumed that such doses would be sufficient to produce a maximum adrenal response and that the increases in cortisol and aldosterone secretion would be similar to those described by Wintour et al (1975) and Liggins et al (1973). In view of the demonstrated effects of exogenous cortisol and aldosterone a simultaneous rise in the secretion of endogenous cortisol and aldosterone is likely to result in conflicting influences on electrolyte excretion by the foetal kidney. In such circumstances the nett effect on foetal kidney function would depend on the relative increases of the two hormones and their relative potency with respect to Na^+ and water excretion or reabsorption.

In the youngest foetus infused with ACTH there was a progressive decrease in urine flow rate during the infusion period and a significant fall in Na^+ excretion rate, after a latent period of 3 hours. In the remaining experiments these trends were reversed with urine flow rate and Na^+ excretion rate exhibiting substantial rises. The K^+ excretion rates were

more variable, but in general they followed similar patterns to Na^+ excretion. If it is accepted that from about the 100th day of gestation onwards, the adrenal glands of foetal sheep become less responsive to ACTH in terms aldosterone secretion (Wintour et al 1975) and more responsive in terms of cortisol production then the ratio of aldosterone output to cortisol output following ACTH stimulation, will change with foetal age. In the youngest foetus (113 days) treated with ACTH the response may have been predominantly a rise in aldosterone secretion, resulting in Na^+ and water reabsorption. However in the older foetuses the response may have been predominantly a rise in cortisol secretion with less aldosterone secretion; in which case the primary influence on kidney function would have been cortisol, resulting in increased Na^+ and water excretion. In support of this proposal it was seen that with mature foetuses the changes in electrolyte excretion rate after ACTH treatment were comparable with the changes in foetuses of a similar age treated with cortisol. Finally, in foetuses 223 and 233 the endogenous creatinine clearance rates increased during ACTH treatment from 1.90 to 2.86 ml/min and from 1.36 to 2.23 ml/min respectively, yet the youngest foetus showed no consistent trend in endogenous creatinine clearance.

The ability of progestins to influence foetal kdiney function was also examined in this survey since there is some evidence that progesterone and 17α HP inhibit the activity of mineralo-corticoids in the renal tubules (Visser et al 1964). In fact Visser et al (1964) have studied salt metabolism in human infants and claim that the natriuretic effects of ACTH in infants are due to aldosterone antagonism by 17α HP. However when foetal sheep were injected with substantial doses of progesterone and 17α HP no evidence that either progestin had a direct or indirect effect on kidney function was obtained.

The final hormone used in foetal sheep was angiotensin II. In adults, angiotensin has intense vasoconstrictor and pressor properties plus a direct effect on the adrenal cortex leading to a relatively selective stimulation of aldosterone secretion. Such stimulation of aldosterone secretion and the resultant retention of Na^+, can be obtained with low doses of angiotensin that have little or no pressor effect (Gross 1958). It is not known with certainty how angiotensin exerts its effect on the adrenal cortex. There is evidence, that it acts early in the biosynthetic pathway for aldosterone, possibly before the cholesterol forming step (Kaplan 1965; Lommer and Wolff, 1966).

In addition to promoting a hormonal effect on kidney function, angiotensin given intra-venously can effect urine formation by several other mechanisms. Low doses of angiotensin cause vasoconstriction leading to a decrease in RBF, GFR and Na^+ excretion rate (Louis and Doyle 1964). Larger doses may saturate tubular receptors and inhibit Na^+ reabsorption (Leyssac et al 1961, Brown and Peart 1962). Finally angiotensin can produce a natriuretic effect by increasing filtration pressure and GFR (Selkurt 1951). There is no information currently available concerning the effect of angiotensin treatment on foetal kidney function.

In the present work three experiments were carried out in which foetuses aged 116, 125 and 140 days were infused with angiotensin II. All foetuses exhibited a rapid pressor response with the MAP increasing by 30% within minutes of beginning the infusion and returning to pre-treatment levels immediately after completing the infusion. With respect to urine production, all foetuses showed prompt reductions in urine flow rate and electrolyte excretion rate. These changes persisted for about 1½ hours and the probable cause was a fall in GFR resulting from the constriction of the afferent arterioles. The ability of vasoconstrictor drugs to reduce foetal

GFR has been confirmed in section 5.4.1. The fall in flow rate and Na^+ excretion rate is unlikely to have been due to increased aldosterone secretion since the response was immediate and aldosterone activity in foetal sheep has been shown to have a latent period of about one hour. Following the initial responses, the trends in urine flow rate and electrolyte excretion rate were quite different. The youngest foetus had a relatively uniform flow rate during the latter part of the infusion period, but the electrolyte concentrations and excretion rates varied so widely that no conclusions concerning angiotensin influence could be drawn. In the 140 day-old foetus, the flow rate during treatment was variable but increased overall as did urinary $[Na^+]$, resulting in a substantial natriuresis which persisted after treatment.

The diuresis and natriuresis that occurred during angiotensin treatment may have been due to a rise in filtration pressure and GFR, following intra-renal accommodation for the initial increase in vascular resistance. This is consistent with the observed changes in creatinine excretion rate but if a rise in GFR was responsible, it is difficult to understand why it persisted for two hours after treatment since the MAP returned to normal within minutes. In general these experiments do not permit firm conclusions to be drawn regarding the influence of angiotensin on foetal kidney function, except that angiotensin appears to alter vascular resistance.

6.3.2 Hormone Case Studies

Apart from hormone infusion experiments, the involvement of hormones in foetal kidney function was considered by comparing the plasma concentration or activity of a variety of endogenous hormones with urine flow rate and composition, in individual foetuses. These case studies could only yield correlations that may or may not indicate causative

relationships but it was anticipated that this would add to the information obtained in the infusion experiments.

In the first case study using foetus 66-345 there was an increase in plasma cortisol concentration and a decrease in total progestins during the last 8 days of pregnancy. These hormonal changes were associated with a rise in the excretion of most of the urinary solutes analysed and a rise in urine flow rate. Since it has been found that cortisol has a diuretic and natriuretic effect in foetal sheep it is tempting to speculate that the rise in cortisol caused the observed changes in kidney function by increasing GFR. The general nature of the excretion rate changes supports this possibility as does the fact that the rises in urinary $[Na^+]$ and $[K^+]$ were consistent with the short-term effects of increased cortisol concentration, seen in the infusion experiments. The relationship of reduced plasma progestin concentration with increased urine flow rate and electrolyte excretion rate is less likely to be significant. Progesterone and 17α HP had no apparent effect on kidney output when injected into foetal sheep. Also the only documented effect of these hormones on water and electrolyte excretion is the ability to act as aldosterone antagonists in which case a decrease in plasma progestin concentration would lead to reduced urine flow and Na^+ excretion, not the reverse.

The association between endogenous cortisol concentration and kidney activity was also evident in the second case study (foetus W177). In that study the cortisol level did not begin to rise until two days before delivery and a particularly high concentration was recorded on the last day of pregnancy. This rise in cortisol concentration was associated with high levels of urinary $[Na^+]$ and Na^+ excretion rate. The final case study involving steroid measurements, was carried out on foetus W38 between the 115th and 133rd days of gestation. This period precedes the usual time of cortisol increase and the only hormone that showed any significant

fluctuation was 20α HP. Predictably no significant changes in the concentration or excretion rate of the urinary solutes occurred and certainly none that could be related to the changes in hormone concentration.

In the remaining three case studies foetal PRA was compared with kidney output. In all cases, foetal PRA began to rise about 14 days before delivery and reached maximum levels within 2-3 days. Thereafter PRA declined erratically until term. This pattern is consistent with the overall trend in foetal PRA described in section 4.13.1. However in none of these studies was there a reliable association between PRA and urinary electrolyte concentration or excretion rate.

6.3.3. Foetal Hormone Concentrations

The third approach to the study of hormonal involvement in foetal kidney function was the determination of normal ranges for the concentration or activity of specific hormones in the plasma of foetal sheep throughout the period of gestation in which kidney activity had been studied. It was anticipated that in this way, statistically significant trends in hormone concentration which may be related to similar trends in urine output or composition, would be revealed.

Of the hormones considered, progesterone, 20α HP and cortisol showed changes in concentration that were significantly correlated with gestational age, as was PRA. Progesterone and 20α HP both increased in concentration approximately four fold between days 115 and 140 of gestation, but thereafter their concentrations decreased. Near-term, the progesterone level was about 75% of its peak value and 17α HP concentration about 35% of its maximum level. In contrast the positive correlation between plasma cortisol concentration and foetal age was due to large increases in concentration that begin sometime during the last 10 days of gestation and continue until term. Finally PRA increased regularly from about the 90th day of gestation

until 7 days before delivery and in that time the daily average for PRA increased 100%, but over the last 7 days of pregnancy PRA fell by 15%.

On the basis of the evidence previously discussed, the changes in plasma cortisol concentration and PRA are the most likely to influence foetal kidney function. However the possibility of subtle or-indirect effects caused by other hormonal changes cannot be dismissed. There is considerable evidence that cortisol will induce natriuresis and diuresis, possibly by raising GFR. Similarly the pressor effect of angiotensin in foetal sheep has been clearly established. Thus the secretion of angiotensin resulting from PRA may influence GFR in two ways. It may reduce GFR by constricting the afferent arterioles and raising vascular resistance or if the increase is vascular resistance is compensated for, the pressor effect of angiotensin may increase GFR. It has been shown in the present work that both MAP and GFR increase with foetal age and the trend in PRA during the last 35 days of gestation parallels very closely the trend in GFR.

6.4 Gestational Variations in Foetal Urine Composition

In section 4 the results obtained from the analysis of urine samples collected from untreated foetuses each day during the last 35 days of pregnancy are recorded. Also recorded are the daily averages for the percentage reabsorption of Na^+, K^+ and water on each day of the same period. With respect to the Na^+ content of foetal urine it was found that overall $[Na^+]$ was negatively correlated with foetal age. However, in reality this relationship only applied between days 115 and 145 during which time there was a 50% decrease in urinary $[Na^+]$. When those foetuses which lambed were considered alone, a five fold increase in urinary $[Na^+]$ during the last five days of gestation was revealed and this was reflected in a lesser increase in Na^+ excretion rate. The pre-parturient rise in urinary $[Na^+]$ and Na^+ excretion rate was so similar in timing and magnitude, to the rise in

cortisol concentration that a causative relationship is proposed. Such a relationship is consistent with the observed effects of exogenous cortisol on urinary [Na^+] and Na^+ excretion rate and with the association between these urinary parameters and endogenous cortisol concentration in the individual case studies. It appears likely that this association involves the effect of cortisol on foetal GFR. Inulin clearance was not measured in foetuses older than 146 days, but 25 estimates of endogenous creatinine clearance were made in the last six days of pregnancy. These indicate that a rise in GFR, which is independent of kidney size, does occur. Therefore it is probable that the rises in urinary [Na^+] and Na^+ excretion rate are due, in part, to a pre-parturient increase in GFR.

The data concerning water and electrolyte reabsorption revealed changes that are obviously the result of major changes in kidney function during the last five days of pregnancy. The fractional reabsorption of water is positively correlated with foetal age over the last 35 days of pregnancy, however the increase is particularly rapid during the last five days possibly because of additional water reaching the distal tubules following an increase in GFR. There was also a 6% reduction of Na^+ reabsorption during the five days before birth which would contribute to the observed increase in urinary [Na^+]. This fall in percentage Na^+ reabsorption may have been due to an increase in the filtered Na^+ load following a rise in GFR or to a change in the hormonal influences on tubular function. All of the alterations in the renal handling of Na^+ and water occurred after day 145 when PRA was reduced. If angiotensin stimulates aldosterone secretion in mature foetuses, as it may, despite the failure of exogenous angiotensin to produce a renal response in younger

foetuses; the fall in PRA would have resulted in reduced aldosterone secretion. This in turn may have been responsible for the fall in the percentage reabsorption of Na^+ and thus have contributed to the rise in Na^+ excretion.

Prior to day 145 there was a negative relationship between urinary [Na^+] and foetal age, but Na^+ excretion rate showed no significant trend. The rise in foetal PRA during this period may have contributed to the decrease in urinary [Na^+] by stimulating increased aldosterone secretion. This possibility is consistent with the evidence obtained in the diuretic experiments which suggests that Na^+ - H^+ and Na^+ - K^+ exchange in the distal tubules of foetal sheep increases until at least the 140th day of gestation. Also the furosemide and sodium ethacrynate experiments suggest that the capacity of the foetal tubules to reabsorb Na^+, increases with increasing foetal age. Since no foetus treated with either diuretic was older than 140 days, these experiments give no information concerning Na^+ reabsorption in the immediate prenatal period where urinary [Na^+] increased dramatically. But the fact that the Na^+ reabsorption mechanism that is inhibited by furosemide and ethacrynate seems to increase its capacity in foetuses aged between 118 and 140 days, is consistent with the observed decrease in urinary [Na^+] between days 115 and 145. All of these arguments appear to contradict the fact that the percentage reabsorption of Na^+ was negatively correlated with foetal age, yet in reality the only significant decrease in fractional Na^+ reabsorption occurred during the last five days of pregnancy. Further the percentage reabsorption of Na^+ between days 115 and 140 remained basically unaltered despite an overall increase in GFR. It therefore appears that the rise in filtered Na^+ is equalled and probably surpassed by additional Na^+ reabsorption in the ascending limb of the loop of Henle and by aldosterone mediated, Na^+ reabsorption in the distal tubules.

In summary, the gestational changes in urinary $[Na^+]$ and Na^+ excretion rate showed two distinct phases. The most significant phase was a rapid increase in $[Na^+]$ and Na^+ excretion during the last five days of gestation that is probably due to cortisol induced increases in GFR plus a decrease in Na^+ reabsorption due to reduced PRA. Earlier in gestation there was a gradual fall in urinary $[Na^+]$. This was probably due to a rise in the activity of the RAS leading to additional aldosterone secretion and also to the expanding capacity of the renal tubules to reabsorb Na^+. The second factor may be due to the maturation of enzymes within the tubule cells of the foetus or simply to an increase in the length of these tubules which would increase the surface area available for reabsorption.

The $[Na^+]$ of foetal plasma was positively correlated with foetal age over the last 35 days of gestation, although examination of the daily averages reveals a slight reversal of this trend in the five days before delivery. The gradual rise in plasma $[Na^+]$, was probably due to increasing Na^+ reabsorption by the foetal tubules prior to day 145. After day 145 there was a substantial increase in Na^+ excretion rate and although plasma $[Na^+]$ decreased slightly the changes were not proportional. It is possible that the loss of plasma Na^+ was minimised by non-renal homeostatic mechanisms in the foetus, or that there was an influx of maternal Na^+.

The $[K^+]$ of foetal urine was found to be positively correlated with foetal age and the correlation was independent of increasing kidney size. However the increase in $[K^+]$ was not consistent over the entire period of gestation examined. For example there was a decrease in concentration between days 132 and 137 followed by a rise until day 143 and a further decrease thereafter. With the exception of the period between days 132 and 137 the pattern for urinary $[K^+]$ was almost the exact converse of the pattern for urinary $[Na^+]$. The daily changes in the fractional reabsorption of K^+ are consistent with the observed changes in urinary $[K^+]$. Between days 115 and 144 there was an 11% decrease in the fractional reabsorption of K^+, but

after day 144 this trend was reversed and in the remaining five days there was a 5% increase in K^+ reabsorption.

Obviously the pre-parturient rise in the fractional reabsorption of K^+ was consistent with the decrease in urinary $[K^+]$ that occurred at the same time. It was also closely related to the pre-parturient decrease in the fractional reabsorption of Na^+ and the rise in urinary $[Na^+]$. In fact an inverse relationship between the reabsorption and excretion of Na^+ and K^+ is evident throughout most of the gestational period examined which confirms that Na^+ - K^+ exchange occurs in the foetal tubules. Further the simultaneous reversal of the trends in Na^+ and K^+ reabsorption and urinary concentration, that occur near day 144 confirms that the pre-parturient increase in urinary $[Na^+]$ and Na^+ excretion rate involves changes in tubular activity as well as a rise in GFR. However the relative magnitude of the changes in percentage reabsorption and excretion rate suggest that the increase in GFR still makes a significant contribution.

It has already been proposed that because of the close correlation of urinary $[Na^+]$ and the fractional reabsorption of Na^+ with PRA, that the RAS influences these parameters by altering aldosterone secretion rate. Thus if the observed changes in the fractional reabsorption of K^+ and urinary $[K^+]$ are due to Na^+ - K^+ exchange in the foetal tubules, then indirectly the RAS also influences the K^+ parameters. Finally if foetal GFR increases between days 115 and 140, as indicated by inulin and creatinine clearance, then the filtered Na^+ load would increase. If under those circumstances proximal reabsorption was unaltered, then the Na^+ load reaching the loops of Henle and the distal tubules would be increased. However it has been found that urinary $[Na^+]$ decreased marginally during that period, therefore Na^+ - K^+ exchange must have increased. If as is likely this readjustment was mediated by the RAS the increased Na^+ - K^+ exchange

would occur in the distal tubule which is known to be the site of aldosterone activity. Therefore there is evidence in the normal data that Na^+ - K^+ exchange in the distal tubules increases in foetuses aged between 115 and 140 days. Since a similar conclusion was arrived at from the diuretic experiments there seems little doubt that Na^+ - K^+ exchange occurs not only in the loops of Henle but also in the distal tubules. There is evidence that the Na^+ - K^+ exchange in the loops of Henle increases during gestation because of the increasing length of the tubules and because of greater Na^+ - K^+ ATPase activity. In the distal tubule there appears to be an increase in Na^+ - K^+ exchange prior to day 140 due to the influence of the RAS.

Plasma $[K^+]$ did not increase significantly during the last third of gestation. Generally the changes in plasma $[K^+]$ could not be related to the changes in urinary $[K^+]$, although a decrease in urinary $[K^+]$ between days 134 and 137 resembled a similar change in plasma $[K^+]$ that commenced five days later.

The first organic solute to be considered is uric acid which in adult mammals and presumably in foetuses, is formed by the breakdown of purines and by direct synthesis from glycine. In humans and other mammals uric acid enters the urine via glomerular filtration and secretion by the cell of the proximal tubules. Hyperuricemia is not uncommon in humans treated with diuretic agents because the diuretic competes with uric acid for secretion. The ethacrynate experiments conducted in the present study, indicate that proximal secretion of uric acid also occurs in foetal sheep as early as the 114th day of gestation.

The data from untreated foetuses revealed that the urinary concentration of uric acid is positively correlated with foetal age. More

significantly the data from foetuses which lambed revealed a three fold increase in the uric acid concentration of foetal urine during the last five days of pregnancy. The excretion rate of uric acid showed a pattern of change similar to concentration, although the increase in excretion rate before day 135 was lost when the values were corrected for kidney weight. Thus the observed changes in the concentration and excretion rate of uric acid before day 135 were probably due to the increasing number of functioning glomerulii in the growing kidney and secondly to the rise in GFR that occurs between days 115 and 135. However after day 135 the uric acid parameters increased sharply and the rises were independent of kidney size. If the plasma concentration of uric acid is examined, the reason for the urinary change is apparent.

There was no significant rise in the plasma concentration of uric acid between days 115 and 135 but there was a substantial rise in the last 15 days of gestation. The uric acid concentration of foetal plasma doubled during that period. If it is considered that this would lead to a proportional increase in the quantity of uric acid filtered at the glomerulii plus an increase in the amount secreted by the proximal tubules, the three fold increase in the urinary concentration of uric acid is readily explained. The increase in foetal GFR during the last 15 days of pregnancy would also contribute to the rises in the concentration and excretion rate of uric acid during that period. A study of the reasons for the rise in the plasma concentration of uric acid in near-term foetuses is outside the scope of this work since it presumably involves maturation of the enzyme mechanisms involved in purine metabolism.

Urinary urea concentration and urea excretion rate showed patterns of change similar to those exhibited by the corresponding uric acid parameters. Urinary urea concentration was positively correlated with

foetal age throughout the last 35 days of gestation and during the last 15 days, the urea concentration increased 100%. Predictably, urea excretion rate showed a similar association with foetal age. Kidney weight was found to be a factor in these correlations, however with both urea parameters about 75% of the correlation with foetal age was independent of kidney weight. Other factors involved in these urea changes would be changes in GFR and the increasing ability of the foetal liver to produce urea. It has been seen that all estimates of GFR indicate an increase in filtration rate up until the 135th day of gestation, while measurements of endogenous creatinine clearance suggest that there is a further increase from then until term. Secondly Kennan and Cohen (1959) and Colombo and Richterich (1968) have studied urea cycle enzymes in mammalian foetuses and concluded that the capacity to produce urea is a function of gestational age. Together these factors would explain the observed changes in urinary urea concentration and urea excretion rate. Perhaps more important than the cause of the rise in urea excretion is its possible significance in terms of overall kidney function.

The results obtained in foetal sheep certainly show that as gestation proceeds urea forms an increasing proportion of the total solute excreted and this is particularly so in the last 15 days of gestation. If the adult model is applied it can be argued that this increase will be of significance to the urinary concentrating ability of the foetal kidney. In adults, urea escaping from the collecting ducts accumulates in the interstitia fluid of the renal medulla because of the low effective blood flow in that region. This results in the development of a steep cortico-medullary concentration gradient which in the event of normal permeability of the distal tubules and collecting ducts will facilitate water reabsorption and increased urine concentration. It is likely that this also occurs in the

foetal kidney. Therefore as the amount of urea cleared by the foetal kidney increases, as indicated by the rise in urinary urea concentration, an increasingly steep cortico-medullary concentration gradient may develop. Stanier (1972) has reported the existence of a steep urea gradient in the renal tissue of mature foetal lambs. It now appears that this may be due to the substantial increase in the amount of urea reaching the distal tubules of foetal sheep during the last 15 days of gestation. This proposal would explain the rise in the percentage reabsorption of water that occurs between days 115 and 140. More specifically it is noticeable, that the exponential increase in urinary urea concentration that occurs in the last 15 days of foetal life is paralleled by a particularly rapid rise in the percentage reabsorption of water. This is probably due to the progressive development of the intra-renal urea gradient. Similarly the progressive development of such a gradient would account for the reported decrease in urine flow rate with foetal age (Alexander et al 1958b) and the reported increase in the hypertonicity of foetal urine as term approaches (Mellor and Slater 1972b). The flow rates reported in the present work were characteristically variable, but nevertheless there was a small negative correlation with foetal age. The diuretic studies indicate that Na^+ is transferred into the renal interstitium, but it appears that the transfer is limited. Accordingly the development of an effective intra-renal concentration gradient may have to await the increase in urea clearance that occurs in the last 15 days of gestation.

The concentration of the remaining organic solute, creatinine, was positively correlated with foetal age. However over the greater part of the last 35 days of gestation this trend was proportional to the increase in kidney weight. In contrast, the data obtained from foetuses which lambed revealed that during the last 15 days of pregnancy there was a three fold rise in urinary creatinine concentration that was independent of changes in

kidney weight. The available evidence concerning the foetal kidney suggests that as in adults, creatinine is neither secreted nor reabsorbed in significant amounts by the renal tubules. Therefore the changes in urinary creatinine concentration and creatinine excretion rate must be due to a rise in the amount of creatinine cleared by the foetal glomerulii. Since there is no increase in the concentration of creatinine in foetal plasma the rise in creatinine clearance must be due to a rise in GFR.

The morphological evidence previously discussed indicates that the number of functioning nephrons increases with foetal age. So also does foetal MAP. Accordingly the increase in creatinine excretion rate between days 115 and 135 is presumably due to a progressive increase in GFR resulting from structural changes within the foetal kidney and changes in filtration pressure. However after day 135 the rate of increase of creatinine excretion is greater than can be explained by these structural and pressure changes. Nevertheless because of the nature of creatinine excretion and its neutrality with respect to tubular function, this increase in creatinine concentration and excretion rate is still presumably the result of increased creatinine clearance and therefore increased GFR. In seeking a likely cause for such a rise in GFR that is independent of kidney size, the most obvious choice is the pre-parturient rise is plasma cortisol. The rise in cortisol concentration is similar to the changes in creatinine concentration and excretion rate and there is substantial evidence that cortisol has the ability to increase GFR, although the mechanism involved is ill-defined. Therefore it is proposed that the changes in urinary creatinine concentration and creatinine excretion rate prior to day 135 are due to a gradual increase in the number of functioning glomerulii in the foetal kidneys and possibly to a rise in filtration pressure due to changes

in MAP. After day 135 it is suggested that the rising level of plasma cortisol induces an additional rise in GFR that is independent of kidney structure and MAP and which further increases urinary creatinine concentration and creatinine excretion rate.

The final parameter examined in untreated foetuses was urine pH. The results obtained were variable and it was difficult to extract significant trends over the period of gestation considered. However when those foetuses that lambed were considered separately a pre-parturient decrease in urine pH was revealed. The decline commenced as early as 25 days before term and was obviously independent of changes in kidney weight. Mellor and Slater (1972a) have reported similar changes in the pH of foetal urine and they observed that the decrease in pH could commence as early as the 130th day of gestation or as late as the 145th day. Those authors offered no explanation for these changes except the vague proposal that "the pre-parturient decrease in urine pH results from changes in foetal plasma hormone concentrations".

The pattern of pH change is inversely related to the trend in plasma cortisol concentration but it is difficult to conceive of a mechanism whereby a rise in plasma cortisol would lead to an increase in H^+ excretion or perhaps to a change in the activity of urine buffers, resulting in a fall in pH. Nevertheless the possibility that cortisol is involved in the pH change cannot be dismissed, particularly since cortisol appears to be involved in other pre-parturient changes. Neither carbonic anhydrase activity, nor Na^+ - H^+ exchange is likely to be responsible for the increase in H^+ excretion. The acetazolamide experiments suggest that there would be no sudden increase in carbonic anhydrase activity near birth, since it appears to be near maximum in younger foetuses. Secondly the rise in urinary $[Na^+]$ and Na^+ excretion rate in the late stages of pregnancy, is not

consistent with an increase in H^+ excretion as a result of additional $Na^+ - H^+$ exchange. Accordingly no explanation for the pre-parturient decrease in foetal urine pH can be offered other than to suggest that an increase in metabolic acidosis occurs which may or may not be connected to the rise in plasma cortisol. If it is assumed that the observed rise in plasma cortisol concentration is associated with stimulation of intermediary metabolism in the foetus there may well be a tendency toward metabolic acidosis. It has been established that the foetal lamb has the capacity to respond to acidosis by increasing the excretion of titratable acid, ammonium and phosphate thereby producing a decrease in foetal urine (Smith and Schwartz, 1970). Further this capacity appears to be related to the number of functioning nephrons in the kidney and the renal blood flow which increases as the kidney matures. Together these factors may explain the progressive fall in the pH of foetal urine during the 25 days before birth. Also the fact that very low urinary pH levels were recorded on days 149 and 150 in foetuses that did not deliver normally may be a reflection of renal compensation for metabolic acidosis induced by stress.

6.5 Gestational Variations in Amniotic Fluid Composition

In addition to analysing the composition of foetal urine samples collected over the last 35 days of gestation, the composition of amniotic fluid samples collected over the same period was also examined. This was done in an attempt to assess the relationship between the composition of foetal urine and the composition of amniotic fluid. The concentration of specific solutes was found to vary widely between foetuses and from day to day in individual foetuses. This is not surprising in view of the variety of mechanisms that are thought to be involved in the formation and dynamics of amniotic fluid. Minor changes in any of these mechanisms could be responsible for transient or prolonged changes in the composition of amniotic fluid. Despite this variability, some solutes did show trends in concentration that were correlated with foetal age.

The concentration of uric acid in amniotic fluid was correlated with foetal age and the correlation was primarily due to a large rise between day 140 and term. Similarly the creatinine level in amniotic fluid was positively correlated with foetal age, although in this case there was a regular increase in concentration between days 115 and 135 of gestation, followed by a small decrease between days 135 and 140 and a further rise from then until term. With respect to the concentration of electrolytes in amniotic fluid; $[Na^+]$ showed a small negative correlation (-0.211) with foetal age but $[K^+]$ was not significantly correlated with foetal age. There was however, a small overall increase in the $[K^+]$ of amniotic fluid as gestation proceeded.

The changes in the concentration of uric acid in amniotic fluid are almost certainly due to the flow of foetal urine into the amniotic sac. In the last 10 days of pregnancy, the uric acid concentration of foetal urine increases exponentially and at this time, foetal urine is discharged primarily through the urethra into the amniotic sac. The trend in the uric acid level of amniotic fluid follows so closely the changes in urinary uric acid concentration that subsequent modification of the uric acid level of amniotic fluid appears to be slight. The levels of creatinine in urine and amniotic fluid are both positively correlated with foetal age which again suggests that the increase in urinary creatinine concentration contributes to the rise in the creatinine level of amniotic fluid. However, in addition, the creatinine concentration of amniotic fluid is considerably greater than that of foetal plasma and the difference widens as gestation proceeds. Therefore it may be that as this gradient widens a point is reached at which creatinine begins to diffuse from the amniotic fluid into the blood perfusing the amnion and foetal skin. Between days 125 and 135 the rate of decrease of plasma creatinine concentration is reduced, as is the rate of increase in the level of creatinine in amniotic fluid. Thus it may be that the increase in renal creatinine clearance, prior to day 125, reduces plasma creatinine concentration and contributes to the rise in amniotic fluid

creatinine. However after day 125, it is possible that the creatinine gradient between foetal plasma and amniotic fluid is so great that the recirculation of creatinine from the amniotic fluid into the foetal blood commences.

With respect to the concentration of electrolytes in amniotic fluid, the trends in $[Na^+]$ and $[K^+]$ generally reflected changes in the urinary concentration of those electrolytes. Both urinary $[Na^+]$ and the $[Na^+]$ of amniotic fluid decreased during gestation, although urinary $[Na^+]$ showed a pre-parturient rise that was not apparent in the $[Na^+]$ of amniotic fluid. Mellor and Slater (1972b) have proposed that one of the major effects of foetal urine on the $[Na^+]$ of amniotic fluid is dilution, because the $[Na^+]$ of foetal urine is considerably less than that of amniotic fluid. In the case of K^+, both the amniotic fluid and urinary concentrations increase during gestation, although the level of K^+ in amniotic fluid fluctuates widely from day to day. Nevertheless foetal urine apparently contributes K^+ to the amniotic fluid, but in this case there would be no dilution since the concentration of K^+ in urine is similar to the concentration in amniotic fluid.

Mellor and Slater (1972b) point out that foetal urine is not the only source of the Na^+ and K^+ in amniotic fluid as these ions also appear to enter the amniotic sac by diffusion. Clearly the Na^+ concentration gradient between foetal blood and amniotic fluid would favour the diffusion of Na^+ from the blood vessels in the amnion and foetal skin into the amniotic fluid. Similarly Na^+ could diffuse from maternal blood into the amniotic fluid across the amnio-allantois, although Mellor (1970) suggests that this is unlikely. For K^+, the concentration gradient between foetal blood and amniotic fluid would favour the movement of K^+ out of the amniotic fluid into the foetal blood.

6.6 Foetal Homeostasis and Maternal - Foetal Relationships

In section 5.1 experiments were described in which foetal sheep were subjected to various stimulii such as salt loading and haemorrhage, in an attempt to disrupt the foetal environment. It was hoped that under

these circumstances, the homeostatic capacity of the foetal kidney would be revealed.

In all but one of the salt loading experiments, the foetuses responded to treatment by increasing urine output and Na^+ excretion rate. There was no well defined association between the salt load administered and the response induced, which indicates that within the physiological limitations of the individual foetuses the maximum homeostatic response was always induced. However there does appear to be certain age-related factors in the renal response to salt loading. The first is the extent to which K^+ excretion rate increases in parallel with Na^+ excretion rate. In the youngest foetuses (111-118 days) the rises in K^+ excretion rate were comparable with the rises in Na^+ excretion rate, but in the 125 day-old foetus the rise in K^+ excretion rate was less than for Na^+ and in the oldest foetus (146 days) there was a decrease in K^+ excretion rate. The second age-related factor was the amount of Na^+ excreted in the two hours following salt loading. In the youngest foetuses this was between 2.6 and 3.7 times the quantity of Na^+ administered, compared with only 11-34% in the older foetuses. This suggests that there is a lack of precision in the mechanisms regulating salt excretion by the younger foetuses.

On the basis of these results and other evidence obtained in this research, it is proposed that in foetuses younger than about 120 days, the homeostatic response to salt loading is primarily an increase in GFR, although some decrease in Na^+ reabsorption by the loops of Henle and distal tubules would also occur. The fact that in younger foetuses the rises in K^+ excretion rate were comparable with the increases in Na^+ excretion implies that they were non-specific responses. Certainly there was no evidence of a decrease in K^+ excretion rate as would be expected if the homeostatic response was primarily due to inhibition of Na^+ reabsorption and reduced

Na^+ - K^+ exchange in the foetal nephrons. Secondly in the younger foetuses the response to the salt load is excessive, mainly because it is more prolonged than in the older foetuses and not because the percentage increase in Na^+ excretion rate is significantly greater. This is consistent with the proposal that in younger foetuses the renal response to salt loading primarily involves an increase in GFR because it was observed in section 5.4 that when a rise in GFR is stimulated along with changes in tubular function, the GFR change persists longer. Finally, the changes in endogenous creatinine clearance provide direct evidence in support of this proposal. In the younger foetuses, the maximum values for endogenous creatinine clearance, measured during or after the saline infusion were between 30% and 80% greater than the pre-treatment average for each foetus. In comparison the rise in the 125 day-old foetus was 11% while in the oldest foetus (146 days) endogenous creatinine clearance was unchanged (see appendix tables 29 to 34).

Therefore it is also proposed that in foetuses older than about 125 days, the homeostatic response to fluid and electrolyte imbalance primarily involves re-adjustment of tubular reabsorption or secretion rather than a change in GFR. This seems likely because under normal conditions GFR increases with increasing foetal age so the potential for additional rises in response to salt loading may be reduced. Secondly it appears that the capacity for Na^+ reabsorption by the foetal nephrons, increases with foetal age, possibly due to elongation of the loops of Henle and development of increased enzyme activity. Therefore it is probable that the inhibition of Na reabsorption in older foetuses will result in a more significant rise in Na^+ excretion than would occur in less mature foetuses, so the need to increase GFR to eliminate water and salt would be reduced.

One experiment was carried out in which 9% of the blood volume of a 125 day-old foetus was removed, resulting in a 15% decrease in PCV. Foetal blood pressure fell from 45 to 42 mm Hg within ten minutes of the blood loss

but had returned to 45 mm Hg, 20 minutes later. Urine flow rate fell by 43% in the half hour after haemorrhage and the excretion rates of Na^+ and K^+ fell by 87% and 71% respectively. Urine flow rate, $[K^+]$ and K^+ excretion rate had returned to normal by the end of the experiment (3½ hr. after treatment) but $[Na^+]$ and Na^+ excretion rate were only 50% of their respective pre-treatment averages. The initial decreases in flow rate, electrolyte concentration and electrolyte excretion rate were so prompt, having commenced within ten minutes of the blood loss, that they were unlikely to have been due to hormone induced changes in foetal tubule function. It is more likely that they were the result of a fall in foetal GFR, following the fall in systemic blood pressure.

The fact that the changes in flow rate and excretion rate persisted after the MAP returned to normal may indicate that the mechanisms for maintaining renal perfusion pressure are less effective, at this stage of gestation, than those for maintaining general systemic blood pressure. This suggestion is supported by the fact that prior to haemorrhage endogenous creatinine clearance was 2.2 ml/min compared with 0.71 ml/min in the 30 minutes after treatment. By the end of the experiment endogenous creatinine clearance had risen to 1.71 ml/min. Not only did endogenous creatinine clearance follow this pattern of change, but so did the excretion rates of all urinary solutes except Na^+. Since it is such a broad effect it is almost certainly due to changes in GFR.

Accordingly, two obvious features of the foetal response to blood loss are the ability to maintain systemic blood pressure, apart from a transient decrease immediately after haemorrhage and the ability to restore GFR to near a pre-treatment level within 3½ hours of the blood loss. The latter ability probably involves a decrease in the pre-glomerular vascular

resistance due to dilation of afferent arterioles, but may also involve an increase in the proportion of total RBF that is channeled through the cortex. These vascular changes may be mediated by the RAS with the reduced renal perfusion pressure following haemorrhage, stimulating a rise in PRA. The pituitary-adrenal axis may also be involved. The reduction in blood volume or plasma electrolyte concentration may have stimulated the pituitary to release ACTH and the resultant increase in plasma cortisol possibly induced a rise in GFR. No direct evidence concerning these possibilities was obtained, however Alexander et al (1971 a and b) have reported that haemorrhage in foetal sheep is followed by an increase in ACTH secretion.

A third significant feature of the foetal response to haemorrhage was that even when GFR had apparently been restored, urinary [Na^+] and Na^+ excretion rate were only 50% of their pre-treatment levels. In fact it was not until 2½ hours after haemorrhage that the [Na^+] began to increase. This implies that in addition to the change in GFR, Na^+ excretion rate was limited by an independent mechanism employed to correct the electrolyte imbalance caused by the blood loss. The Na^+ retaining influence was presumably aldosterone and it is very likely that an increase in aldosterone secretion followed a rise in PRA or ACTH secretion or both.

Other experiments were carried out in which attempts were made to disrupt the fluid balance of foetal sheep. In two of these experiments, sucrose was injected into the amniotic fluid and this produced rises in the plasma OP and PCV of the foetus, presumably because of water loss across the amnion and foetal skin. Predictably the response of the foetal kidney under these circumstances was similar to that observed following haemorrhage. In both experiments sucrose treatment was followed by a reduction in urine flow rate. This was more apparent in the youngest foetus where the water loss may have been higher due to the greater permeability of the foetal

skin (Lind et al 1972). Not only did urine output decrease but there was a substantial reduction in the excretion rate of all urinary solutes.

Again the results indicate that the foetal fluid loss caused a reduction of foetal GFR and the changes in the rate of endogenous creatinine clearance support this proposal. In the first experiment, pre-treatment creatinine clearance was 5.38 ml/min but following the sucrose infusion it decreased to a minimum of 3.07 ml/min and then varied between 3.07 and 3.55 ml/min. In the second experiment the pre-treatment creatinine clearance was 3.42 ml/min and following treatment values of 2.29 and 2.11 ml/min were recorded. The most likely cause of these changes in GFR is reduced renal perfusion pressure following the loss of water from the foetus. Nevertheless in such circumstances, where foetal blood volume is decreased, a rise in ADH activity would be anticipated and it is conceivable that the pressor effect of ADH could reduce GFR by increasing renal vascular resistance. In one of the ADH infusion experiments there was a rise in foetal blood pressure and a decrease in urine flow that may have been due in part to a fall in GFR. However the doses of ADH used in those experiments were relatively large and it is doubtful that in the sucrose experiments the water loss would induce the secretion of sufficient endogenous ADH to exert a significant pressor effect.

It could be argued that the changes in urine flow rate that occurred in these experiments were due to a rise in ADH secretion resulting in increased water reabsorption in the distal tubules. If this were the case it is unlikely that there would have been a general decrease in the urinary concentration of K^+, uric acid and creatinine, in fact the reverse would be anticipated. Finally the results of these two experiments and the haemorrhage experiment are consistent with the proposal that in foetuses of about 125 days of gestational age or younger, renal homeostasis results primarily from changes in GFR. Certainly in these three experiments the youngest foetus showed the greatest variation in GFR, as assessed by endogenous creatinine clearance.

The final experiment of this type involved the infusion of sucrose into a ewe in an attempt to establish a trans-placental osmotic gradient which would encourage an efflux of foetal water. Such a gradient was established and a reduction of foetal urine flow did occur, although surprisingly the period of maximum osmotic imbalance and the period of minimum foetal urine flow did not coincide, indicating a latent period in the foetal response to water loss. Also water conservation persisted after the maternal-foetal osmotic gradient was obliterated, again suggesting a lack of precision in the water conserving mechanisms employed by the foetus. As in previous experiments there was some decrease in urinary $[Na^+]$ and Na^+ excretion rate following the sucrose infusion, but the concentrations and excretion rates of the remaining urinary solutes were variable before and after treatment and there was no clear indication that these parameters were reduced. Nevertheless there were significant changes in endogenous creatinine clearance which indicate that the renal response of the foetus involved a decrease in GFR. The average pre-treatment value for creatinine clearance was 3.61 ml/min but 30 minutes after treatment it was 2.25 ml/min and at the end of the experiment it was 2.60 ml/min.

The relationship between the output and composition of maternal and foetal urine was studied in a preparation involving a 122 day-old foetus. Urine samples from mother and foetus were collected simultaneously over a 24 hour period. The urinary flow rate, Na^+ and K^+ excretion rates and urine pH of mother and foetus were compared. Flow rates, Na^+ excretion rates and pH were found to be significantly correlated but maternal and foetal K^+ excretion rates were not correlated. The association between water and Na^+ excretion in mother and foetus was further investigated in three experiments. In two of these experiments the ewe was water depleted using furosemide and in the third experiment the ewe was salt loaded by infusing hypertonic saline.

In the first experiment involving water depletion, the furosemide treatment was insufficient to significantly decrease the total body water (TBW) of the ewe. The total water loss induced by the furosemide treatment

was 413 ml and TBW decreased by only 1.7%. Accordingly the data concerning foetal urine output and composition provides little information on foetal renal homeostasis during maternal dehydration. In the second experiment, involving a 118 day-old foetus, the maternal furosemide treatment was prolonged, resulting in a water loss of 1.8L, a 5.6% decrease in maternal TBW and a 12% increase in the OP of maternal plasma. Of the 1.8L of water lost by the ewe, over half was lost in the first two hours of furosemide treatment. During that time the flow rate of foetal urine decreased by 85% and there was a substantial decrease in the excretion rate of all the urinary solutes considered. However after the first two hours of maternal treatment these trends were reversed and ultimately foetal urine flow reached a level greater than the pre-treatment level. Similarly the $[Na^+]$ and $[K^+]$ of foetal urine increased and in combination with the rise in flow rate resulted in natriuresis and kaliuresis. The natriuresis and kaliuresis were similar to those observed in the first water depletion experiment although on this occasion the increases in Na^+ and K^+ excretion were greater.

It is proposed that the initial decrease in flow rate and solute excretion was again due to reduced GFR following the loss of foetal plasma water to the ewe during the first two hours of treatment. It is further proposed that the reversal of this pattern after three hours of treatment was the result of furosemide crossing the placenta and directly affecting the foetal kidney. Wladimiroff (1974) has provided evidence that furosemide can cross the placenta and affect urine output by the foetal kidney. By the third hour of the experiment, 40 mg of furosemide had been administered to the ewe and since doses as low as 0.8 mg/kg can induce a substantial response in foetuses of this age, it is quite conceivable that sufficient furosemide had entered the foetus to induce the observed response.

The final experiment in this series involved the infusion of hypertonic saline into the jugular vein of a ewe carrying a 122 day-old foetus. This had the effect of altering the Na^+ concentration gradient between maternal and foetal plasma from 1-2 mEq/L in favour of the foetus to 13 mEq/L in favour of the ewe. As a result, foetal urine flow decreased

by about 70% while the urinary concentrations of Na^+, creatinine and uric acid increased between 2 and 8 times and urinary K^+ increased by 70%. Thus apart from a transient decrease in the excretion rate of all solutes at the time of minimum urine flow, the excretion rates were not significantly altered. So it would appear that the changes in the concentration of urinary solutes was due to water retention. This in turn indicates that, on this occasion, the renal response was due to a rise in water reabsorption and not to a decrease in GFR, as in earlier experiments. This is supported by the fact that endogenous creatinine clearance during the pre-treatment period was 2.0 ml/min and one hour after treatment it was 2.58 ml/min.

It can be argued, that in this experiment the treatment resulted in a loss of plasma water from the foetus due to a saline-induced osmotic gradient favouring the ewe and secondly that there was an influx of maternal Na^+ into the foetus as a result of the Na^+ concentration gradient. In fact the $[Na^+]$ of foetal plasma did increase from a pre-treatment average of 144.4 mEq/L to a maximum of 148.6 mEq/L. Under these circumstances it would not be in the best interests of the foetus to compensate for the water loss by reducing GFR. If that occurred it would also reduce the rate of Na^+ excretion and compound the imbalance resulting from the influx of maternal Na^+. Similarly, the rise in plasma $[Na^+]$ could not be overcome by increasing GFR, as occurred when water and salt were administered simultaneously, since that would contribute further to the foetal water loss. The most suitable response would be to reduce urine flow rate by increasing water reabsorption and at the same time maintain or if possible increase urinary $[Na^+]$. This is precisely what occurred in this experiment. The most likely stimulus for the increase in water reabsorption would be ADH released from the foetal pituitary in response to the changes in the degree of hydration and

electrolyte concentration within the foetus. Alexander et al (1971) have demonstrated the ability of foetal sheep, as young as 107 days, to secrete arginine vasopressin (ADH).

In the final phase of this experiment, the ewe was permitted access to water and drank 1½ L within minutes. As a result foetal urine flow doubled within 30 minutes. In the same period the $[Na^+]$ of foetal plasma decreased from 148.5 to 144.1 mEq/L and urinary $[Na^+]$ fell by 50%. Presumably the maternal water intake had rapidly reversed the transplacental osmotic gradient established by the saline infusion allowing water to pass into the foetus. Accordingly foetal plasma $[Na^+]$ and OP would have been reduced to normal levels, thereby removing the need for renal water conservation.

In summary the experiments concerning foetal renal homeostasis and maternal-foetal relationships have indicated quite clearly that foetuses in the last third of gestation have the ability to compensate for disruptions to normal fluid and electrolyte balance. This ability is apparent irrespective of whether the disruptions are the result of direct manipulation of foetal body fluids or indirect disruptions consequent upon changes in the composition of maternal fluids or amniotic fluid. The renal mechanisms involved in these homeostatic activities include both changes in GFR and changes in the reabsorptive activity of the renal tubules. The relative involvement of each appears to be a function of foetal age and the nature of the imbalance in the internal environment of the foetus.

APPENDIX TABLE 1

CONCENTRATION OF ELECTROLYTES IN FOETAL PLASMA

DAY OF GESTATION	[Na+] ($\frac{mEq}{L}$)				[K+] ($\frac{mEq}{L}$)			
115	147.5			(1)	3.9			(1)
116	142.0	±	2.1	(3)	4.4	±	.3	(3)
117	135.7	±	7.0	(3)	3.9	±	.3	(3)
118	104.5			(1)	3.4			(1)
119	132.3	±	1.2	(2)	4.1	±	.4	(2)
120	137.7	±	2.1	(3)	4.4	±	.3	(3)
121	138.9	±	2.1	(5)	4.4	±	.2	(5)
122	141.2	±	2.2	(3)	4.3	±	.1	(3)
123	141.1	±	1.5	(4)	4.8	±	.2	(4)
124	142.3	±	1.0	(4)	4.3	±	.2	(4)
125	140.1	±	1.3	(5)	4.6	±	.2	(5)
126	141.8	±	0.4	(4)	4.6	±	.1	(4)
127	142.9	±	1.1	(8)	4.4	±	.1	(8)
128	141.2	±	1.1	(8)	4.6	±	.1	(8)
129	149.4	±	4.2	(7)	4.8	±	.2	(7)
130	147.5	±	1.2	(7)	4.5	±	.1	(7)
131	146.5	±	2.1	(5)	4.4	±	.2	(6)
132	145.5	±	1.8	(8)	4.7	±	.2	(9)
133	146.3	±	1.0	(9)	4.7	±	.1	(8)
134	146.5	±	1.9	(8)	4.5	±	.2	(10)
135	147.9	±	5.0	(7)	4.8	±	.2	(5)
136	148.1	±	2.1	(6)	4.5	±	.1	(6)
137	145.5	±	2.7	(7)	4.6	±	.1	(7)
138	144.3	±	1.8	(6)	4.4	±	.1	(6)
139	144.1	±	2.0	(7)	4.4	±	.1	(7)
140	132.1	±	2.5	(4)	4.1	±	.1	(4)
141	141.6	±	3.4	(4)	4.3	±	.1	(4)
142	144.6	±	1.7	(4)	4.5	±	.2	(3)
143	142.3	±	1.2	(2)	5.3	±	.1	(2)
144	139.2	±	1.5	(3)	4.5	±	.3	(3)
145	141.8	±	1.2	(2)	4.9	±	.5	(3)
146	150.0	±	3.6	(2)	4.3	±	.1	(2)
147	144.8	±	1.2	(2)	4.3	±	.1	(2)
148	144.8	±	1.6	(2)	4.1	±	.2	(2)

APPENDIX TABLE **2**

CONCENTRATION OF ELECTROLYTES IN FOETAL URINE

DAY OF GESTATION	[Na+] ($\frac{mEq}{L}$)				[K+] ($\frac{mEq}{L}$)			
115	28.0			(1)	13.0			(1)
116	39.5	±	0.4	(2)	5.0	±	0.7	(2)
117	70.8	±	15.4	(2)	10.0	±	5.7	(2)
118	29.3	±	10.1	(2)	6.5	±	3.9	(2)
119	34.0			(1)	4.0			(1)
120	20.3	±	2.5	(3)	10.5	±	4.6	(2)
121	33.6	±	7.0	(4)	10.3	±	2.2	(4)
122	22.1	±	2.3	(4)	17.5	±	5.4	(4)
123	32.6	±	7.3	(7)	9.6	±	2.3	(5)
124	26.2	±	7.6	(7)	11.1	±	2.5	(7)
125	36.9	±	8.0	(7)	14.6	±	2.3	(7)
126	27.9	±	8.1	(8)	12.3	±	2.0	(8)
127	23.9	±	4.1	(8)	14.9	±	2.5	(10)
128	27.2	±	6.4	(9)	17.0	±	4.4	(9)
129	30.1	±	5.2	(8)	14.5	±	2.4	(8)
130	25.4	±	5.3	(8)	10.0	±	1.6	(8)
131	32.8	±	5.8	(6)	15.6	±	2.8	(5)
132	28.6	±	3.9	(5)	10.6	±	3.2	(5)
133	19.8	±	3.9	(9)	27.8	±	8.2	(9)
134	17.1	±	3.6	(7)	17.4	±	3.0	(7)
135	18.8	±	4.4	(8)	15.6	±	2.9	(8)
136	24.7	±	4.4	(6)	10.8	±	2.8	(6)
137	31.0	±	7.4	(5)	9.0	±	1.5	(5)
138	30.9	±	7.5	(8)	12.6	±	2.6	(8)
139	32.0	±	7.6	(9)	16.0	±	3.1	(9)
140	25.7	±	6.3	(6)	15.8	±	4.3	(6)
141	19.3	±	3.6	(6)	35.0	±	14.3	(6)
142	21.9	±	11.3	(5)	29.6	±	9.6	(5)
143	12.5	±	3.5	(3)	30.0	±	3.1	(3)
144	10.4	±	1.9	(4)	27.4	±	9.1	(5)
145	18.1	±	7.1	(4)	16.6	±	3.9	(5)
146	23.3	±	8.1	(3)	18.3	±	4.6	(4)
147	28.1	±	6.3	(4)	17.3	±	5.3	(4)
148	15.6	±	3.1	(4)	29.3	±	6.5	(4)
149	15.3	±	7.3	(2)	13.5	±	4.8	(4)
150	22.0			(1)	74.0			(1)

APPENDIX TABLE **3**

CONCENTRATION OF ELECTROLYTES IN FOETAL URINE

(Foetuses that delivered normally)

DAYS PRIOR TO PARTURITION	[Na+] ($\frac{mEq}{L}$)				[K+] ($\frac{mEq}{L}$)			
25	39.9			(1)	18.0			(1)
24	73.0			(1)	13.0			(1)
23	62.0			(1)	13.0			(1)
22	21.3	±	5.9	(2)	18.0			(1)
21	20.5	±	1.8	(2)	11.67	±	2.0	(3)
20	13.3	±	0.2	(2)	14.7	±	2.9	(3)
19	10.8	±	0.2	(2)	11.3	±	2.3	(3)
18	17.0			(1)	14.0	±	6.4	(2)
17	15.8	±	0.5	(2)	18.0	±	2.6	(3)
16	35.0	±	16.9	(4)	12.6	±	2.1	(5)
15	32.5	±	9.2	(3)	13.0	±	3.5	(4)
14	30.1	±	11.1	(4)	12.0	±	2.9	(4)
13	30.6	±	9.0	(5)	8.3	±	1.8	(4)
12	23.2	±	5.7	(4)	13.4	±	3.6	(5)
11	30.2	±	6.4	(5)	12.8	±	3.9	(4)
10	28.8	±	4.4	(6)	24.7	±	16.1	(6)
9	21.5	±	6.1	(7)	19.2	±	10.1	(6)
8	21.2	±	4.4	(8)	16.3	±	3.4	(7)
7	19.8	±	4.5	(8)	22.4	±	3.7	(7)
6	11.5	±	4.6	(2)	18.5	±	7.5	(2)
5	18.7	±	4.4	(5)	22.0	±	7.1	(5)
4	30.6	±	4.2	(5)	16.4	±	4.1	(5)
3	28.2	±	5.4	(6)	21.67	±	5.4	(6)
2	32.1	±	10.2	(6)	24.8	±	12.4	(6)
1	53.3	±	14.5	(5)	29.0	±	23.6	(5)

APPENDIX TABLE 4

FOETAL ELECTROLYTE EXCRETION RATES

DAY OF GESTATION	NaER (μEq/min)		KER (μEq/min)	
115	13.2	(1)	6.1	(1)
116	16.0 ± 3.1	(2)	2.2 ± 0.7	(2)
117	23.0 ± 9.1	(2)	1.8 ± 0.2	(2)
118	26.4 ± 12.1	(2)	6.3 ± 0.2	(2)
119	11.6	(1)	1.4	(1)
120	9.6 ± 3.4	(2)	5.5 ± 3.1	(2)
121	6.5 ± 1.0	(4)	6.4 ± 3.7	(4)
122	10.0 ± 4.1	(4)	6.2 ± 2.2	(4)
123	11.8 ± 2.3	(4)	5.0 ± 1.5	(4)
124	6.5 ± 1.6	(6)	4.3 ± 1.7	(6)
125	17.7 ± 8.9	(7)	5.2 ± 1.5	(7)
126	7.2 ± 1.7	(7)	5.1 ± 1.7	(8)
127	12.9 ± 2.8	(8)	10.6 ± 2.6	(8)
128	12.1 ± 2.3	(7)	8.7 ± 3.4	(7)
129	18.7 ± 7.5	(7)	8.2 ± 2.7	(7)
130	9.2 ± 1.6	(8)	4.7 ± 1.2	(8)
131	15.6 ± 5.2	(6)	5.3 ± 1.4	(6)
132	9.7 ± 1.0	(5)	3.4 ± 0.3	(5)
133	8.3 ± 2.1	(9)	9.4 ± 2.4	(9)
134	7.1 ± 1.5	(7)	9.0 ± 2.1	(6)
135	11.0 ± 2.8	(9)	7.5 ± 1.7	(9)
136	18.7 ± 6.8	(6)	6.3 ± 1.3	(5)
137	29.5 ± 11.5	(5)	7.2 ± 1.3	(5)
138	21.3 ± 3.2	(8)	8.8 ± 1.4	(8)
139	24.5 ± 9.4	(8)	8.0 ± 2.0	(9)
140	13.2 ± 3.6	(7)	9.4 ± 1.4	(6)
141	9.6 ± 2.9	(5)	11.8 ± 3.1	(6)
142	8.9 ± 1.8	(6)	12.7 ± 3.4	(5)
143	6.4 ± 2.5	(3)	10.6 ± 1.2	(3)
144	9.9 ± 4.0	(5)	12.3 ± 3.4	(5)
145	17.4 ± 5.6	(5)	5.7 ± 1.3	(5)
146	17.3 ± 7.4	(3)	7.3 ± 1.7	(4)
147	40.7 ± 21.5	(4)	8.0 ± 1.6	(4)
148	3.3 ± 0.01	(2)	8.1 ± 2.7	(4)
149	5.3	(1)	7.2 ± 1.4	(2)
150	-		17.8	(1)

APPENDIX TABLE 5

CONCENTRATION OF ELECTROLYTES IN AMNIOTIC FLUID

DAY OF GESTATION	[Na+] (mEq/L)			[K+] (mEq/L)		
115	99.0		(1)	8.1		(1)
116	93.5	± 6.7	(2)	10.5	± 1.3	(2)
117	83.0		(1)	11.2		(1)
118	95.0	± 10.6	(2)	9.1	± .01	(2)
119	95.0	± 9.2	(2)	10.3	± 1.6	(2)
120	80.8	± 5.9	(2)	10.9	± .07	(2)
121	97.0	± 3.6	(2)	9.6	± 2.0	(2)
122	110.5	± 8.9	(2)	12.1	± 0.3	(2)
123	80.0	± 2.1	(2)	11.5	± 0.3	(2)
124	97.8	± 4.1	(5)	12.6	± 0.8	(5)
125	103.0	± 8.0	(4)	13.1	± 0.6	(4)
126	92.0	± 5.2	(5)	13.0	± 1.0	(5)
127	93.0	± 4.2	(4)	13.6	± 2.1	(4)
128	85.0	± 5.4	(6)	12.6	± 2.6	(6)
129	95.1	± 5.5	(5)	10.2	± 1.0	(5)
130	75.3	± 5.9	(3)	11.9	± 1.2	(3)
131	93.7	± 2.7	(3)	10.7	± 0.9	(4)
132	80.0	± 3.2	(4)	14.0	± 3.4	(4)
133	86.8	± 6.8	(4)	14.6	± 1.0	(4)
134	89.3	± 1.8	(3)	10.5	± 0.3	(2)
135	85.8	± 1.4	(4)	13.3	± 1.2	(4)
136	94.3	± 5.2	(3)	11.9	± 0.9	(5)
137	85.0	± 3.7	(5)	11.9	± 0.9	(5)
138	92.2	± 5.1	(5)	12.7	± 0.8	(5)
139	91.0	± 2.2	(4)	11.9	± 0.6	(4)
140	81.5	± 3.2	(2)	13.4	± 1.8	(2)
141	92.3	± 6.0	(3)	12.3	± 1.4	(3)
142	102.0		(1)	10.1		(1)
143	97.1		(1)	8.9		(1)
144	86.0		(1)	9.8		(1)
145	85.2		(1)	13.1		(1)
146	88.2		(1)	12.7		(1)
147	66.0		(1)	13.3		(1)

APPENDIX TABLE **6**

CONCENTRATION OF SOLUTES IN FOETAL PLASMA

DAY OF GESTATION	[Cr] (mg/100 ml)	[UA] (mg/100 ml)	DAYS PRIOR TO PARTURITION	[UA] (mg/100 ml)
115	13.7 (1)	1.4 (1)		
116	4.6 ± 1.8 (3)	0.9 ± 0.2 (3)		
117	4.2 ± 1.6 (3)	1.1 ± 0.3 (3)		
118	4.3 ± 0.3 (2)	1.1 ± 0.4 (2)	22	0.7 (1)
119	4.7 ± 0.9 (2)	1.2 ± 0.4 (2)	21	0.6 (1)
120	3.6 ± 0.6 (3)	0.9 ± 0.3 (2)	20	0.5 (1)
121	2.2 ± 0.7 (5)	0.8 ± 0.3 (4)	19	0.6 (1)
122	3.7 ± 1.8 (3)	1.2 ± 0.4 (3)	18	0.5 (1)
123	1.9 ± 0.6 (4)	0.8 ± 0.2 (4)	17	0.6 ± 0.1 (3)
124	2.2 ± 0.7 (3)	2.0 ± 0.8 (3)	16	0.6 ± 0.1 (3)
125	3.1 ± 1.6 (5)	1.0 ± 0.4 (5)	15	0.6 ± 0.1 (3)
126	1.9 ± 0.3 (4)	2.4 ± 0.9 (4)	14	0.6 ± 0.1 (4)
127	1.9 ± 0.2 (8)	1.4 ± 0.5 (8)	13	0.6 ± 0.1 (5)
128	1.9 ± 0.3 (8)	1.1 ± 0.5 (8)	12	0.5 ± 0.2 (6)
129	1.8 ± 0.3 (7)	0.5 ± 0.1 (7)	11	0.6 ± 0.2 (4)
130	1.6 ± 0.1 (8)	1.0 ± 0.4 (7)	10	0.6 ± 0.2 (7)
131	1.8 ± 0.2 (4)	0.7 ± 0.1 (5)	9	0.7 ± 0.2 (5)
132	2.8 ± 1.2 (7)	1.0 ± 0.3 (6)	8	0.7 ± 0.2 (6)
133	2.9 ± 1.1 (8)	0.8 ± 0.2 (8)	7	1.0 ± 0.9 (6)
134	1.8 ± 0.2 (9)	0.8 ± 0.2 (9)	6	0.6 ± 0.1 (3)
135	1.8 ± 0.2 (6)	0.7 ± 0.1 (7)	5	1.1 ± 0.7 (5)
136	1.6 ± 0.8 (6)	0.6 ± 0.1 (6)	4	0.8 ± 0.1 (3)
137	1.8 ± 0.2 (7)	0.7 ± 0.1 (7)	3	1.0 ± 0.5 (5)
138	1.7 ± 0.3 (6)	0.7 ± 0.1 (6)	2	0.9 ± 0.1 (4)
139	2.0 ± 0.3 (7)	1.0 ± 0.3 (7)	1	1.22 ± 0.4 (5)
140	1.6 ± 0.4 (4)	0.6 ± 0.1 (4)		
141	1.3 ± 0.2 (4)	1.1 ± 0.4 (4)		
142	1.3 ± 0.1 (3)	0.7 (1)		
143	3.2 ± 1.0 (2)	1.2 ± 0.6 (2)		
144	1.3 ± 0.7 (3)	0.7 ± 0.2 (3)		
145	1.0 ± 0.1 (3)	1.03 ± 0.4 (3)		
146	1.2 ± 0.3 (2)	0.6 ± 0.1 (2)		
147	1.0 ± 0.2 (2)	0.6 ± 0.1 (2)		
148	1.4 ± 0.2 (2)	0.9 ± 0.2 (2)		

APPENDIX TABLE 7

CONCENTRATION OF SOLUTES IN FOETAL URINE

DAY OF GESTATION	[Cr] (mg/100 ml)		[UA] (mg/100 ml)		[Urea] (mg/100 ml)	
115	10.0	(1)	1.4	(1)	209	(1)
116	4.3 ± 0.9	(2)	0.8 ± 0.04	(2)	178 ± 16	(2)
117	4.7 ± 0.5	(2)	0.8 ± 0.2	(3)	104 ± 5	(2)
118	3.4 ± 0.3	(2)	0.7 ± 0.2	(2)	113 ± 4	(2)
119	10.0	(1)	1.0	(1)	256	(1)
120	6.8 ± 0.9	(2)	1.0 ± 0.04	(2)	139 ± 53	(2)
121	8.6 ± 2.4	(4)	1.1 ± 0.2	(4)	157 ± 37	(4)
122	14.5 ± 5.4	(5)	1.8 ± 0.7	(5)	132 ± 45	(3)
123	9.3 ± 3.4	(6)	1.2 ± 0.3	(6)	134 ± 53	(3)
124	5.5 ± 1.1	(7)	2.3 ± 1.0	(6)	129 ± 29	(4)
125	9.2 ± 2.3	(8)	2.6 ± 1.2	(8)	175 ± 31	(4)
126	7.8 ± 1.2	(9)	1.9 ± 0.6	(9)	142 ± 35	(5)
127	8.3 ± 0.9	(11)	1.9 ± 0.7	(10)	141 ± 18.6	(6)
128	9.0 ± 1.7	(10)	1.9 ± 0.8	(10)	123 ± 22	(6)
129	7.6 ± 1.0	(9)	1.3 ± 0.3	(9)	141 ± 45	(5)
130	7.3 ± 1.2	(8)	1.2 ± 0.3	(7)	126 ± 19	(5)
131	10.7 ± 2.2	(5)	1.9 ± 0.6	(6)	216 ± 55	(3)
132	10.7 ± 4.0	(6)	2.4 ± 0.8	(5)	147 ± 37	(3)
133	13.2 ± 3.0	(10)	3.1 ± 1.1	(5)	296 ± 112	(7)
134	15.5 ± 4.6	(8)	2.4 ± 1.0	(10)	193 ± 41	(6)
135	6.8 ± 0.9	(8)	1.7 ± 0.4	(8)	144 ± 26	(6)
136	6.1 ± 0.8	(6)	1.2 ± 0.2	(8)	103 ± 23	(4)
137	5.2 ± 0.5	(5)	1.2 ± 0.3	(6)	86 ± 19	(5)
138	8.1 ± 1.4	(8)	1.6 ± 0.3	(5)	165 ± 68	(7)
139	8.4 ± 1.8	(9)	1.9 ± 0.4	(8)	183 ± 67	(8)
140	7.6 ± 2.1	(6)	1.2 ± 0.3	(9)	183 ± 52	(5)
141	21.1 ± 8.9	(6)	2.9 ± 1.3	(6)	400 ± 57	(5)
142	15.4 ± 7.6	(5)	2.6 ± 0.8	(6)	362 ± 61	(4)
143	11.7 ± 3.0	(3)	2.1 ± 0.4	(5)	206 ± 29	(3)
144	9.9 ± 2.4	(5)	1.5 ± 0.4	(3)	229 ± 27	(4)
145	9.6 ± 1.6	(5)	2.6 ± 1.4	(5)	315 ± 163	(4)
146	11.8 ± 3.2	(3)	2.0 ± 0.5	(5)	146 ± 56	(3)
147	7.9 ± 1.8	(4)	3.2 ± 1.7	(4)	289 ± 127	(3)
148	13.3 ± 2.5	(4)	5.8 ± 1.3	(4)	447 ± 172	(3)
149	27.0 ± 4.9	(2)	6.0 ± 3.4	(4)	200	(1)
150	85.0	(1)	23.0	(1)	700	(1)

APPENDIX TABLE **8**

CONCENTRATION OF SOLUTES IN FOETAL URINE

(Foetuses that delivered normally)

DAYS PRIOR TO PARTURITION	[Cr] (mg/100 ml)		[UA] (mg/100 ml)		[Urea] (mg/100 ml)	
25	9.5	(1)	1.3	(1)		
24	4.4	(1)	0.7	(1)		
23	5.2	(1)	0.7	(1)		
22	9.7 ± 1.0	(2)	1.1 ± 0.2	(2)	150	(1)
21	10.2 ± 1.6	(2)	1.2 ± 0.01	(2)	110	(1)
20	7.7 ± 0.4	(2)	1.5 ± 0.4	(2)	95	(1)
19	7.5 ± 3.5	(2)	1.2 ± 0.5	(2)	130	(1)
18	6.6	(1)	1.3	(1)		
17	5.8 ± 2.8	(2)	1.0 ± 0.2	(2)	155	(1)
16	7.5 ± 1.2	(4)	1.1 ± 0.2	(4)	105 ± 4	(3)
15	5.2 ± 0.9	(3)	0.7 ± 0.1	(3)	93 ± 10	(3)
14	4.6 ± 1.7	(4)	1.0 ± 0.3	(4)	148 ± 46	(3)
13	5.2 ± 0.7	(6)	1.7 ± 0.6	(6)	92 ± 13	(4)
12	11.3 ± 3.7	(6)	1.6 ± 0.2	(6)	120 ± 40	(5)
11	8.3 ± 2.8	(5)	2.9 ± 1.1	(5)	127 ± 31	(5)
10	14.9 ± 6.8	(8)	3.8 ± 1.4	(8)	198 ± 72	(6)
9	12.3 ± 5.1	(8)	2.4 ± 0.7	(8)	168 ± 64	(6)
8	10.7 ± 2.4	(9)	2.6 ± 0.7	(9)	144 ± 31	(7)
7	10.8 ± 2.4	(9)	2.8 ± 0.9	(9)	176 ± 41	(7)
6	13.3 ± 1.6	(3)	2.0 ± 0.7	(3)	153 ± 33	(2)
5	14.1 ± 5.6	(5)	2.0 ± 0.4	(5)	234 ± 86	(4)
4	17.6 ± 10.0	(5)	4.4 ± 1.6	(5)	399 ± 178	(4)
3	20.0 ± 11.3	(7)	4.1 ± 0.9	(7)	295 ± 134	(5)
2	21.2 ± 5.8	(7)	2.5 ± 0.6	(7)	144 ± 34	(5)
1	31.0 ± 11.4	(6)	4.3 ± 1.2	(6)	273 ± 129	(4)

APPENDIX TABLE 9

CONCENTRATION OF SOLUTES IN AMNIOTIC FLUID

DAY OF GESTATION	[Cr] (mg/100 ml)		[UA] (mg/100 ml)	
115	44.0	(1)	10.0	(1)
116	-		10.0 ± 0.7	(2)
117	30.0	(1)	10.0	(1)
118	29.1	(1)	8.0 ± 0.7	(2)
119	54.0	(1)	14.0 ± 0.01	(2)
120	51.0	(1)	12.0 ± 0.7	(2)
121	34.0	(1)	14.0 ± 2.1	(2)
122	68.1	(1)	13.5 ± 1.1	(2)
123	47.0	(1)	17.5 ± 1.1	(2)
124	44.3 ± 5.5	(4)	16.6 ± 2.6	(5)
125	49.7 ± 12.1	(3)	19.8 ± 3.4	(4)
126	57.5 ± 13.8	(4)	17.8 ± 2.4	(5)
127	67.0 ± 19.2	(4)	18.0 ± 4.5	(4)
128	71.0 ± 18.1	(5)	19.5 ± 3.1	(6)
129	68.0 ± 5.1	(5)	16.6 ± 1.5	(5)
130	85.0 ± 9.2	(2)	25.3 ± 4.3	(3)
131	78.5 ± 11.0	(4)	16.3 ± 1.7	(4)
132	74.0 ± 24.9	(3)	28.0 ± 7.4	(4)
133	90.7 ± 27.6	(3)	19.8 ± 3.6	(4)
134	70.5 ± 0.4	(2)	16.0 ± 2.9	(3)
135	83.5 ± 10.3	(4)	24.0 ± 3.8	(5)
136	67.7 ± 3.8	(3)	22.7 ± 4.1	(3)
137	67.6 ± 8.7	(5)	21.0 ± 2.0	(5)
138	74.0 ± 7.5	(5)	22.2 ± 3.3	(5)
139	60.3 ± 9.7	(4)	26.0 ± 1.8	(4)
140	60.5 ± 11.7	(2)	18.0 ± 0.01	(2)
141	59.3 ± 3.8	(3)	20.7 ± 0.7	(3)
142	62.0	(1)	32.0	(1)
143	82.4	(1)	28.6	(1)
144	71.1	(1)	30.1	(1)
145	77.0	(1)	28.0	(1)
146	65.0	(1)	29.0	(1)
147	81.0	(1)	32.0	(1)

APPENDIX TABLE **10**

FOETAL SOLUTE EXCRETION RATES

DAY OF GESTATION	CrER (µg/min)		UAER (µg/min)		Urea ER (µg/min)	
115	47.0					
116	18.9 ± 7.2	(2)	3.1 ± 0.8	(2)	450	(1)
117	17.6 ± 8.3	(2)	2.1 ± 0.6	(2)	458 ± 250	(2)
118	28.3 ± 6.9	(2)	6.1 ± 2.1	(2)	1070	(1)
119	34.0	(1)	3.4	(1)	-	
120	27.8 ± 2.7	(2)	4.1 ± 0.8	(2)	368	(1)
121	21.6 ± 5.0	(4)	4.1 ± 1.1	(4)	526 ± 197	(2)
122	42.1 ± 13.1	(5)	5.7 ± 1.8	(5)	400 ± 102	(2)
123	33.9 ± 11.5	(5)	7.9 ± 3.7	(5)	250 ± 123	(3)
124	17.5 ± 4.5	(6)	9.8 ± 6.3	(6)	261 ± 115	(3)
125	28.2 ± 8.2	(8)	7.5 ± 3.7	(8)	184 ± 99	(4)
126	30.4 ± 6.4	(8)	10.2 ± 5.5	(8)	356 ± 82	(5)
127	49.4 ± 11.0	(9)	14.3 ± 6.5	(9)	926 ± 160	(4)
128	48.8 ± 12.3	(8)	11.7 ± 5.0	(9)	747 ± 196	(5)
129	41.9 ± 9.7	(8)	7.3 ± 1.7	(8)	778 ± 224	(4)
130	33.3 ± 6.5	(8)	4.8 ± 0.9	(7)	501 ± 125	(3)
131	25.0 ± 4.7	(6)	4.3 ± 1.2	(6)	942 ± 372	(2)
132	36.0 ± 15.3	(6)	8.8 ± 3.8	(5)	312 ± 28	(6)
133	43.9 ± 8.0	(10)	7.4 ± 1.2	(10)	793 ± 158	(5)
134	52.8 ± 10.1	(8)	8.1 ± 2.3	(8)	751 ± 153	(7)
135	40.5 ± 10.1	(8)	7.9 ± 2.3	(8)	821 ± 171	(4)
136	37.0 ± 5.9	(6)	7.4 ± 2.1	(6)	861 ± 191	(5)
137	47.1 ± 12.3	(5)	6.9 ± 2.5	(5)	814 ± 251	(7)
138	62.7 ± 16.5	(8)	11.2 ± 2.2	(8)	1051 ± 271	(8)
139	39.2 ± 9.2	(9)	10.4 ± 2.6	(9)	1021 ± 441	(5)
140	51.1 ± 9.8	(6)	7.7 ± 1.1	(6)	1493 ± 593	(5)
141	69.0 ± 20.4	(6)	8.6 ± 1.9	(6)	1846 ± 644	(4)
142	54.1 ± 15.2	(5)	11.1 ± 3.2	(5)	1929 ± 783	(3)
143	37.3 ± -8.4	(3)	7.4 ± 1.6	(3)	766 ± 159	(4)
144	80.7 ± 25.5	(5)	6.5 ± 0.8	(5)	941 ± 17	(4)
145	35.7 ± 7.8	(5)	10.1 ± 6.6	(5)	346 ± 755	(3)
146	26.7 ± 0.7	(3)	9.5 ± 4.2	(4)	832 ± 426	(3)
147	84.6 ± 27.6	(4)	12.2 ± 4.0	(4)	1181 ± 189	(3)
148	86.5 ± 26.7	(4)	25.4 ± 12.5	(4)	1606 ± 322	(3)
149	24.1 ± 1.4	(2)	10.9 ± 2.2	(2)	1300	(1)
150	20.4	(1)	55.2	(1)	1680	(1)

APPENDIX TABLE 11

FOETAL URINE pH

DAY OF GESTATION	pH		DAYS PRIOR TO PARTURITION	pH	
115	7.5		25	8.8	(1)
116	7.6 ± 0.01	(2)	24	8.8	(1)
117	8.1 ± 0.1	(3)	23	8.9	(1)
118	7.4 ± 0.2	(2)	22	-	
119	7.4	(1)	21	7.9 ± 0.3	(2)
120	7.3	(1)	20	8.4	(1)
121	7.8 ± 0.2	(3)	19	7.9 ± 0.8	(2)
122	7.1 ± 0.3	(3)	18	8.9	(1)
123	7.5 ± 0.3	(5)	17	8.1 ± 1.0	(2)
124	7.9 ± 0.4	(6)	16	7.9 ± 0.7	(4)
125	7.6 ± 0.4	(7)	15	8.1 ± 0.4	(3)
126	7.6 ± 0.4	(7)	14	8.1 ± 0.6	(4)
127	7.2 ± 0.3	(7)	13	7.7 ± 0.8	(6)
128	7.6 ± 0.3	(10)	12	7.4 ± 0.6	(6)
129	7.7 ± 0.3	(8)	11	7.6 ± 0.4	(5)
130	7.6 ± 0.3	(8)	10	7.5 ± 0.7	(8)
131	8.1 ± 0.4	(6)	9	7.5 ± 0.6	(8)
132	8.1 ± 0.4	(6)	8	7.6 ± 0.6	(9)
133	7.5 ± 0.3	(10)	7	7.7 ± 0.7	(8)
134	7.4 ± 0.3	(8)	6	6.9 ± 0.2	(3)
135	7.3 ± 0.3	(8)	5	7.9 ± 0.6	(5)
136	7.8 ± 0.3	(6)	4	7.5 ± 0.8	(5)
137	7.4 ± 0.1	(5)	3	7.5 ± 1.1	(7)
138	7.5 ± 0.2	(7)	2	7.3 ± 0.9	(7)
139	7.7 ± 0.2	(9)	1	7.3 ± 0.8	(6)
140	7.8 ± 0.2	(6)			
141	7.8 ± 0.2	(6)			
142	8.0 ± 0.2	(5)			
143	7.5 ± 0.1	(3)			
144	7.6 ± 0.3	(5)			
145	7.6 ± 0.3	(5)			
146	8.1 ± 0.4	(4)			
147	7.6 ± 0.5	(4)			
148	7.3 ± 0.4	(4)			
149	6.0 ± 0.01	(2)			
150	6.1	(1)			

APPENDIX TABLE **12**

FOETAL GFR AND FOETAL URINE FLOW RATE PER GM OF KIDNEY

DAY OF GESTATION	FLOW RATE ml/min/gm		GFR (ml/min)	
115	31 x 10^{-3}	(1)		
116	26 x " ± 4 x 10^{-3}	(2)		
117	26 x " ± 14 x "	(2)		
118	49 x " ± 7 x "	(2)		
119	20 x "	(1)		
120	17 x "	(1)		
121	36 x " ± 19 x "	(3)		
122	22 x " ± 7 x "	(4)		
123	29 x " ± 7 x "	(5)		
124	19 x " ± 3 x "	(7)		
125	17 x " ± 5 x "	(9)		
126	38 x " ± 17 x "	(9)		
127	57 x " ± 29 x "	(10)		
128	29 x " ± 4 x "	(10)	3.9 ± 0.3	(2)
129	29 x " ± 6 x "	(9)		
130	23 x " ± 6 x "	(8)	2.8 ± 0.4	(5)
131	21 x " ± 4 x "	(6)		
132	17 x " ± 3 x "	(7)	4.3	(1)
133	16 x " ± 3 x "	(11)		
134	17 x " ± 4 x "	(9)		
135	28 x " ± 5 x "	(9)		
136	29 x " ± 5 x "	(7)		
137	30 x " ± 6 x "	(6)		
138	33 x " ± 5 x "	(9)		
139	23 x " ± 4 x "	(10)	4.6	(1)
140	29 x " ± 5 x "	(7)	5.1 ± 0.2	(2)
141	23 x " ± 5 x "	(7)		
142	24 x " ± 6 x "	(5)	7.4 ± 0.3	(3)
143	15 x " ± 2 x "	(3)		
144	23 x " ± 4 x "	(5)	6.8 ± 0.2	(2)
145	15 x " ± 3 x "	(5)		
146	18 x " ± 3 x "	(4)	6.2 ± 0.2	(2)
147	23 x " ± 5 x "	(4)		
148	18 x " ± 7 x "	(4)		
149	16 x " ± 8 x "	(2)		
150	10 x "	(1)		

APPENDIX TABLE **13**

FOETAL MAP

DAY OF GESTATION	MAP (mm.Hg)	
114	49.0	(1)
115	35.3 ± 0.8	(2)
117	38.5	(1)
119	38.0 ± 1.0	(2)
121	37.2 ± 0.4	(2)
122	37.0	(1)
124	41.5	(1)
125	39.0	(1)
128	48.5	(1)
129	40.5 ± 0.5	(2)
130	53.0 ± 8.0	(2)
132	54.0	(1)
134	42.5 ± 0.5	(2)
137	45.5 ± 1.5	(2)
141	44.0 ± 1.0	(2)
144	49.0	(1)
145	53.3 ± 1.8	(2)
148	52.0 ± 1.0	(2)
149	58.0	(1)

APPENDIX TABLE **14**

PERCENTAGE REABSORPTION OF WATER AND ELECTROLYTES BY THE FOETAL KIDNEYS

DAY OF GESTATION	WATER REABSORPTION (%)		K+ REABSORPTION (%)		DAYS PRIOR TO PARTURITION	Na+ REABSORPTION (%)	
115	-						
116	70.1	(1)	68.6	(1)	22	98.8	(1)
117	77.5	(1)	86.3	(1)	21	98.4 ± 0.5	(3)
118	-		-		20	98.6	(1)
119	-		-		19	99.3	(1)
120	69.1	(1)	60.9 ± 11.5	(2)	18	-	
121	87.5 ± 2.5	(2)	65.9 ± 21.7	(2)	17	98.0	(1)
122	73.2 ± 3.0	(2)	78.2	(1)	16	97.4 ± 2.3	(2)
123	75.0	(1)	56.2 ± 12.4	(2)	15	87.0	(1)
124	72.0 ± 16.9	(2)	47.9 ± 34.7	(2)	14	95.1 ± 4.2	(2)
125	87.1 ± 2.7	(3)	78.1	(1)	13	90.5 ± 4.9	(3)
126	80.2 ± 4.9	(4)	52.3 ± 10.4	(4)	12	94.9 ± 2.4	(4)
127	82.3 ± 2.2	(6)	46.1 ± 11.7	(6)	11	93.8 ± 2.5	(5)
128	82.8 ± 4.9	(9)	49.6 ± 15.1	(4)	10	95.0 ± 1.4	(4)
129	75.4 ± 6.8	(7)	59.4 ± 6.6	(8)	9	95.5 ± 1.1	(7)
130	71.5 ± 5.4	(8)	43.8 ± 9.5	(4)	8	96.5 ± 1.3	(5)
131	82.1 ± 4.8	(4)	65.0 ± 3.9	(6)	7	96.8 ± 1.0	(7)
132	80.0 ± 4.7	(7)	35.4 ± 10.0	(5)	6	98.3	(1)
133	80.9 ± 4.4	(7)	44.1 ± 9.2	(6)	5	94.6 ± 2.4	(6)
134	83.0 ± 3.5	(6)	37.1 ± 9.0	(5)	4	91.9 ± 3.2	(3)
135	78.9 ± 2.1	(7)	55.0 ± 11.0	(4)	3	94.7 ± 2.1	(2)
136	73.7 ± 4.8	(5)	46.2 ± 10.6	(5)	2	91.3 ± 6.1	(4)
137	66.9 ± 5.5	(5)	40.9 ± 9.7	(6)	1	82.9 ± 9.9	(3)
138	78.1 ± 3.4	(7)	40.3 ± 8.7	(7)			
139	82.7 ± 2.5	(7)	40.0 ± 11.6	(6)			
140	80.8 ± 4.4	(7)	23.3 ± 11.3	(4)			
141	79.1 ± 7.6	(4)	43.4 ± 7.3	(6)			
142	79.4 ± 3.2	(8)	23.6	(1)			
143	89.7 ± 4.6	(3)	57.1 ± 10.5	(5)			
144	83.9 ± 2.4	(6)	44.3 ± 14.4	(3)			
145	85.1 ± 1.9	(3)	42.5 ± 9.3	(3)			
146	75.2 ± 0.1	(3)	51.25 ± 23.3	(2)			
147	90.0 ± 4.3	(3)	60.3	(1)			
148	90.6 ± 3.9	(3)					
149	94.7	(1)					
150	97.9	(1)					

APPENDIX TABLE **15**

HORMONE CONCENTRATIONS IN FOETAL BLOOD

DAY OF GESTATION	17α HP (ng/ml)		20α HP (ng/ml)		PROGESTERONE (ng/ml)	
115	0.3	(1)	5.8	(1)	4.6	(1)
116	0.7 ± 0.4	(3)	5.7 ± 2.2	(3)	2.5 ± 0.4	(2)
117	1.8 ± 0.7	(3)	2.6 ± 0.7	(2)	1.6 ± 0.2	(2)
118	0.8 ± 0.3	(3)	3.1 ± 1.7	(3)	3.4 ± 1.0	(4)
119	0.6 ± 0.2	(2)	8.9 ± 3.8	(3)	2.4 ± 0.8	(2)
120	0.2 ± 0.1	(2)	6.3 ± 1.7	(2)	3.5 ± 0.9	(2)
121	1.0 ± 0.3	(3)	10.2 ± 0.5	(2)	2.8 ± 0.1	(2)
122	0.3	(1)	12.0	(1)	2.6	(1)
123	0.6 ± 0.3	(3)	12.5	(1)	3.1 ± 1.8	(3)
124	0.7 ± 0.4	(3)	14.1 ± 4.0	(3)	10.9 ± 5.0	(2)
125	0.5 ± 0.1	(6)	25.0 ± 5.2	(6)	4.8 ± 2.0	(6)
126	0.6 ± 0.3	(6)	20.7 ± 9.0	(5)	7.0 ± 3.2	(5)
127	0.6 ± 0.2	(4)	12.9 ± 5.4	(3)	9.2 ± 3.8	(4)
128	1.2 ± 0.6	(4)	21.3 ± 7.4	(3)	3.6 ± 2.1	(4)
129	0.3 ± 0.1	(3)	8.6 ± 4.7	(3)	11.8 ± 4.2	(3)
130	0.4 ± 0.2	(4)	11.6 ± 3.4	(4)	9.3 ± 2.8	(4)
131	0.2	(1)	28.0	(1)	3.0	(1)
132	0.4 ± 0.1	(4)	13.5 ± 0.9	(3)	4.1 ± 1.4	(4)
133	0.3 ± 0.1	(3)	10.3 ± 2.5	(3)	2.9 ± 4.7	(3)
134	1.5 ± 0.6	(4)	18.0 ± 6.2	(4)	9.9 ± 4.2	(4)
135	2.1 ± 1.4	(3)	24.5 ± 4.7	(3)	9.4 ± 5.1	(3)
136	3.3 ± 1.6	(3)	17.1 ± 6.5	(3)	8.0 ± 2.1	(3)
137	0.4 ± 0.2	(2)	24.0 ± 6.4	(2)	6.5 ± 0.7	(2)
138	0.1	(1)	18.0	(1)	3.2	(1)
139	5.2	(1)	19.9	(1)	13.1	(1)
140	-		-		-	
141	0.1	(1)	9.3	(1)	10.2	(1)
142	-		-		-	
143	0.1	(1)	7.1	(1)	9.0	(1)
144	0.1	(1)	12.0	(1)	5.5	(1)
145	0.1	(1)	5.4	(1)	5.4	(1)
146	0.1	(1)	5.9	(1)	1.6	(1)

APPENDIX TABLE **16**

CORTISOL CONCENTRATION IN FOETAL PLASMA

DAY OF GESTATION	[CORTISOL] (ng/ml)		DAYS PRIOR TO PARTURITION	[CORTISOL] (ng/ml)	
116	22.0	(1)	17	22.0	(1)
117	27.9	(1)	16	27.9	(1)
118	20.9	(1)	15	20.9	(1)
119	30.0	(1)	14	30.0	(1)
120	39.9	(1)	13	26.3 ± 9.6	(2)
121	52.0	(1)	12	30.6 ± 15.1	(2)
122	-		11	9.5	(1)
123	24.5	(1)	10	15.1 ± 2.9	(4)
124	-		9	13.4 ± 0.9	(3)
125	8.8	(1)	8	19.0 ± 2.3	(3)
126	26.3 ± 10.1	(2)	7	25.3 ± 4.9	(4)
127	13.1 ± 0.1	(2)	6	22.1 ± 0.6	(2)
128	27.4 ± 9.2	(3)	5	39.1 ± 7.2	(3)
129	16.3 ± 4.8	(2)	4	22.0	(1)
130	13.4 ± 0.1	(2)	3	47.4 ± 12.5	(2)
131	15.5	(1)	2	75.4 ± 9.7	(3)
132	60.3 ± 17.6	(3)	1	66.6 ± 14.3	(4)
133	44.9 ± 21.9	(3)			
134	24.6 ± 2.7	(2)			
135	21.5	(1)			
136	18.1 ± 2.7	(2)			
137	21.2 ± 6.1	(2)			
138	22.8 ± 8.5	(3)			
139	44.42 ± 12.2	(3)			
140	29.4	(1)			
141	41.5 ± 4.4	(2)			
142	50.0	(1)			
143	-				
144	59.4	(1)			
145	50.4	(1)			

APPENDIX TABLE 17

FOETAL PRA

DAY OF GESTATION	PRA ng/ml/hr	
70	6.7 ± 0.8	(3)
75	8.9 ± 1.2	(3)
76	8.0	(1)
80	7.3 ± 2.1	(2)
90	8.5 ± 0.3	(3)
96	10.9 ± 1.1	(2)
98	11.5	(1)
100	12.4 ± 1.8	(5)
108	12.1 ± 0.2	(2)
110	17.7 ± 1.4	(3)
120	13.3 ± 2.3	(4)
129	15.8	(1)
130	13.3 ± 1.5	(4)
131	16.3	(1)
132	14.7 ± 3.8	(2)
133	12.7 ± 0.2	(2)
134	10. ± 0.3	(2)
135	12.7 ± 1.2	(5)
136	16.9 ± 1.7	(3)
137	17.8 ± 3.8	(3)
138	17.8 ± 2.7	(6)
139	20.3 ± 2.2	(2)
140	19.9 ± 5.5	(3)
141	20.8 ± 1.5	(5)
142	17.4 ± 4.1	(3)
143	12.9 ± 4.9	(3)
144	15.5 ± 2.9	(3)
145	14.2 ± 1.2	(7)
146	15.5 ± 3.0	(3)
147	19.3 ± 0.8	(2)
148	11.4 ± 2.4	(3)
149	13.2 ± 3.0	(2)
150	14.4 ± 2.4	(2)
151	12.2 ± 5.1	(2)

APPENDIX TABLE **18**

FOETUS 66-345: PLASMA HORMONE CONCENTRATIONS

DAYS PRIOR TO PART-URITION	[E_1] (ng/ml)	[$E_2 17\alpha$] (ng/ml)	[$E_2 17\beta$] (ng/ml)	[PROG] (ng/ml)	[17αHP] (ng/ml)	[20αHP] (ng/ml)	[CORT.] (ng/ml)
12	7.0	5.0	10.0	7.5	3.6	7.0	-
11	-	-	-	-	-	-	-
10	12.0	5.0	13.0	12.4	0.6	20.0	14.3
9	6.1	6.0	14.1	7.5	0.1	14.9	12.6
8	14.1	4.0	14.1	3.2	0.1	18.0	22.8
7	8.0	6.0	10.0	13.1	5.2	19.9	13.9
6	-	-	-	-	-	-	-
5	14.0	12.0	18.0	10.2	0.1	9.3	47.8
4	-	-	-	-	-	-	-
3	5.0	1.0	8.0	9.0	0.1	7.1	46.5
2	7.0	4.0	10.0	5.5	0.1	12.0	59.4
1	8.0	5.0	12.0	5.4	0.1	5.4	50.4

PLASMA SOLUTE CONCENTRATIONS

DAYS PRIOR TO PARTURITION	[Na+] (mEq/1)	[K+] (mEq/L)	[Cr] (mg/100 ml)	[UA] (mg/100 ml)
12	144.5	4.5	1.6	0.4
11	-	-	-	-
10	143.0	4.4	1.4	0.3
9	140.5	4.8	1.7	0.6
8	142.5	4.7	1.2	0.7
7	142.5	4.7	3.7	3.0
6	-	-	-	-
5	140.0	4.8	1.5	2.5
4	-	-	-	-
3	143.0	5.4	4.6	2.0
2	140.5	5.0	1.5	1.0
1	143.0	6.0	1.0	1.9

APPENDIX TABLE 19

FOETUS 66-345: URINE PARAMETERS

DAYS PRIOR TO PARTU-RITION	[Na+] (mEq/1)	[K+] (mEq/1)	[Cr] (mg/100ml)	[UA] (mg/100ml)	NaER (µEq/min)	KER (µEq/min)	FLOW (ml/mi
12	9.0	28.0	16.0	0.8	2.0	6.2	.22
11	-	-	-	-	-	-	-
10	17.0	5.0	6.0	0.4	8.5	2.5	.50
9	5.0	4.0	4.0	0.3	2.8	2.2	.55
8	4.0	9.0	7.0	0.6	2.2	4.9	.54
7	6.0	17.0	12.0	0.7	1.3	3.2	.43
6	-	-	-	-	-	-	-
5	7.0	52.0	37.0	3.0	2.6	9.8	.19
4	-	-	-	-	-	-	-
3	16.0	23.0	5.0	2.5	7.0	10.1	.44
2	29.0	66.0	46.0	2.0	11.6	26.4	.40
1	38.0	27.0	15.0	1.0	12.2	8.6	.32

APPENDIX TABLE **20**

FOETUS W-177: PLASMA HORMONE CONCENTRATIONS MEASURED DURING GESTATION

DAYS PRIOR TO PARTURITION	[PROGESTERONE] (ng/ml)	[17 α HP] (ng/ml)	[20 α HP] (ng/ml)	[CORTISOL] (ng/ml)
13	2.0	0.1	1.1	12.8
12	1.9	0.2	1.6	9.3
11	5.4	0.3	0.8	9.5
10	1.3	0.8	1.7	13.0
9	4.7	0.1	3.9	15.5
8	2.6	1.1	10.8	20.8
7	-	-	-	21.8
6	1.5	0.1	21.3	21.3
5	0.1	0.2	22.8	21.5
4	0.1	0.3	26.0	22.0
3	0.1	0.7	39.0	29.8
2	0.6	0.8	12.7	68.3
1	0.8	0.2	11.5	93.0

PLASMA SOLUTE CONCENTRATIONS

DAYS PRIOR TO PARTURITION	[Na+] (mEq/1)	[K+] (mEq/1)	[Cr] (mg/100ml)	[UA] (mg/100ml)
13	143.5	4.0	1.7	0.7
12	142.0	4.6	1.9	0.9
11	143.0	5.3	2.2	0.9
10	147.0	4.8	1.8	0.9
9	141.0	4.7	2.3	0.9
8	140.0	4.9	2.2	0.8
7	147.5	5.0	2.0	0.5
6	154.0	5.0	2.4	0.5
5	144.5	6.6	2.2	0.6
4	158.0	5.0	2.0	0.9
3	147.0	5.0	2.3	0.9
2	145.0	4.7	2.2	0.8
1	146.0	4.7	2.4	1.5

APPENDIX TABLE **21**

FOETUS W-177: URINE PARAMETERS MEASURED DURING GESTATION

DAYS PRIOR TO PARTURITION	[Na+] (mEq/1)	[K+] (mEq/1)	[Cr] (mg/100ml)	[UA] (mg/100ml)	NaER (uEq/min)	KER (uEq/min)	FLOW (ml/mir
13	30.0	6.0	8.0	1.9	14.4	2.9	.48
12	29.0	4.0	3.8	1.2	21.8	3.0	.75
11	46.0	4.0	3.9	1.3	62.6	5.4	1.36
10	48.0	8.0	8.7	2.5	15.4	2.6	.32
9	49.0	13.0	15.0	3.6	42.6	11.3	.87
8	38.0	24.0	25.0	4.0	5.7	3.6	.15
7	27.0	40.0	28.0	4.5	5.7	8.4	.21
6	18.0	29.0	16.8	3.6	7.9	12.8	.44
5	29.0	22.0	10.7	2.5	24.6	18.7	.85
4	35.0	12.0	6.5	1.6	33.3	11.4	.95
3	30.0	11.0	6.3	1.9	34.8	12.8	1.16
2	29.0	18.0	9.4	3.7	19.1	11.9	.66
1	74.0	7.0	3.3	2.2	88.1	8.3	1.19

APPENDIX TABLE **22**

FOETUS W-38 PLASMA HORMONE CONCENTRATIONS

DAY OF GESTATION	[PROGESTERONE] (ng/ml)	[17 α HP] (ng/ml)	[20 α HP] (ng/ml)	[CORTISOL] (ng/ml)
115	4.6	0.3	5.8	9.5
116	3.0	0.3	5.6	6.8
117	-	-	-	-
118	3.0	0.3	7.3	10.8
119	3.5	0.3	7.5	11.6
120	2.2	0.3	8.6	11.8
121	3.0	0.3	9.5	13.1
122	2.6	0.3	12.0	12.8
123	0.9	0.3	12.5	10.6
124	3.8	0.3	7.5	9.7
125	1.9	0.5	9.4	13.3
126	2.7	0.5	7.1	15.8
127	3.5	0.5	-	14.3
128	1.8	0.5	9.2	16.5
129	-	-	-	-
130	3.8	0.5	0.1	18.7
131	-	-	-	-
132	3.9	0.5	6.3	-
133	4.0	0.5	6.4	21.8

APPENDIX TABLE **23**

FOETUS W38 PLASMA SOLUTE CONCENTRATIONS

DAY OF GESTATION	[Na+] (mEq/1)	[K+] (mEq/1)	[Cr] (mg/100ml)	[UA] (mg/100ml)
115	147.5	3.9	13.7	1.9
116	137.0	4.6	8.3	1.3
117	134.0	3.6	7.7	1.9
118	-	-	4.7	1.7
119	130.5	3.7	5.9	1.7
120	134.5	3.6	5.0	1.5
121	134.5	3.5	5.2	1.9
122	145.5	3.8	8.0	1.9
123	142.5	4.8	3.8	1.2
124	143.0	3.8	3.9	1.3
125	135.5	3.9	8.7	2.5
126	141.0	4.1	15.0	3.6
127	141.5	3.9	20.0	4.0
128	141.5	4.3	20.0	4.5
129	-	-	-	-
130	147.5	4.0	16.8	3.6
131	-	-	-	-
132	149.0	4.5	10.7	2.5
133	149.5	4.6	11.7	2.8

APPENDIX TABLE **24**

FOETUS W38: URINE PARAMETERS

DAY OF GESTATION	[Na+] (mEq/1)	[K+] (mEq/1)	[Cr] (mg/100ml)	[UA] (mg/100ml)	NaER (µEq/min)	KER (µEq/min)	FLOW (ml/min)
115	28.0	13.0	10.0	1.4	13.2	6.1	.47
116	39.0	6.0	5.6	0.8	20.3	3.1	.52
117	-	-	-	-	-	-	-
118	15.0	1.0	3.0	0.5	9.3	0.6	.62
119	34.0	4.0	10.0	1.0	11.6	1.4	.34
120	16.0	4.0	8.0	1.0	4.8	1.2	.30
121	22.0	7.0	12.0	1.5	6.4	2.0	.29
122	22.0	6.0	10.0	1.4	7.3	2.0	.33
123	13.0	5.0	7.0	1.0	9.1	3.5	.70
124	12.0	5.0	8.0	1.0	4.8	2.0	.40
125	21.0	7.0	12.0	1.5	11.6	3.9	.55
126	15.0	7.0	10.0	1.4	9.0	4.2	.60
127	25.0	5.0	9.0	0.9	25.0	5.0	1.02
128	11.0	6.0	8.0	1.0	5.1	2.8	.46
129	-	-	-	-	-	-	-
130	15.0	6.0	10.0	1.2	9.3	3.7	.62
131	-	-	-	-	-	-	-
132	20.0	6.0	4.0	0.6	9.6	2.9	.48
133	42.0	17.0	10.0	1.6	20.6	8.3	.49

APPENDIX TABLE **25**

FOETUS 66-323: PLASMA AND URINE PARAMETERS MEASURED DURING GESTATION

	PLASMA			URINE			
DAY OF GESTATION	PRA (mg/ml/hr)	[Na+] (mEq/1)	[K+] (mEq/1)	[Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/min)	FLOW (ml/min)
135	12.3	148.6	5.1	21.5	7.3	8.5	.34
136	13.8	147.2	5.3	20.5	12.9	11.5	.63
137	21.1	148.1	5.5	27.3	12.0	10.7	.44
138	16.7	148.3	4.9	18.5	12.0	6.6	.65
139	18.1	146.2	4.7	23.6	8.7	4.1	.37
140	8.9	145.3	4.9	22.1	7.1	4.6	.32
141	17.5	147.1	5.1	20.5	7.8	5.8	.38
142	12.3	147.4	5.2	18.2	8.9	8.2	.49
143	5.6	146.8	5.3	12.7	7.9	9.2	.62
144	10.1	148.1	5.0	21.3	9.0	7.3	.42
145	11.0	148.3	4.9	24.5	14.2	7.4	.58
146	9.5	147.1	5.1	25.5	8.2	4.4	.32
147	-	-	-	21.2	8.2	5.6	.39
148	7.8	148.2	5.0	30.5	12.8	11.8	.42
149	10.2	148.6	4.9	20.2	12.3	22.6	.61
150	12.0	146.1	4.8	19.5	11.3	11.1	.58
(Birth)	7.1	143.0	5.0	18.5	11.1	9.9	.60

APPENDIX TABLE **26**

FOETUS 66-478: PLASMA AND URINE PARAMETERS MEASURED DURING GESTATION

	PLASMA			URINE			
DAY OF GESTATION	PRA (mg/ml/hr)	[Na+] (mEq/1)	[K+] (mEq/1)	[Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/min)	FLOW (ml/min)
129	15.8	142.5	4.1	22.7	12.3	8.8	.54
130	17.5	143.5	4.2	25.3	9.1	6.5	.36
131	16.3	142.1	4.2	28.4	7.1	4.5	.25
132	18.4	145.2	4.1	33.2	9.6	4.2	.29
133	12.8	142.3	3.9	30.1	14.2	6.0	.47
134	10.2	144.6	3.9	27.2	18.2	8.4	.67
135	11.5	146.3	3.8	25.3	9.6	4.6	.38
136	19.6	145.4	3.9	22.4	8.1	5.0	.36
137	22.1	146.7	4.1	21.6	5.2	2.2	.24
138	29.2	146.3	4.3	23.7	8.5	4.6	.36
139	-	-	-	19.8	9.7	7.0	.49
140	25.5	145.2	4.1	27.3	10.7	5.2	.39
141	23.4	143.1	4.3	30.8	8.6	4.9	.28
142	14.5	142.2	4.4	28.6	15.2	14.9	.53
143	11.0	143.5	4.1	26.2	11.8	7.7	.45
144	16.3	145.1	4.3	22.3	9.6	6.3	.43
145	18.4	146.1	4.1	40.9	19.6	7.8	.48
146	18.8	143.2	3.9	52.8	24.8	5.3	.47
147	20.1	145.7	3.8	48.7	22.4	6.6	.46
148 (Birth)	10.5	145.1	4.0	36.1	14.1	4.9	.39

APPENDIX TABLE **27**

FOETUS 70-409: PLASMA AND URINE PARAMETERS MEASURED DURING GESTATION

	PLASMA			URINE			
DAY OF GESTATION	PRA (mg/ml/hr)	[Na+] (mEq/1)	[K+] (mEq/1)	[Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/min)	FLOW (ml/min)
132	10.9	146.1	4.8	25.2	27.7	18.0	1.10
133	12.5	144.2	4.3	28.3	24.1	14.7	.85
134	10.8	145.1	4.2	32.4	31.1	14.6	.96
135	17.1	144.3	4.4	47.1	35.8	3.3	.76
136	17.4	144.2	4.8	26.3	14.7	2.9	.56
137	10.3	145.8	5.1	28.2	17.5	4.8	.62
138	17.5	146.1	5.2	31.0	22.0	7.3	.71
139	22.5	146.3	5.6	28.7	21.2	10.4	.74
140	25.3	144.2	5.1	23.6	19.4	14.9	.82
141	-	-	-	32.4	30.8	15.6	.95
142	25.5	142.3	4.9	24.1	18.6	10.1	.77
143	22.1	143.8	4.8	24.8	19.8	11.8	.80
144	20.1	148.1	4.4	25.3	20.8	15.7	.82
145	11.5	146.2	4.7	18.6	14.3	-	.77
146	18.3	147.3	4.9	26.1	25.1	12.8	.96
147	18.5	144.1	4.8	14.2	14.3	14.7	1.01
148	15.9	142.3	4.3	24.3	27.2	15.9	1.12
149	16.1	143.8	4.7	21.6	19.7	11.7	.91
150	16.8	144.2	4.7	29.8	16.7	11.3	.56
151	17.3	142.6	4.5	26.9	23.4	12.0	.87
152	10.9	145.1	4.3	34.3	28.1	14.1	.82

APPENDIX TABLE **28**

FOETUS 69-464 (111 DAYS) SALT LOAD (Na+, 0.39 mEq/kg/hr; 1 hr)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	[K+] (mEq/l)	KER (µEq/min)
0 - ½	.07	36	2.52	3.6	.25
½ - 1	.08	35	2.80	4.4	.35
1 - 1½	.11	33	3.63	4.1	.45
1½ - 2	.12	35	4.20	3.6	.43
2 - 2½	.11	36	3.96	3.5	.39
2½ - 3	.13	38	4.94	3.4	.44
3 - 3½	.14	40	5.60	4.2	.59
3½ - 4	.13	45	5.85	5.4	.70
4 - 4½	.16	53	8.48	5.6	.90
4½ - 5	.16	58	9.28	6.4	1.02
5 - 5½	.18	64	11.52	6.3	1.13
5½ - 6	.16	63	10.08	7.9	1.26
6 - 6½	.24	70	16.80	8.2	1.97
6½ - 7	.22	70	15.40	8.1	1.78

In all appendix tables listing the data from experiments carried out on individual foetuses, the horizontal lines indicate either the time of injection of the drugs or hormones involved or the times between which these agents were infused.

APPENDIX TABLE **29**

FOETUS 69-539 (111 DAYS) SALT LOAD (Na+, 0.39 mEq/kg/hr; 1 hr)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	[K+] (mEq/l)	KER (µEq/min)	CrCl (ml/min)
0 - ½	.10	34	3.40	12.2	1.22	
½ - 1	.09	37	3.33	11.9	1.07	2.12
1 - 1½	.07	31	2.17	10.2	.71	
1½ - 2	.11	32	3.52	12.4	1.36	2.28
2 - 2½	.14	33	4.62	13.1	1.83	
2½ - 3	.20	38	7.60	15.1	3.02	
3 - 3½	.18	44	7.92	14.7	2.65	1.92
3½ - 4	.16	42	6.72	14.8	2.37	
4 - 4½	.22	51	11.22	15.2	3.34	2.36
4½ - 5	.28	54	15.12	15.8	4.42	
5 - 5½	.24	60	14.40	16.2	3.89	2.00
5½ - 6	.29	69	20.01	18.9	5.48	
6 - 6½	.33	69	22.77	19.3	6.37	2.68
6½ - 7	.37	70	25.90	20.1	7.44	
7 - 7½	.39	77	30.03	19.9	7.76	2.75

APPENDIX TABLE **30**

FOETUS 69-443 (114 DAYS) SALT LOAD (Na+, 0.12 mEq/kg/hr; 3 hr)

TIME (Hrs)	URINE FLOW (ml/min)	URINE [Na+] (mEq/l)	NaER (μEq/min)	KER (μEq/min)	CrCl (ml/min)
0 - ½	.08	32	-	-	
½ - 1	.07	33	2.31	.34	
1 - 1½	.10	34	3.40	.69	1.25
1½ - 2	.22	22	4.84	.84	
2 - 2½	.12	33	3.96	.82	1.08
2½ - 3	.33	15	4.95	.66	
3 - 3½	.39	25	9.75	1.21	1.50
3½ - 4	.37	24	8.88	.70	
4 - 4½	.35	25	8.75	.70	1.85
4½ - 5	.36	26	9.36	.79	
5 - 5½	.23	26	5.98	.53	1.58
5½ - 6	.21	21	4.41	.48	
6 - 6½	.24	23	5.52	.77	2.25
6½ - 7	.21	25	5.25	.86	
7 - 7½	.26	29	7.54	.99	1.95
7½ - 8	.24	29	6.96	.82	
8 - 8½	.33	35	11.55	1.22	

APPENDIX TABLE 31

FOETUS 69-467 (116 DAYS) SALT LOAD (Na+, 0.87 mEq/kg/hr; 1 hr)

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/L)	NaER (μEq/min)	[K+] (mEq/1)	KER (μEq/min)	CrCl (ml/min)
0 - ½	.81	49	39.69	4.1	3.32	
½ - 1	.72	32	23.04	2.5	1.80	
1 - 1½	.83	39	32.37	2.5	2.08	
1½ - 2	.80	50.5	40.40	1.7	1.36	
2 - 2½	1.03	60.2	62.01	1.4	1.44	
2½ - 3	.97	66	64.02	1.5	1.46	
3 - 3½	.98	69	67.62	1.5	1.47	1.69
3½ - 4	.97	68	65.96	1.5	1.46	
4 - 4½	.93	59	54.87	1.4	1.30	2.86
4½ - 5	.79	61	48.19	1.6	1.26	
5 - 5½	.81	56	45.36	1.6	1.30	2.49
5½ - 6	.83	64	53.12	1.3	1.08	
6 - 6½	.75	57	42.75	1.5	1.13	2.36
6½ - 7	.68	58	39.44	1.8	1.22	
7 - 7½	.68	78	53.04	2.2	1.50	2.67
7½ - 8	.74	91	67.34	2.3	1.70	
8 - 8½	1.42	97	137.74	2.4	3.41	4.29
8½ - 9	1.73	124	214.52	1.2	2.08	
9 - 9½	1.68	125	210.00	1.1	1.85	3.80
9½ - 10	1.35	121	163.35	1.1	1.49	
10 - 10½	1.14	123	140.22	1.3	1.48	

APPENDIX TABLE 32

FOETUS 69-613 (118 DAYS) SALT LOAD (Na+, 0.33 mEq/kg/hr; 3 hr)

	URINE					
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (μEq/min)	[K+] (mEq/1)	KER (μEq/min)	CrCl (ml/min
0 - ½	.21	29	6.09	9.2	1.93	
½ - 1	.12	35	4.20	9.1	1.09	2.55
1 - 1½	.19	37	7.03	12.2	2.32	3.56
1½ - 2	.18	34	6.12	15.1	2.72	
2 - 2½	.16	33	5.28	13.7	2.19	2.92
2½ - 3	.35	47	16.45	19.2	6.72	
3 - 3½	.41	50	20.50	23	9.43	4.58
3½ - 4	.37	60	22.20	22.5	8.33	
4 - 4½	.50	69	34.50	22.5	11.25	3.61
4½ - 5	.33	68	22.44	22.6	7.46	
5 - 5½	.75	70	52.50	23.1	17.33	5.42
5½ - 6	.81	67	54.27	21.3	17.25	
6 - 6½	.69	77	53.13	22.3	15.39	3.83
6½ - 7	.55	70	38.50	24.4	12.32	
7 - 7½	.73	79	57.67	22.9	16.72	4.06
7½ - 8	.93	93	86.49	20.1	18.69	

APPENDIX TABLE 33

FOETUS (125 DAYS) SALT LOAD (Na+ 3.08 mEq/kg/hr; ½ hr)
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TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/L)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	CrCl (ml/min)
0 - ½	.03	24.5	.73	18.0	.54	1.03
½ - 1	.04	31.5	1.26	23.1	.92	
1 - 1½	.04	25.	1.00	18.1	.72	
1½ - 2	.03	36.5	.73	25.2	.50	
2 - 2½	.03	-	-	-	-	
2½ - 3	.04	34.0	1.36	22.0	.88	
3 - 3½	.09	42.5	3.82	24.1	2.16	1.03
3½ - 4	.18	27.0	4.86	12.0	2.16	
4 - 4½	.15	41.0	6.15	19.0	2.85	1.15
4½ - 5	.13	48.0	6.24	22.1	2.86	
5 - 5½	.07	43.5	3.01	18.9	1.33	1.01
5½ - 6	.04	51.1	2.04	24.1	.96	
6 - 6½	.02	-	-	-	-	
6½ - 7	.03	-	-	-	-	
7 - 7½	.13	51.5	6.69	20.1	2.6	

APPENDIX TABLE 34

FOETUS 66-310 (146 DAYS) SALT LOAD (Na+, 0.85 mEq/kg/hr; 3½ hr)

	URINE					
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	CrCl (ml/min
0 - ½	.30	15	4.50	10	3.00	
½ - 1	.24	26	6.24	16	3.84	2.40
1 - 1½	.25	15	3.75	12	3.00	
1½ - 2	.25	20	5.00	17	4.25	
2 - 2½	.29	10.5	3.05	10	2.90	
2½ - 3	.30	14	4.20	15	4.50	2.78
3 - 3½	.30	8	2.40	9	2.70	
3½ - 4	.38	6.5	2.47	6.5	2.47	2.52
4 - 4½	.30	9.0	2.70	9	2.70	
4½ - 5	.29	8.5	2.47	8	2.32	
5 - 5½	.45	12	5.40	10	4.50	
5½ - 6	.29	5	1.45	6	1.74	
6 - 6½	.59	15	8.85	6	3.54	
6½ - 7	.61	20	12.20	3	1.83	
7 - 7½	.88	27	23.76	4	3.52	
7½ - 8	.52	22	11.44	5	2.60	2.68
8 - 8½	.48	27	10.16	5	2.40	
8½ - 9	.30	27	8.10	9	2.70	

APPENDIX TABLE **35**

FOETUS 12 (125 DAYS) HAEMORRHAGE (40 ml blood loss)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	.62	98.0	60.76	7.0	4.34		
½ - 1	.88	95.0	83.60	7.1	6.16	142.5	4.6
1 - 1½	.97	89.5	86.82	7.0	6.79		
1½ - 2	.72	-	-	-	-	143.3	4.2
2 - 2½	.92	89.0	85.88	5.2	4.60		
2½ - 3	.80	81.5	83.20	5.5	4.40		
3 - 3½	.50	19.5	9.75	3.0	1.50		
3½ - 4	.52	22.0	11.44	3.3	1.56	139.6	3.9
4 - 4½	.68	17.5	11.90	4.8	7.48		
4½ - 5	.68	18.5	12.58	3.5	2.38	141.7	4.2
5 - 5½	.66	26.5	17.49	5.0	3.30		
5½ - 6	.55	35.5	19.53	8.0	4.40		
6 - 6½	.88	18.0	15.84	7.1	6.16		
6½ - 7	.82	47	38.54	6.1	4.92	144.5	4.2

APPENDIX TABLE **36**

FOETUS 220 (124 DAYS) INTRA-AMNIOTIC SUCROSE INJECTIONS
(10 ml, 2.5 M Sucrose & 10 ml, 2.5 M Sucrose)

		URINE				PLASMA	
TIME (Hrs)	FLOW (ml/min)	NaER (μEq/min)	KER (μEq/min)	Crcl (ml/min)	UAER (μg/min)	PCV (%)	OP (mosm/
0 - ½	.79	28.13	5.82		16.49		
½ - 1	.73	16.06	2.92		9.49		
1 - 1½	.85	27.20	4.25	5.38	16.15	42	316
1½ - 2	.76	17.48	2.66		11.40		
2 - 2½	.73	23.36	3.29		14.60		
2½ - 3	.57	14.25	2.28		9.69		
3 - 3½	.53	17.49	2.65	4.95	11.13	41	335
3½ - 4	.69	22.08	3.45		13.80		
4 - 4½	.56	8.96	1.96		6.16		
4½ - 5	.63	11.34	1.89		8.19		
5 - 5½	.61	11.59	2.14		8.54		
5½ - 6	.60	13.2	3.3	3.07	10.20	44	317
6 - 6½	.59	11.8	2.36		9.44		
6½ - 7	.45	14.4	2.70		10.35		
7 - 7½	.48	17.28	3.36	3.37	12.96	44	328
7½ - 8	.41	12.3	2.46		9.84		
8 - 8½	.47	13.16	2.82	3.55	11.28	46	316
8½ - 9	.52	14.04	3.12		12.48		
9 - 9½	.65	11.05	2.60		9.75		
9½ - 10	.68	8.84	2.04		8.84		
10 - 10½	.67	9.38	2.35		9.38		
10½ - 11	.71	9.23	2.49		9.23		

APPENDIX TABLE 37

FOETUS 253 (116 days) INTRA-AMNIOTIC SUCROSE INJECTION

(25 ml of 2.5 M sucrose)

URINE

TIME (Hrs)	FLOW (ml/min)	(Na+) (mEq/l)	NaER (μEq/min)	(K+) (mEq/l)	KER (μEq/l)	CrER (μg/min)	UAER (μg/min)	Cr (mg/100 ml)
0 - ½	.19	23.5	4.47	15.0	2.85	22.23	3.99	10.7
½ - 1	.16	20	3.20	14.0	2.24	16.48	2.24	10.3
1 - 1½	.17	23.5	4.00	18.0	3.06	23.29	3.23	13.7
1½ - 2	.20	19.5	3.90	14.0	2.8	21.60	2.60	10.8
2 - 2½	.16	21.5	3.44	12.0	1.92	14.88	1.92	9.3
2½ - 3	.21	23.5	4.94	12.0	2.52	19.95	2.31	9.5
3 - 3½	.23	26	5.98	13.0	2.99	22.77	2.30	9.9
3½ - 4	.14	24	3.36	12.0	1.68	12.74	1.40	9.1
4 - 4½	.20	27	5.40	13.0	2.60	18.00	2.20	9.0
4½ - 5	.07	24	1.68	11.0	.77	5.74	.77	8.2
5 - 5½	.06	24	1.44	11.0	.66	4.98	.66	8.3
5½ - 6	.03	23.5	.71	11.0	.33	2.52	.30	8.4
6 - 6½	.02	24	.48	12.0	.24	1.56	.22	7.8
6½ - 7½	.02	22	.44	11.0	.22	1.64	.18	8.2
7½ - 8½	.01	23	.23	9.0	.09	.69	.10	6.9
8½ - 9½	.04	30.8	1.23	8.0	.32	1.80	.32	4.5
9½ - 10½	.06	40.5	2.43	8.0	.48	2.94	.42	4.9

PLASMA

TIME Hrs.)	PCV (%)	OP (mOsm/kg)	(Na+) (mEg/L)	(K+) (mEq/L)	CrCl (ml/min)
3 - 3½	41	299	140.5	3.8	3.42
5 - 5½	38	293	138.5	3.7	2.29
9 - 9½	39	293	138.0	3.6	2.11

APPENDIX TABLE **38**

EWE CARRYING FOETUS 271 (120 DAYS)

MATERNAL SUCROSE INFUSION (100 ml 2.5 M Sucrose at 2 hours followed by 20 ml/hr 2.5 M Sucrose for 3 hr)

TIME (Hr)	PLASMA				URINE	
	FOETAL O.P. (mOsm/kg)	MATERNAL O.P. (mOsm/kg)	FOETAL [Na+] (mEq/1)	MATERNAL [Na+] (mEq/1)	FOETAL FLOW (ml/min)	MATERNAL FLOW (ml/min)
0 - ½	295	-	143.5	-	0.58	-
½ - 1	-	-	-	-	0.56	3.30
1 - 1½	-	-	-	-	0.47	-
1½ - 2	293	287	143.3	147.5	0.65	0.80
2 - 2½	-	310	-	144.5	0.59	-
2½ - 3	-	-	-	-	0.58	1.27
3 - 3½	-	314	-	135.6	0.59	-
3½ - 4	295	304	139.6	135.7	0.56	2.17
4 - 4½	-	-	-	-	0.52	-
4½ - 5	-	301	-	136.0	0.55	2.27
5 - 5½	-	295	-	137.3	0.56	-
5½ - 6	296	292	139.5	138.5	0.50	3.00
6 - 6½	-	-	-	-	0.48	-
6½ - 7	-	-	-	-	0.41	1.63
7 - 7½	-	-	-	-	0.38	-
7½ - 8	296	296	140.1	137.8	0.57	0.70

APPENDIX TABLE 39

FOETUS 271 (120 DAYS) MATERNAL SUCROSE INFUSION
(100 ml 2.5 M Sucrose, plus 20 ml/hr 2.5 M Sucrose; 3 hr)

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (μEq/min)	[K+] (mEq/l)	KER (μEq/min)	CrCl (ml/min)	[UA] (mg/100ml
0 - ½	.58	34.5	20.01	8.0	4.64	3.83	1.6
½ - 1	.56	25.0	14.0	6.0	3.36		1.6
1 - 1½	.47	24.5	11.52	5.0	2.35		1.5
1½ - 2	.65	33.5	21.45	6.1	3.9	3.39	1.7
2 - 2½	.59	38.1	22.42	7.0	4.13		1.9
2½ - 3	.58	30.2	11.6	3.0	1.74		.4
3 - 3½	.59	40.3	23.6	6.0	3.54		2.3
3½ - 4	.56	31.5	17.64	6.1	3.36	4.04	1.6
4 - 4½	.52	31.0	16.12	8.1	4.16		1.8
4½ - 5	.55	25.0	13.75	7.0	3.85		1.7
5 - 5½	.56	28.5	15.68	6.1	3.36		1.8
5½ - 6	.50	30.5	15.25	5.2	2.50	2.25	1.8
6 - 6½	.48	25.0	12.0	3.9	1.92		1.5
6½ - 7	.41	22.1	9.02	5.2	2.05		1.5
7 - 7½	.38	22.1	8.36	7.0	2.66		1.7
7½ - 8	.57	30.0	17.10	9.0	5.13	2.60	1.8

APPENDIX TABLE **40**

SIMULTANEOUS MEASUREMENT OF MATERNAL AND FOETAL URINE PARAMETERS
(EWE AND FOETUS 69-826) (MATERNAL VALUES IN BRACKETS)

TIME (Hrs)	FLOW (ml/min)		pH	
0 - ½	.21	(1.69)	6.05	(8.43)
½ - 1	.28	(1.75)	5.95	(8.48)
1 - 1½	.24	(1.55)	6.00	(8.90)
1½ - 2	.19	(1.54)	5.82	(8.42)
2 - 2½	.30	(1.73)	5.85	(8.70)
2½ - 3	.22	(1.46)	5.95	(8.62)
3 - 3½	.24	(1.48)	5.45	(8.55)
3½ - 4	.40	(1.42)	6.05	(8.76)
4 - 4½	.25	(1.09)	6.17	(8.65)
4½ - 5	.21	(1.20)	6.03	(8.70)
5 - 5½	.23	(1.12)	5.95	(8.45)
5½ - 6	.20	(1.14)	5.97	(8.70)
6 - 6½	.24	(1.06)	6.10	(9.05)
6½ - 7	.13	(0.84)	6.10	(8.33)
7 - 7½	.27	(0.90)	5.90	(8.43)
7½ - 8	.34	(1.14)	5.95	(8.48)
8 - 8½	.27	(1.16)	5.95	(8.45)
8½ - 9	.17	(1.30)	5.78	(8.30)
9 - 9½	.21	(1.27)	5.90	(8.25)
9½ - 10	.20	(1.32)	5.75	(8.35)
10 - 10½	.19	(1.09)	5.85	(8.22)
10½ - 11	.50	(1.15)	5.87	(8.31)
11 - 11½	.16	(1.15)	5.90	(8.42)
11½ - 12	.16	(0.74)	5.60	(8.40)

APPENDIX TABLE CONT. 40

TIME (Hrs)	FLOW (ml/min)		pH	
12 - 12½	.20	(0.82)	6.10	(8.62)
12½ - 13	.28	(0.51)	5.85	(8.53)
13 - 13½	.12	(1.21)	5.85	(8.48)
13½ - 14	.25	(1.17)	5.80	(8.48)
14 - 14½	.52	(1.44)	5.95	(8.60)
14½ - 15	.27	(0.59)	5.85	(8.58)
15 - 15½	.27	(0.54)	5.81	(8.42)
15½ - 16	.26	(0.36)	5.95	(8.43)
16 - 16½	.42	(0.60)	6.03	(8.45)
16½ - 17	.44	(0.72)	6.03	(8.51)
17 - 17½	.29	(0.68)	6.15	(8.61)
17½ - 18	.36	(0.53)	6.15	(8.63)
18 - 18½	.24	(0.54)	6.13	(8.58)
18½ - 19	.46	(0.36)	6.05	(8.43)
19 - 19½	.18	(0.66)	6.00	(8.68)
19½ - 20	.20	(0.49)	6.03	(8.61)
20 - 20½	.31	(0.47)	6.08	(8.63)
20½ - 21	.27	(0.44)	6.15	(8.42)
21 - 21½	.30	(0.68)	6.20	(8.48)
21½ - 22	.36	(0.54)	6.05	(8.57)
22 - 22½	.22	(0.25)	6.15	(8.61)
22½ - 23	.22	(0.65)	6.08	(8.58)
23 - 23½	.30	(0.29)	6.11	(8.61)
23½ - 24	.33	(0.55)	6.10	(8.60)

APPENDIX TABLE **41**

SIMULTANEOUS MEASUREMENT OF MATERNAL AND FOETAL URINE PARAMETERS
(EWE AND FOETUS 69-826) (MATERNAL VALUES IN BRACKETS)

TIME (Hrs)	NaER (µEq/min)		KER (µEq/min)	
0 - ½	17.0	(180.3)	4.0	(287.3)
½ - 1	23.7	(250.8)	4.4	(379.2)
1 - 1½	20.1	(227.3)	4.4	(320.3)
1½ - 2	16.9	(189.9)	4.3	(364.5)
2 - 2½	28.1	(178.8)	7.1	(369.1)
2½ - 3	16.1	(177.6)	3.9	(355.3)
3 - 3½	16.5	(166.7)	4.2	(370.0)
3½ - 4	23.6	(156.2)	5.8	(336.1)
4 - 4½	11.5	(96.0)	3.0	(360.0)
4½ - 5	12.4	(108.3)	3.7	(365.9)
5 - 5½	13.5	(118.4)	3.6	(342.8)
5½ - 6	10.9	(106.4)	3.0	(338.2)
6 - 6½	16.2	(111.3)	4.3	(282.7)
6½ - 7	11.4	(93.8)	3.0	(277.2)
7 - 7½	18.2	(121.7)	5.4	(366.2)
7½ - 8	24.0	(135.8)	7.5	(335.3)
8 - 8½	17.4	(128.6)	5.0	(365.7)
8½ - 9	11.6	(96.5)	3.6	(345.1)
9 - 9½	15.1	(108.4)	4.3	(256.9)
9½ - 10	16.2	(118.7)	4.9	(292.7)
10 - 10½	10.6	(128.4)	3.1	(288.7)
10½ - 11	28.0	(166.4)	8.0	(301.8)
11 - 11½	8.6	(182.1)	2.6	(323.7)
11½ - 12	12.1	(164.1)	3.8	(279.3)

APPENDIX TABLE CONT. 41

TIME (Hrs)	NaER (μEq/min)		KER (μEq/min)	
12 - 12½	15.4	(168.2)	4.9	(285.6)
12½ - 13	21.0	(181.7)	6.7	(298.4)
13 - 13½	7.3	(105.6)	2.0	(307.6)
13½ - 14	13.5	(104.7)	4.9	(341.9)
14 - 14½	28.2	(134.7)	5.3	(362.2)
14½ - 15	19.6	(118.6)	4.8	(326.1)
15 - 15½	17.4	(128.6)	4.0	(318.8)
15½ - 16	15.7	(142.8)	5.6	(354.2)
16 - 16½	24.4	(113.7)	8.1	(288.7)
16½ - 17	19.1	(103.6)	6.0	(264.4)
17 - 17½	13.4	(145.7)	4.2	(272.2)
17½ - 18	17.1	(203.8)	5.0	(301.2)
18 - 18½	10.4	(202.3)	3.6	(333.7)
18½ - 19	21.9	(185.8)	7.8	(342.8)
19 - 19½	18.0	(168.4)	5.2	(318.3)
19½ - 20	12.4	(145.7)	4.4	(342.1)
20 - 20½	16.2	(162.4)	5.3	(340.2)
20½ - 21	14.0	(151.2)	4.5	(315.2)
21 - 21½	17.1	(148.3)	4.5	(288.7)
21½ - 22	14.0	(166.1)	4.3	(276.3)
22 - 22½	10.3	(95.1)	3.7	(298.7)
22½ - 23	12.1	(101.1)	3.7	(264.4)
23 - 23½	15.1	(122.3)	4.7	(281.2)
23½ - 24	16.2	(168.1)	5.7	(302.2)

APPENDIX TABLE **42**

FOETUS 253 (117 DAYS) MATERNAL FUROSEMIDE TREATMENT (2 DOSES 10 mg)

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (uEq/min)	[K+] (mEq/1)	KER (µEq/min)
0 - ½	.60	71.0	50.94	6.0	3.66
½ - 1	.61	83.5	27.73	4.0	1.88
1 - 1½	.47	59.0	49.56	6.0	3.36
1½ - 2	.56	88.5	28.67	4.0	1.96
2 - 2½	.49	58.5	28.32	5.1	2.40
2½ - 3	.48	59.5	39.35	7.0	3.01
3 - 3½	.43	91.5	17.29	7.0	1.33
3½ - 4	.19	91.0	19.44	6.0	1.62
4 - 4½	.27	72.0	23.58	5.0	2.05
4½ - 5	.41	57.5	25.16	6.0	2.34
5 - 5½	.39	64.5	40.98	7.0	3.85
5½ - 6	.55	74.5	32.37	9.0	3.51
6 - 6½	.39	83.0	30.42	5.1	2.60
6½ - 7	.52	58.5	23.79	6.1	2.34

APPENDIX TABLE **43**

EWE CARRYING FOETUS 275: MATERNAL FUROSEMIDE TREATMENT (10 mg/hr; 11 hr)

MATERNAL RESPONSES

TIME (Hrs)	URINE FLOW (ml/min)	URINE [Na+] (mEq/l)	URINE NaER (µEq/min)	URINE KER (µEq/min)	PLASMA [Na+] (mEq/l)	PLASMA [K+] (mEq/l)	PLASMA OP (mOsm/kg)
0 - 2	0.69	5.5	3.8	29.6	143.5	3.8	279
2 - 4	6.34	27.5	174.35	431.1	143.9	4.0	283
4 - 6	4.42	24.0	160.08	256.4	143.5	3.8	309
6 - 8	2.92	30.0	87.60	183.9	-	-	-
8 - 10	2.42	22.1	53.24	104.1	143.5	3.4	303
10 - 12	1.82	14.0	25.48	101.9	144.0	3.9	313
12 - 14	1.28	4.2	5.12	71.7	-	-	-

APPENDIX TABLE **44**

FOETUS 275 (118 DAYS) MATERNAL FUROSEMIDE TREATMENT (10 mg/hr; 11 hrs)

	URINE					
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (μEq/min)	[K+] (mEq/1)	KER (μEq/min)	CrER (μg/min)
0 - ½	.26	-	-	-	-	-
½ - 1	.30	53	14.84	24.0	6.72	5.70
1 - 1½	.23	-	-	-	-	-
1½ - 2	.20	55.5	12.21	21.4	4.71	4.23
2 - 2½	.25	-	-	-	-	-
2½ - 3	.19	54.5	13.09	21.0	4.62	4.98
3 - 3½	.07	-	-	-	-	-
3½ - 4	.02	78	3.12	27.1	1.08	0.90
4 - 4½	.13	-	-	-	-	-
4½ - 5	.08	78.5	8.64	36.0	3.96	3.73
5 - 5½	.31	-	-	-	-	-
5½ - 6	.14	76.5	17.60	44.0	10.12	10.41
6 - 6½	.09	-	-	-	-	-
6½ - 7	.18	98	13.72	54.0	7.56	4.75
7 - 7½	.07	-	-	-	-	-
7½ - 8	.23	51.5	7.73	26.0	3.90	6.79
8 - 8½	.30	-	-	-	-	-
8½ - 9	.24	102.5	27.68	52.0	14.04	6.11
9 - 9½	.28	-	-	-	-	-
9½ - 10	.55	98.5	54.18	54.0	22.68	33.25
10 - 10½	.43	-	-	-	-	-
10½ - 11	.23	70.5	23.27	34.0	11.22	33.59
11 - 11½	.23	-	-	-	-	-
11½ - 12	.35	97.5	28.28	55.0	15.95	19.68
12 - 12½	.38	-	-	-	-	-
12½ - 13	.08	52.5	12.08	32.0	7.36	10.41
13 - 13½	.18	-	-	-	-	-
13½ - 14	.25	83	18.26	75.0	16.5	42.30

APPENDIX TABLE **45**

EWE CARRYING FOETUS 275: MATERNAL SALT LOAD (Na^+ & Cl^-, 1.6 mEq/min: 2½ hr)

MATERNAL RESPONSES

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	PH	[Na+] (mEq/l)	[K+] (mEq/l)
0 - ½	-	-	-	-	-	142.0	4.3
½ - 1	2.17	4.5	9.77	436.2	7.65	-	-
1 - 1½	-	-	-	-	-	-	-
1½ - 2	1.42	6.5	9.23	227.2	7.70	142.0	4.4
2 - 2½	-	-	-	-	-	146.5	4.4
2½ - 3	0.18	5.5	0.99	33.5	8.01	157.6	4.0
3 - 3½	-	-	-	-	-	155.3	4.1
3½ - 4	0.08	12.0	0.96	17.4	8.15	158.2	3.9
4 - 4½	-	-	-	-	-	158.5	4.1
4½ - 5	0.15	-	-	-	-	-	-
5 - 5½	-	-	-	-	-	-	-
5½ - 6	0.09	123.5	11.12	20.7	8.15	159.6	4.0
6 - 6½	-	-	-	-	-	-	-
6½ - 7	2.17	131.0	284.27	312.5	7.80	160.5	4.0
7 - 7½	-	-	-	-	-	-	-
7½ - 8	1.05	86.1	90.30	201.6	7.55	158.9	3.9
8 - 8½	-	-	-	-	-	-	-
8½ - 9	0.15	71.0	10.65	20.4	7.35	157.8	4.1
9 - ½	-	-	-	-	-	-	-
9½ - 10	0.47	46.5	21.86	62.5	6.81	159.2	4.1

APPENDIX TABLE **46**

FOETUS 275 (122 DAYS) MATERNAL SALT LOAD (Na^+ & Cl^-, 1.6mEq/min; 2½ hr)

		URINE				PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (μEq/min)	KER (μEq/min)	[Cr] (mg/100ml)	[Na+] (mEq/l)	[K+] (mEq/l)
0 - ½	.25	13.5	3.38	2.00	7.6	145.5	4.6
½ - 1	.42	4.5	4.92	2.52	6.2		
1 - 1½	.50	15.5	7.75	4.00	8.7		
1½ - 2	.56	13.5	7.56	3.36	5.9	143.3	4.5
2 - 2½	.53	17.5	9.28	4.24	7.5		
2½ - 3	.33	14.5	4.79	2.00	6.1		
3 - 3½	.45	12.5	5.63	1.80	5.2		
3½ - 4	.32	17.1	5.44	1.92	8.0	148.6	3.8
4 - 4½	.27	17.1	4.59	1.89	9.3		
4½ - 5	.11	27.0	2.97	.99	13.5		
5 - 5½	.21	36.0	7.56	2.52	19.5		
5½ - 6	.26	28.5	7.28	2.34	14.9	147.5	3.9
6 - 6½	.27	29.1	7.83	2.16	13.2		
6½ - 7	.22	27.5	6.55	2.52	8.9		
7 - 7½	.51	17.5	8.93	2.04	4.5		
7½ - 8	.62	17.5	10.85	1.86	3.8	149.1	4.1
8 - 8½	.57	16.0	9.12	1.71	2.2		
8½ - 9	.54	31.0	16.74	3.24	4.4		
9 - 9½	.60	16.5	9.90	1.80	2.2		
9½ - 10	.64	29.5	18.88	3.84	4.0	146.2	4.4

APPENDIX TABLE **47**

FOETUS 51 (118 DAYS) FUROSEMIDE INJECTION (0.8 mg/kg)

	URINE					
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml)
0 - ½	.29	46.0	13.34	1.89	8.8	1.9
½ - 1	.35	26.0	9.10	1.75	7.4	2.0
1 - 1½	.45	34.5	15.30	2.25	6.0	.7
1½ - 2	.63	44.5	28.04	3.15	5.7	.7
2 - 2½	.53	35.0	18.55	1.59	2.4	.5
2½ - 3	.62	68.5	42.47	3.10	3.8	.6
3 - 3½	1.28	44.5	56.32	3.20	1.4	.4
3½ - 4	1.2	96.5	115.80	6.00	3.0	.6
4 - 4½	1.03	18.5	18.54	1.03	.5	.4
4½ - 5	1.37	13.0	17.81	2.06	.9	.4
5 - 5½	1.42	48.5	68.87	2.84	1.4	.5
5½ - 6	1.05	26.0	27.30	1.05	.6	.5
6 - 6½	.97	36.0	34.92	1.94	1.2	.6
6½ - 7	1.02	73.0	74.46	4.08	3.3	.9
7 - 7½	.97	32.5	31.53	1.94	1.3	.7
7½ - 8	.95	73.5	69.35	3.80	2.9	.9
8 - 8½	1.05	81.0	82.05	4.20	2.8	1.0

APPENDIX TABLE **48**

FOETUS 69-559 (121 DAYS) FUROSEMIDE INJECTION (1.0 mg/kg)

URINE

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	UAER (µg/min)	PH
0 - ½	.07	63	4.41	35	2.45	4.20	6.7
½ - 1	.11	77	8.47	33	3.63	6.27	8.7
1 - 1½	.06	60	3.60	18	1.08	2.58	6.0
1½ - 2	.16	66	10.56	30	4.8	9.12	8.7
2 - 2½	.14	38.5	5.39	15	2.10	3.22	8.4
2½ - 3	.13	-	-	4	-	1.04	9.1
3 - 3½	.53	79.5	42.14	9	4.77	6.36	8.7
3½ - 4	.97	30	29.10	3	2.91	7.76	9.2
4 - 4½	1.10	49	53.9	5	5.5	11.00	9.4
4½ - 5	1.38	35.5	48.99	4	5.52	12.42	8.7
5 - 5½	1.35	27	40.5	3	4.05	9.45	9.4
5½ - 6	1.45	33.5	48.58	3	4.35	13.05	9.4
6 - 6½	1.07	33	35.31	5	5.35	8.56	8.9
6½ - 7	1.32	60	79.20	9	11.88	17.16	8.8
7 - 7½	1.13	46.5	52.55	8	9.04	14.69	9.0
7½ - 8	.73	56	40.88	14	10.22	13.87	8.7
8 - 8½	.78	54	42.12	15	11.7	14.04	8.8
8½ - 9	.75	58	43.50	7	5.25	12.00	8.8
9 - 9½	.53	57	30.21	13	6.89	14.31	8.9
9½ - 10	.51	56.5	28.82	9	4.59	11.22	8.5

APPENDIX TABLE **49**

FOETUS 107 (121 DAYS) FUROSEMIDE INJECTION (1.0 mg/kg)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	.12	84	10.08	1.6	.19		
½ - 1	.06	83	4.98	1.5	.09	146.1	4.8
1 - 1½	.11	82	9.02	1.5	.17		
1½ - 2	.11	75	8.25	1.7	.19		
2 - 2½	.08	75	6.00	1.8	.14		
2½ - 2¾	.35	99	34.65	1.1	.39		
2¾ - 3	.43	99	52.47	1.1	.58	142.2	5.1
3 - 3¼	.28	102	28.56	1.1	.31		
3¼ - 3½	.36	104	37.44	1.2	.43		
3½ - 3¾	.45	103	46.35	1.2	.54		
3¾ - 4	.19	98	18.62	1.2	.23	142.3	5.4
4 - 4¼	.35	101	35.35	1.2	.42		
4¼ - 4½	.46	112	51.52	1.2	.55		
4½ - 4¾	.39	109	42.51	1.2	.47		
4¾ - 5	.37	106	39.22	1.1	.41	144.4	4.9
5 - 5½	.37	103	38.11	1.0	.37		
5½ - 6	.33	111	36.63	1.1	.36	145.5	5.0
6 - 6¼	.35	107	37.45	1.2	.42		
6¼ - 6½	.29	105	30.45	1.3	.38		
6½ - 6¾	.25	103	25.75	1.5	.38		
6¾ - 7	.33	104	34.32	1.5	.50	146.6	5.1
7 - 7¼	.31	107	33.17	1.5	.47		
7¼ - 7½	.31	108	33.48	1.5	.47		
7½ - 7¾	.33	109	35.97	1.6	.53		
7¾ - 8	.33	109	35.97	1.7	.56	147.5	4.7

APPENDIX TABLE 50

FOETUS 150 (122 DAYS) FUROSEMIDE INJECTION (1.0 mg/kg)

URINE

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)
0 - ½	.48	40.5	19.44	25	12.0
½ - 1	.42	44.5	18.69	24	10.08
1 - 1½	.28	32	8.96	11	3.08
1½ - 2	.43	19	8.17	13	5.59
2 - 2½	.52	20	10.4	12.5	6.50
2½ - 3	.70	15	10.5	3	2.10
3 - 3½	.50	14	7.0	2	1.00
3½ - 4	1.02	39	39.78	3.5	3.57
4 - 4½	1.17	-	-	-	-
4½ - 5	1.17	31	36.27	3	3.51
5 - 5½	1.17	21.5	25.16	2	2.34
5½ - 6	.87	30	26.10	3	2.61
6 - 6½	1.02	39.5	40.29	4	4.08
6½ - 7	.95	21	19.95	2	1.90
7 - 7½	.95	82.5	78.38	11	10.45
7½ - 8	.85	23.5	19.98	3	2.55
8 - 8½	.87	28	24.36	4	3.48
8½ - 9	.75	32.5	24.38	4	3.00
9 - 9½	.68	34.5	23.46	5	3.40
9½ - 10	.68	34	23.12	7	4.76

APPENDIX TABLE **51**

FOETUS 230 (122 DAYS) FUROSEMIDE INJECTION (1.3 mg/kg)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	.47	38	17.86	5.5	2.58	141.5	4.5
½ - 1	.35	28	9.8	6	2.10		
1 - 1½	.37	33.5	12.39	6	2.22	140.5	4.6
1½ - 2	.37	37.5	13.87	6	2.22		
2 - 2½	.36	49.5	17.82	8.5	3.06		
2½ - 3	1.05	51.5	54.07	3	3.15	143.5	4.7
3 - 3½	.9	70	63.0	4	3.6		
3½ - 4	.83	85	70.55	5	4.15	146.5	4.5
4 - 4½	.73	44.5	32.48	3	2.19		
4½ - 5	.75	46.5	34.87	3	2.25	142	4.6
5 - 5½	.65	49	31.85	4	2.6		
5½ - 6	.58	33.5	19.43	3	1.74	147.5	4.5
6 - 6½	.49	57	27.93	5	2.45		
6½ - 7	.40	42.5	17.6	4	1.60	145.5	4.7

APPENDIX TABLE **52**

FOETUS 66-310 (139 DAYS) FUROSEMIDE INJECTION (0.8 mg/kg)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	.28	32.5	9.10	35	9.80		
½ - 1	.30	21	6.30	26	7.80		
1 - 1½	.23	22	5.06	27	6.21	144.5	5.0
1½ - 2	.29	17.5	5.08	21	6.09		
2 - 2½	.20	15	3.00	19	3.80	147.5	4.8
2½ - 3	.28	11.5	3.22	16	4.48		
3 - 3½	.25	55	13.75	23	5.75		
3½ - 4	.62	75	46.50	15	9.30	148.2	5.0
4 - 4½	.61	45	27.45	11	6.71		
4½ - 5	.34	35	11.90	11	3.74	146.0	4.7
5 - 5½	.36	35	12.60	13	4.68		
5½ - 6	.28	35	9.80	13	3.64		
6 - 6½	.31	55	17.05	23	7.13	147.0	4.2
6½ - 7	.30	30	9.00	13	3.90		

APPENDIX TABLE **53**

FOETUS 66-440 (140 DAYS) FUROSEMIDE INJECTION (0.9 mg/kg)

TIME (Hrs)	FLOW (ml/min)	URINE [Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	CrER (µg/min)
0 - ½	.09	52	4.68	6.4	.58	11.07
½ - 1	.09	60	5.40	7.7	.69	14.40
1 - 1½	.10	45	4.50	4.7	.47	15.50
1½ - 2	.13	30	3.90	3.5	.46	18.85
2 - 2½	.17	44	7.48	5.0	.85	28.05
2½ - 3	.08	70	5.60	7.6	.61	12.80
3 - 3½	.33	49.5	16.34	4.5	1.49	54.72
3½ - 4	.40	100	40.00	2.7	1.08	40.00
4 - 4½	.41	95	38.95	2.2	.90	37.72
4½ - 5	.26	87.5	22.75	2.4	.62	29.38
5 - 5½	.11	138	15.18	3.5	.39	12.87
6 - 6½	.21	86.5	18.17	2.3	.48	24.15

APPENDIX TABLE **54**

FOETUS 223 (114 DAYS) ETHACRYNATE INJECTION (2.1 mg/kg)

	URINE						PLASMA
TIME (Hrs)	FLOW (ml/min)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH	[Na+] (mEq/1)
0 - ½	.11	10.01	.99	2.05	1.64	6.6	
½ - 1	.07	5.95	.70	1.08	.79	6.7	135.7
1 - 1½	.14	13.02	1.12	2.55	1.67	6.6	
1½ - 2	.10	9.5	.90	1.95	1.37	6.5	
2 - 2½	.12	11.52	1.08	2.34	1.72	6.5	138.5
2½ - 3	.16	12.8	1.12	2.46	1.90	6.5	
3 - 3½	1.06	104.94	4.24	2.33	5.09	7.1	
3½ - 4	1.05	100.80	4.20	2.31	4.94	7.1	134.0
4 - 4½	.85	92.65	4.25	2.30	4.59	7.3	
4½ - 5	.53	50.35	2.65	1.70	3.18	7.0	
5 - 5½	.51	68.85	3.57	2.40	3.32	6.8	133.5
5½ - 6	.54	62.10	2.70	2.00	3.24	6.9	
6 - 6½	.45	34.2	1.35	1.13	2.70	7.1	
6½ - 7	.52	58.76	2.60	2.18	3.38	6.7	133.0
7 - 7½	.41	35.26	1.64	1.27	2.46	6.6	
7½ - 8	.50	56.0	2.50	1.95	3.25	6.6	

APPENDIX TABLE **55**

FOETUS 230 (123 DAYS) ETHACRYNATE INJECTION (1.7 mg/kg)

TIME (Hrs)	FLOW (ml/min)	NaER (µEq/min)	URINE KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH	PLASMA [Na+] (mEq/1)
0 - ½	.40	10.20	.40	19.60	4.0	6.5	142.5
½ - 1	.36	10.44	.36	23.76	4.68	6.5	
1 - 1½	.38	17.29	1.14	36.86	6.84	6.4	144.7
1½ - 2	.43	13.33	.43	26.23	5.16	6.5	
2 - 2½	.43	14.62	.43	27.95	6.02	6.4	
2½ - 3	1.36	98.6	4.08	35.36	8.16	6.8	142.5
3 - 3½	1.70	185.3	6.8	49.30	13.60	7.3	140.6
3½ - 4	1.33	86.45	3.99	26.60	6.65	7.2	
4 - 4½	.95	69.35	2.85	27.55	6.65	6.8	139.8
4½ - 5	.78	79.56	3.12	37.44	7.80	6.7	
5 - 5½	.33	19.14	.66	9.90	2.31	6.8	
5½ - 6	.72	30.96	1.08	17.28	3.60	6.6	
6 - 6½	.22	22.33	.88	13.20	2.64	6.6	
6½ - 7	.58	40.89	1.16	24.94	5.80	6.6	
7 - 7½	.47	27.26	.82	16.81	3.69	6.7	

APPENDIX TABLE **56**

FOETUS 230 (129 DAYS) ETHACRYNATE INJECTION (1.6 mg/kg)

		URINE					PLASMA
TIME (Hrs)	FLOW (ml/min)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH	[Na+] (mEq/1)
0 - ½	.19	3.80	2.47	3.00	3.84	6.4	
½ - 1	.21	6.51	3.99	4.64	5.00	6.6	142.5
1 - 1½	.19	5.51	3.42	4.03	4.64	6.6	
1½ - 2	.18	5.58	3.42	3.98	4.61	6.6	
2 - 2½	.21	6.09	3.57	4.28	5.00	6.6	
2½ - 3	.20	5.40	2.80	3.50	4.40	6.5	141.5
3 - 3½	.18	6.30	2.88	3.19	4.07	6.9	
3½ - 4	.07	2.10	.70	1.30	1.67	6.7	
4 - 4½	.13	9.88	1.95	1.57	.70	6.6	
4½ - 5	2.08	128.96	12.48	5.62	13.52	7.2	
5 - 5½	1.63	215.16	19.56	5.38	12.55	7.1	139.0
5½ - 6	1.35	109.35	9.45	3.24	10.40	6.7	
6 - 6½	.53	71.02	6.36	2.65	7.26	6.5	
6½ - 7	.19	22.23	2.66	1.67	3.95	6.4	140.5
7 - 7½	.13	16.77	1.95	1.56	3.25	6.5	
7½ - 8	.14	16.80	2.66	2.56	4.41	6.5	

APPENDIX TABLE **57**

FOETUS 223 (130 DAYS) ACETAZOLAMIDE INJECTIONS (50 mg/kg & 50 mg/kg)

TIME (Hrs)	FLOW (ml/min)	URINE [Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH
0 - ½	.51	29.5	15.05	2.04	12.69	7.72	7.25
½ - 1	.40	37.5	15.0	1.60	10.86	7.40	7.3
1 - 1½	.77	83.1	63.91	10.01	45.29	14.24	7.6
1½ - 2	.34	103.5	35.19	4.42	19.61	5.72	7.75
2 - 2½	.33	97.5	32.18	4.29	18.29	7.21	7.8
2½ - 3	.60	124.0	74.4	7.80	40.72	12.10	7.65
3 - 3½	.74	70.5	52.17	5.18	29.21	8.71	7.9
3½ - 4	1.46	97.5	142.35	11.68	82.56	24.54	7.9
4 - 4½	1.28	66.0	84.48	3.84	26.06	12.91	7.95
4½ - 5	1.33	103.2	136.99	6.65	46.63	17.89	7.8
5 - 5½	.61	93.5	55.04	4.27	22.77	10.25	8.0
5½ - 6	.38	72.5	27.55	1.52	9.03	6.39	8.15
6 - 6½	.49	89.1	43.61	4.9	21.06	10.71	8.05
6½ - 7	.80	79.2	63.20	6.40	28.05	14.79	8.0
7 - 7½	.65	82.5	53.63	5.85	27.20	12.02	8.05
7½ - 8	.56	105.0	58.8	8.40	36.73	11.30	8.05
8 - 8½	.45	60.5	27.23	3.15	16.80	6.05	8.05
8½ - 9	.53	82.5	43.73	3.18	19.18	9.80	8.0
9 - 9½	.47	84.0	39.48	2.82	19.67	7.90	8.0
9½ - 10	.51	107.6	54.57	4.08	28.26	10.29	7.9
10 - 10½	.65	103.5	67.28	6.50	39.70	14.20	7.95

APPENDIX TABLE **58**

FOETUS 271 (145 DAYS) ACETAZOLAMIDE INJECTIONS (28 mg/kg & 56 mg/kg)

	URINE						
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH
0 - ¼	.45	16	7.20	4.05	32.85	1.35	7.55
¼ - ½	.71	15	10.65	6.39	55.38	5.68	7.45
½ - ¾	.59	15	8.85	5.31	48.38	5.31	7.55
¾ - 1	.66	17	11.22	5.94	53.46	3.30	7.45
1 - 1¼	.66	15.5	10.23	5.94	56.76	5.28	7.6
1¼ - 1½	1.06	21.5	22.79	12.72	85.86	8.48	8.0
1½ - 1¾	.96	34	32.64	10.56	56.64	4.80	8.2
1¾ - 2	.94	35	32.90	10.34	53.58	3.76	8.05
2 - 2¼	.84	35	29.40	12.60	47.04	1.68	8.25
2¼ - 2½	.87	33	28.71	13.92	50.46	3.48	8.3
2½ - 2¾	.99	31	30.69	10.89	58.41	4.95	8.4
2¾ - 3	.90	33	29.70	9.00	48.60	3.60	8.3
3 - 3¼	.85	32.5	27.63	7.65	47.60	2.55	8.1
3¼ - 3½	.73	30.5	22.27	7.30	43.80	2.92	8.4
3½ - 3¾	1.00	30.5	30.50	10.00	60.00	5.00	8.3
3¾ - 4	1.01	34	34.34	9.09	52.52	4.04	8.2
4 - 4¼	.69	25.5	17.60	6.90	36.57	2.76	8.2
4¼ - 4½	.60	32	19.20	6.60	37.80	3.00	8.35
4½ - 4¾	.66	29.5	19.47	7.92	46.86	1.98	8.3
4¾ - 5	.49	29.5	14.46	5.88	29.40	2.94	8.45
5 - 5¼	.65	31	20.15	7.80	44.85	3.25	8.25
5¼ - 5½	.60	28.5	17.10	7.80	43.80	4.20	8.4
5½ - 5¾	.54	31.5	17.01	7.02	40.50	3.78	8.45

APPENDIX TABLE 59

FOETUS 275 (113 DAYS) CHLOROTHIAZIDE INJECTIONS (83 mg/kg & 166 mg/kg)

TIME (Hrs)	FLOW (ml/min)	URINE [Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	PH
0 - ½	.06	17.5	1.05	9.0	.54	-
½ - 1	.04	38.0	1.52	15.0	.60	7.15
1 - 1½	.02	44.0	.88	14.0	.28	7.3
1½ - 2	.10	47.0	4.7	9.0	.90	7.95
2 - 2½	.24	63.5	15.24	14.0	3.36	7.45
2½ - 3	.13	65.0	8.45	13.0	1.69	7.65
3 - 3½	.04	36.0	1.44	7.0	.28	7.55
3½ - 4	.02	-	-	-	-	-
4 - 4½	.35	56.5	19.78	2.0	.70	7.45
4½ - 5	.07	50.5	3.50	6.0	.42	7.75
5 - 5½	.06	74.0	4.44	5.0	.30	7.75

APPENDIX TABLE **60**

FOETUS 257 (129 DAYS) CHLOROTHIAZIDE INJECTIONS (13 mg/kg & 26 mg/kg)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/1)	Cr (mg/100ml)	UA (mg/100ml)	PH
0 - ½	.13	30	3.90	1.95	5.5	1.3	7.1
½ - 1	.08	30.5	2.44	1.12	5.3	1.4	6.9
1 - 1¼	.29	39	11.31	3.77	5.5	1.3	7.05
1¼ - 1½	.39	72	28.08	3.90	4.4	1.0	7.45
1½ - 1¾	.20	79	15.80	2.00	4.6	1.1	7.65
1¾ - 2	.49	74.5	36.51	4.90	4.5	1.0	7.9
2 - 2¼	.43	60.5	26.02	3.44	3.2	.8	7.6
2¼ - 2½	.43	51.5	22.15	3.01	2.5	.7	7.55
2½ - 2¾	.35	72.5	25.38	3.85	3.5	.8	7.6
2¾ - 3	.35	72.5	25.38	4.20	3.8	1.0	7.6
3 - 3¼	.43	71	29.82	4.62	4.1	1.1	7.6
3¼ - 3½	.24	72	17.28	2.64	3.9	1.1	7.65
3½ - 4	.15	71.5	10.73	1.65	4.1	1.1	7.6
4 - 4½	.24	67	16.08	2.40	3.9	1.1	7.45
4½ - 5	.23	68	15.64	2.53	4.3	1.1	7.45
5 - 5½	.17	66.5	11.31	1.87	4.4	1.2	7.35
5½ - 6	.23	68	15.64	2.53	4.2	1.1	7.65
6 - 6½	.38	43.5	16.53	2.28	1.8	.6	7.9
6½ - 7	.27	74	19.98	2.70	3.1	.9	7.9
7 - 7½	.26	74	19.24	2.86	3.4	1.1	7.9
7½ - 8	.27	73.5	19.85	3.24	4.2	1.1	7.95
8 - 8½	.19	73.5	13.97	2.28	4.3	1.2	7.8
8½ - 9	.32	70.5	22.56	3.52	4.0	1.1	8.0
9 - 9½	.30	71	21.3	3.00	3.8	1.1	7.5
9½ - 10	.31	66.5	20.62	2.79	3.33	.9	8.0
10 - 10½	.41	47	19.27	2.46	2.3	.6	7.9
10½ - 11	.22	72	15.84	1.98	3.6	1.0	7.7
11 - 11½	.16	70.5	11.28	1.44	3.9	1.2	7.65

APPENDIX TABLE 61

FOETUS 3 (143 DAYS) CHLOROTHIAZIDE INJECTIONS (30 mg/kg & 15 mg/kg)

TIME (mins)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	Cr (mg/100ml)	UA (mg/100ml)	PH
0 - 10	.21	56.5	11.87	1.05	7.3	2.1	7.1
10 - 20	.36	58.5	21.06	1.80	8.0	2.2	7.1
20 - 30	.21	54	11.34	1.26	8.7	2.5	7.05
30 - 40	.58	56	32.48	4.06	9.8	2.7	6.9
40 - 50	.58	52.5	30.45	4.06	9.9	2.7	6.9
50 - 60	.64	49.5	31.68	4.48	10.2	2.9	6.7
60 - 70	1.09	55.5	60.5	7.63	9.5	2.6	6.75
70 - 80	1.44	58	83.52	10.08	9.0	2.4	6.9
80 - 90	2.12	68.5	145.22	12.72	7.4	2.0	7.1
90 - 100	1.12	77	86.24	6.72	5.9	1.3	7.3
100 - 110	.93	75.5	70.22	4.65	6.0	1.4	7.6
110 - 120	.87	71	61.77	3.48	6.3	1.5	7.45
120 - 130	.68	78.5	53.38	2.72	5.6	1.4	7.65
130 - 140	.86	80	68.80	3.44	5.0	1.3	8.05
140 - 150	.95	81.5	77.43	2.85	4.9	1.3	7.85
150 - 160	-	-	-	-	-	-	-
160 - 170	.69	79.5	54.86	4.83	5.1	1.3	7.95
170 - 180	.76	78.5	59.66	3.04	5.3	1.4	7.7
180 - 190	1.37	80	109.60	5.48	5.3	1.5	7.5
190 - 200	1.59	80	127.20	4.77	5.2	1.4	7.55
200 - 210	1.57	80.5	126.39	4.71	5.0	1.3	7.5
210 - 220	1.08	78.5	84.78	3.24	4.9	1.2	7.65
220 - 230	1.04	79.5	82.68	3.12	4.8	1.2	7.6
230 - 240	.73	79	57.67	2.92	4.8	1.1	7.8
240 - 250	1.27	79	100.33	5.08	4.8	1.3	7.8
250 - 260	.55	76	47.30	2.75	4.9	.6	

APPENDIX TABLE **62**

FOETUS 3 (135 DAYS) AMILORIDE HYDROCHLORIDE INJECTION (2.0 mg/kg)

URINE

TIME (Hrs)	FLOW (ml/Min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	PH
0 - ½	.83	25.3	20.99	7.1	5.89	7.11
½ - 1	.99	26.2	25.94	7.2	7.13	7.31
1 - 1½	1.06	32.1	34.03	7.4	7.84	7.20
1½ - 2	1.35	34.3	46.31	7.1	9.59	7.32
2 - 2½	.64	32.3	20.61	6.8	4.35	7.40
2½ - 3	.81	28.7	23.24	6.7	5.43	7.51
3 - 3½	.77	29.1	22.41	6.9	5.31	7.42
3½ - 4	.67	28.6	19.16	7.1	4.76	7.62
4 - 4½	.53	26.5	14.05	7.2	3.82	7.74
4½ - 5	.74	26.5	19.61	7.7	5.70	7.31
5 - 5½	.71	26.8	19.03	7.1	5.04	7.41

APPENDIX TABLE **63**

FOETUS 3 (137 DAYS) AMILORIDE HYDROCHLORIDE INJECTION (2.0 mg/kg)

URINE

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (μEq/min)	[K+] (mEq/1)	KER (μEq/min)	PH
0 - ½	.44	22	9.68	7.1	3.08	6.65
½ - 1	.31	26	8.06	7.1	2.50	7.01
1 - 1½	.56	26.5	14.84	8.0	4.48	6.95
1½ - 2	.86	29	24.94	7.2	6.02	7.05
2 - 2½	.86	36	30.96	4.1	3.44	7.05
2½ - 3	.63	35	22.05	2.2	1.26	7.15
3 - 3½	.51	42	21.42	3.0	1.53	7.20
3½ - 4	.46	42	19.32	3.0	1.38	7.10
4 - 4½	.36	43.5	15.66	3.1	1.08	7.10
4½ - 5	.55	42	23.10	3.1	1.65	7.05
5 - 5½	.77	40	30.8	3.0	2.31	7.0
5½ - 6	.27	40.5	10.94	3.0	.81	6.95
6 - 6½	.31	38.5	11.94	3.2	.93	7.05
6½ - 7	.55	37.5	20.63	3.3	1.65	7.05
7 - 7½	.31	36	11.16	3.1	.93	7.05
7½ - 8	.40	37.5	15.0	2.0	.80	7.15
8 - 8½	.30	37	11.10	2.1	.60	7.01
8½ - 9	.51	35.5	18.11	2.1	1.02	7.03
9 - 9½	.30	34.5	10.35	2.0	.60	7.01

APPENDIX TABLE **64**

FOETUS 3 (139 DAYS) AMILORIDE HYDROCHLORIDE INJECTION (2.0 mg/kg)

URINE

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)
0 - ¼	.40	32.5	13.00	1.5	.60	28.80	8.80
¼ - ½	.33	35.0	11.55	1.9	.63	26.73	7.26
½ - ¾	.38	35.0	13.30	2.0	.76	32.30	8.36
¾ - 1	.19	33.5	6.37	1.8	.34	14.25	3.04
1 - 1¼	.30	35.5	10.65	2.0	.60	24.00	5.70
1¼ - 1½	.20	34.5	6.90	1.9	.38	16.00	4.00
1½ - 1¾	.29	-	-	-	-	-	-
1¾ - 2	.42	39.0	16.38	2.2	.92	33.60	9.66
2 - 2¼	.49	35.5	17.40	1.9	.93	42.14	9.80
2¼ - 2½	.29	36.5	10.59	2.1	.61	25.81	5.80
2½ - 2¾	.61	35.5	21.66	2.1	1.28	55.50	9.76
2¾ - 3	.81	36.0	29.16	2.1	1.70	73.71	15.39
3 - 3¼	.73	36.0	26.28	2.0	1.46	64.24	14.60
3¼ - 3½	.87	35.0	30.45	2.0	1.74	73.08	18.27
3½ - 3¾	.21	36.0	7.56	2.0	.42	18.48	4.41
3¾ - 4	.09	37.5	3.38	2.0	.18	7.29	1.98
4 - 4¼	.25	38.5	9.63	2.0	.50	20.00	5.00
4¼ - 4½	.25	34	8.50	1.9	.48	21.00	5.00

APPENDIX TABLE **65**

FOETUS 66-440 (133 DAYS) CORTISOL INFUSION (5 µg/kg/hr; 5 hr)

	URINE						
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Cr] (mg/100ml)	CrER (µg/min)
0 - ½	.35	20.5	7.18	7	2.45	14	49.00
½ - 1	.41	31.5	12.92	12	4.92	4	16.40
1 - 1½	.58	28.5	16.53	8	4.64	8	46.40
1½ - 2	.66	65.5	43.23	22	14.52	8	52.80
2 - 2½	.69	59.0	40.71	21	14.49	16	110.40
2½ - 3	.52	16.5	8.58	7	3.64	13	67.60
3 - 3½	.53	17.5	9.28	7	3.71	3	15.90
3½ - 4	.49	19.1	9.31	8	3.92	4	19.60
4 - 4½	.63	20.0	12.60	7	4.41	4	25.20
4½ - 5	.70	15.5	10.85	4	2.8	4	28.00
5 - 5½	.65	23.5	15.28	7	4.55	1	6.50
5½ - 6	.73	38.5	28.11	11	8.03	3	21.90
6 - 6½	.76	12.5	13.26	4	3.05	6	45.60

APPENDIX TABLE **66**

FOETUS 66-440 (137 DAYS) CORTISOL INFUSION (24 µg/kg/hr; 4 hr)

	URINE						
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml)
0 - ½	.18	11.5	2.07	12	2.16	7.0	2.1
½ - 1	.18	17	3.06	20	3.60	12.0	2.6
1 - 1½	.04	33.5	1.34	28	1.12	15.0	-
1½ - 2	.14	14	1.96	18	2.52	9.0	2.5
2 - 2½	.10	25.5	2.55	38	3.80	-	4.2
2½ - 3	.10	25.2	2.52	41	4.1	24.0	4.2
3 - 3½	.11	25.5	2.81	38	4.18	23.0	4.0
3½ - 4	.11	15	1.65	23	2.53	21.0	3.8
4 - 4½	.08	32	2.56	52	4.16	12.0	5.5
4½ - 5	.14	21.5	3.01	38	5.32	33.0	5.0
5 - 5½	.11	32.5	3.58	57	6.27	24.0	6.3
5½ - 6	.37	32	11.84	58	21.46	38.0	6.8
6 - 6½	.21	29.5	6.20	50	10.50	44.0	5.8
6½ - 7	.23	36.5	3.80	24	5.52	35.0	3.8
7 - 7½	.15	39	5.85	43	6.45	15.0	5.3
7½ - 8	.19	23	5.37	24	4.56	28.0	3.4

APPENDIX TABLE **67**

FOETUS 220 (145 DAYS) CORTISOL INFUSION (20 μg/kg/hr; 4 hr)

	URINE						
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (μEq/min)	[K+] (mEq/l)	KER (μEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml)
0 - ½	.58	63	36.54	19.9	11.54	18.0	2.8
½ - 1	.66	54	35.64	16.6	10.96	11.5	2.2
1 - 1½	.88	67	58.96	15.4	13.55	9.8	2.9
1½ - 2	1.13	55	62.15	9.6	10.85	5.8	2.0
2 - 2½	.97	47.5	46.08	12.1	11.74	9.5	2.7
2½ - 3	.82	47.5	38.95	13.3	10.91	5	1.7
3 - 3½	1.18	44	51.92	8.3	9.79	5.2	1.7
3½ - 4	1.40	43.5	60.90	8.7	12.18	4	1.3
4 - 4½	1.33	38.5	51.21	7.2	9.58	5.7	2.0
4½ - 5	1.37	54.5	74.67	9.5	13.02	6	2.0
5 - 5½	1.00	42	42	10.2	10.02	7.8	2.5
5½ - 6	1.03	87.5	90.13	14.6	15.04	16	5.5
6 - 6½	.88	107	94.16	15.8	13.90	9.7	3.6
6½ - 7	.41	103.5	42.44	26	10.66	9.4	3.0
7 - 7½	.62	105.5	65.41	18.8	11.66	9.1	3.5
7½ - 8	.53	107	56.71	18	9.54	14.8	3.4
8 - 8½	.55	121	66.55	15.2	8.36	10.1	3.7

APPENDIX TABLE **68**

FOETUS 157 (148 DAYS) CORTISOL INFUSION (20 µg/kg/hr; 6 hr)

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	[K+] (mEq/l)	KER (µEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml)
0 - ½	.38	96.5	36.67	15.6	5.93	16.8	4.3
½ - 1	.55	78	42.90	7.2	3.96	6.6	1.7
1 - 1½	1.02	122.5	124.95	8.3	8.47	9.1	2.0
1½ - 2	1.30	109	141.70	8.1	10.53	6.1	2.0
2 - 2½	1.73	122.5	211.93	10	17.30	7.2	2.0
2½ - 3	1.35	124.5	168.08	10.4	14.04	7.0	2.2
3 - 3½	1.42	64.5	91.59	5.1	7.24	3.8	1.0
3½ - 4	1.88	69.5	130.66	5.6	10.53	3.8	.9
4 - 4½	2.20	-	-	-	-	-	-
4½ - 5	1.29	55	70.95	5.3	6.84	3.6	1.0
5 - 5½	1.33	112.5	149.63	12.8	17.02	8.4	2.0
5½ - 6	1.00	55.5	55.5	21.1	21.1	19.0	4.0
6 - 6½	1.35	109	147.15	11.3	15.26	11.0	3.0
6½ - 7	1.22	86	104.92	9.6	10.82	7.0	2.3
7 - 7½	1.20	93.5	112.20	10.1	12.12	6.2	2.1
7½ - 8	1.22	113.5	138.47	11.3	13.79	6.8	2.1
8 - 8½	1.47	111.5	163.91	10.3	15.14	6.8	2.0

APPENDIX TABLE **69**

FOETUS 223 (127 DAYS) ALDOSTERONE INFUSION (130 µg/kg/hr; 2½ hr)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	PH	[Na+] (mEq/l)	[K+] (mEq/l)
0 - ½	.27	68	18.36	2.86	7.2		
½ - 1	.41	72	29.52	4.51	7.3	141.5	3.9
1 - 1½	.11	84	9.24	1.34	7.4		
1½ - 2	.03	90	2.70	.37	7.8		
2 - 2½	.05	81	4.05	.54	7.9		
2½ - 3	.20	72	1.44	1.94	7.6		
3 - 3½	.11	80	8.80	1.16	7.6		
3½ - 4	.68	97	65.96	8.3	7.4	140.2	3.7
4 - 4½	.23	60	13.80	1.61	7.5		
4½ - 5	.32	58	18.56	4.00	7.4		
5 - 5½	.46	38	17.48	4.92	7.3		
5½ - 6	.19	78	14.82	4.18	7.4		
6 - 6½	.31	80	24.80	7.44	7.2		
6½ - 7	.21	80	16.80	5.46	7.3	139.8	4.1
7 - 7½	.48	58	27.84	11.04	7.1		
7½ - 8	.38	75	28.5	11.40	7.2		
8 - 8½	.37	78	28.86	11.10	7.2		
8½ - 9	.63	77	48.51	12.60	7.3		
9 - 9½	.24	85	20.40	3.84	7.4		
9½ - 10	.34	90	30.60	6.46	7.2	141.0	4.1

APPENDIX TABLE **70**

FOETUS 220 (128 DAYS) ALDOSTERONE INFUSION (460 µg/kg/hr; 3½ hr)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/min)	PH	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	.27	64.5	17.42	.81	6.75	141.5	4.3
½ - 1	.26	50.5	13.13	.52	7.0		
1 - 1½	.23	50	11.50	.46	6.75		
1½ - 2	.27	49	13.23	.54	6.70		
2 - 2½	.22	49.5	10.89	.44	6.6	141.5	4.5
2½ - 3	.23	66	15.18	.69	6.65		
3 - 3½	.22	56	12.32	1.10	6.6		
3½ - 4	.25	42	10.50	1.25	6.4		
4 - 4½	.23	46	10.58	1.38	6.45		
4½ - 5	.19	42.5	8.08	1.52	6.55		
5 - 5½	.20	41.5	8.30	1.80	6.4		
5½ - 6	.19	38	7.22	1.14	6.55	140.8	4.7
6 - 6½	.19	49.5	9.41	2.66	6.4		
6½ - 7	.16	51	8.16	2.08	6.5		
7 - 7½	.20	30	6.00	1.60	6.4		
7½ - 8	.18	44	7.92	1.98	6.4	139.5	4.3

APPENDIX TABLE 71

FOETUS 275 (111 DAYS) PROGESTERONE INJECTION (10 mg/hr; 6 hr)

	URINE					
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)
0 - ½	.16	71	11.36	1.12	8.96	1.44
½ - 1	.12	60	7.20	.36	8.52	1.80
1 - 1½	.16	58	9.28	.48	12.00	2.56
1½ - 2	.12	60.5	7.26	.36	8.40	1.80
2 - 2½	.28	36	10.08	.28	11.76	2.52
2½ - 3	.17	-	-	-	-	-
3 - 3½	.05	57	2.85	.10	3.25	.65
3½ - 4	.05	56.5	2.83	.10	2.95	.60
4 - 4½	.07	60.5	4.24	.14	4.90	.91
4½ - 5	.06	56.5	3.39	.12	3.66	.72
5 - 5½	.02	57.5	1.15	.06	.98	.22
5½ - 6	.08	59	4.72	.16	4.24	.88
6 - 6½	.04	59	2.36	.08	2.24	.48
6½ - 7	.09	56.5	5.09	.27	5.31	1.08
7 - 7½	.11	55.5	6.11	.33	6.82	1.43
7½ - 8	.05	39	1.95	.10	2.40	.60
8 - 8½	.06	51.5	3.09	.18	4.02	1.02
8½ - 9	.14	49.4	6.93	.56	9.38	2.10
9 - 9½	.06	41.5	2.49	.24	2.88	.60
9½ - 10	.09	48	4.32	.45	5.31	1.08

APPENDIX TABLE **72**

FOETUS 275 (116 DAYS) 17 ∝ HP INJECTION (8 mg/hr; 5 hr)

URINE

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)
0 - ½	.18	67	12.06	.54	9.72	2.34
½ - 1	.35	51	17.85	.70	13.65	3.50
1 - 1½	.33	34	11.22	.33	7.59	2.31
1½ - 2	.20	46.5	9.30	.20	6.40	2.00
2 - 2½	.19	66.5	12.64	.38	9.50	2.28
2½ - 3	.15	50	7.50	.30	4.95	1.65
3 - 3½	.38	47.5	18.05	.76	11.78	3.04
3½ - 4	.61	45.5	27.76	1.22	15.25	6.10
4 - 4½	.34	40	13.60	.34	6.12	2.04
4½ - 5	.54	40.5	21.87	.54	9.18	2.16
5 - 5½	.49	45	22.05	.98	10.29	3.43
5½ - 6	.67	75	50.25	2.01	21.44	6.03
6 - 6½	.50	42	21.00	.50	8.00	3.00
6½ - 7	.65	36	23.40	.65	9.10	3.25
7 - 7½	.45	55.5	24.98	.90	11.25	3.60
7½ - 8	.24	52	12.48	.48	6.96	2.16
8 - 8½	.35	37	12.95	.70	8.40	2.80
8½ - 9	.17	62	10.54	.68	8.16	2.21
9 - 9½	.18	71.5	12.87	.90	8.82	2.34
9½ - 10	.24	41.5	9.96	.72	6.96	2.40
10 - 10½	.26	43.5	11.21	.78	7.28	2.60

APPENDIX TABLE **73**

FOETUS 220 (133 DAYS) DEXAMETHASONE INFUSION (0.4 mg/kg/hr; 2½ hr)

	URINE						PLASMA
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	CrCl (ml/min)
0 - ½	.37	11.5	4.26	1.11	13.69	.74	
½ - 1	.35	21.6	7.35	1.40	23.80	1.05	1.98
1 - 1½	.35	12.5	4.38	1.70	11.90	.70	
1½ - 2	.25	15.1	3.75	1.75	14.75	.50	
2 - 2½	.17	21.5	3.66	1.02	18.36	.68	
2½ - 3	.21	10.1	2.10	1.84	10.92	.63	
3 - 3½	.70	17.0	11.9	2.10	19.60	1.40	
3½ - 4	1.07	16.5	17.66	2.14	14.98	2.14	2.50
4 - 4½	.87	22.5	19.58	2.61	16.53	1.74	
4½ - 5	.69	28.5	19.67	3.45	18.63	2.07	
5 - 5½	.73	35.5	25.92	4.30	39.42	2.92	
5½ - 6	.37	24.1	8.88	2.96	16.28	1.11	
6 - 6½	.68	9.5	6.46	4.08	19.04	1.36	1.83
6½ - 7	.52	16.0	8.32	2.60	13.52	1.04	
7 - 7½	.24	16.5	3.96	1.92	9.84	.48	
7½ - 8	.37	14.5	5.37	2.07	20.35	1.48	
8 - 8½	.25	14.0	3.50	2.25	13.25	.75	1.02

APPENDIX TABLE **74**

FOETUS 242 (121 DAYS) DEXAMETHASONE INFUSION (3 mg/kg & 0.8 mg/kg/hr; 2 hr)

	URINE						PLASMA
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	CrCl (ml/min)
0 - ½	.23	18.5	4.26	.23	4.83	.46	
½ - 1	.31	24.5	7.60	1.24	8.68	.93	
1 - 1½	.16	29.5	4.72	1.16	4.96	.48	
1½ - 2	.25	26.5	6.63	1.25	7.75	.50	1.70
2 - 2½	.10	30.5	3.05	1.10	3.30	.30	
2½ - 3	.26	31.5	8.19	1.52	9.62	.78	
3 - 3½	.43	40.5	17.42	1.86	13.33	.86	
3½ - 4	1.07	30	32.10	1.07	13.91	1.07	
4 - 4½	1.16	30	34.80	1.16	13.92	1.16	2.07
4½ - 5	1.46	43.5	63.51	2.92	24.82	1.46	
5 - 5½	1.36	28	38.08	2.72	13.60	1.36	
5½ - 6	1.38	40.5	55.89	4.02	22.08	1.38	
6 - 6½	1.14	38.5	43.89	3.60	19.38	1.14	
6½ - 7	1.15	64	73.60	3.20	33.35	3.45	
7 - 7½	.77	28.5	21.95	3.85	13.09	1.54	2.09
7½ - 8	.60	24.5	14.70	3.0	10.20	1.20	
8 - 8½	.91	36.5	33.22	4.55	16.38	3.64	
8½ - 9	.43	28	12.04	1.72	6.02	1.72	1.55

APPENDIX TABLE **75**

FOETUS 188 (124 DAYS) METYRAPONE INFUSION (24 mg/kg plus 24 mg/kg/hr; 8 hr)

URINE

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (μEq/min)	[K+] (mEq/l)	KER (μEq/min)
0 - ½	1.22	57.5	70.15	11	13.42
½ - 1	1.36	59.5	80.92	10.2	13.87
1 - 1½	1.21	50.5	61.11	9	10.89
1½ - 2	1.31	45.5	59.61	7.8	10.22
2 - 2½	1.43	34.5	49.34	6.2	8.87
2½ - 3	1.45	50	72.50	6.3	9.14
3 - 3½	2.07	54.5	112.82	5	10.35
3½ - 4	2.01	52.5	105.53	4.3	8.64
4 - 4½	1.26	39	49.14	6.6	8.32
4½ - 5	1.66	55	91.30	7.3	12.12
5 - 5½	1.15	40.5	46.58	8.3	9.55
5½ - 6	1.14	43.5	49.59	8.2	9.35
6 - 6½	1.23	44	54.12	7.4	9.10
6½ - 7	1.22	46.5	56.73	7.6	9.27
7 - 7½	1.47	51	74.97	6.8	10.00
7½ - 8	1.04	40	41.6	7.6	7.90
8 - 8½	.96	40.5	38.88	7.3	7.01
8½ - 9	1.03	35.5	36.57	5.5	5.67
9 - 9½	.87	24.5	21.32	5.8	5.05
9½ - 10	.84	41	34.44	8.5	7.14
10 - 10½	.78	23	17.94	5.8	4.52
10½ - 11	.85	37.5	31.88	6.8	5.78
11 - 11½	.74	38	28.12	9.8	7.25
11½ - 12	.58	30.5	17.69	10.2	5.92
12 - 12½	.61	26	15.86	8.7	5.21
12½ - 13	.76	27.5	20.90	8.4	6.38

APPENDIX TABLE **76**

FOETUS 250 (115 DAYS) METYRAPONE INFUSION (34 mg/kg plus 34 mg/kg/hr; 20 hr)

TIME (Hrs)	FLOW (ml/min)	URINE [Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)
0 - ½	.70	23.5	16.45	.7
½ - 1	.58	42.8	24.36	1.16
1 - 1½	.43	42.0	18.06	.86
2 - 2½	.52	40.1	20.8	.52
2½ - 3	.52	30.1	15.6	1.04
3 - 3½	.46	19.0	8.74	.46
3½ - 4½	.61	23.5	14.34	1.21
4½ - 5½	.66	38.5	25.08	.66
5½ - 6½	.37	36.0	13.32	.74
6½ - 7½	.34	32.5	11.05	.68
7½ - 8½	.44	36.0	15.84	.88
8½ - 9½	.29	30.0	8.7	.29
9½ - 10½	.52	26.0	13.52	.52
10½ - 11½	.42	34.1	14.28	.42
11½ - 12½	.66	19.5	12.87	.66
12½ - 13½	.31	32.1	9.92	.62
13½ - 14½	.38	15.5	5.89	.38
14½ - 15½	.38	30.5	11.59	.76
15½ - 16½	.35	25.1	8.75	.35
16½ - 17½	.33	30.0	9.9	.33
17½ - 18½	.30	28.0	8.4	.30
18½ - 19½	.34	28.5	9.69	.68
19½ - 20½	.23	26.0	5.98	.46
20½ - 21½	.24	24.5	5.88	.48
21½ - 22½	.27	19.5	5.27	.27
22½ - 23½	.42	18.5	7.77	.42

APPENDIX TABLE **77**

FOETUS 69-825 (123 DAYS) METYRAPONE + DEXAMETHASONE INFUSION

[metyrapone: 26 mg/kg plus 26 mg/kg/hr; 8 hr]
[dexamethasone: 0.12 mg/kg plus 0.12 mg/kg/hr; 8 hr]

TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	[K+] (mEq/l)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)
0 - ½	.96	30	28.80	4.5	4.32	25.92	7.68
½ - 1	1.00	44	44.00	8.0	8.00	10.00	3.00
1 - 1½	1.34	25.5	34.17	4.7	6.30	22.78	6.70
1½ - 2	1.13	44	49.72	5.0	5.65	20.34	5.65
2 - 2½	1.38	59	81.42	5.2	7.18	26.22	6.90
2½ - 3	1.11	52	57.72	4.7	5.22	16.65	5.55
3 - 3½	1.21	39.5	47.80	4.2	5.08	10.89	2.42
3½ - 4	1.60	34	54.40	2.7	4.32	38.40	9.60
4 - 4½	.80	57.5	46.0	7.5	6.00	16.00	4.00
4½ - 5	.98	39.5	38.71	7.7	7.55	11.76	2.94
5 - 5½	1.90	38	72.2	8.8	16.72	13.30	3.80
5½ - 6	2.07	36.5	75.56	10.1	12.91	20.70	6.21
6 - 6½	2.11	49	103.39	8.6	18.15	29.54	8.44
6½ - 7	2.04	74	150.96	11.2	12.85	16.12	4.08
7 - 7½	2.23	60.5	45.72	2.3	5.13	35.68	11.15
7½ - 8	1.77	71.5	126.56	13.3	13.54	27.17	12.39
8 - 8½	1.16	71	82.36	10.9	12.64	12.76	4.64
8½ - 9	1.59	43	68.37	5.7	9.06	30.21	9.54
9 - 9½	1.10	50.5	55.55	8.6	9.46	19.80	6.60
9½ - 10	1.05	44	46.20	8.6	9.03	17.85	5.25
10 - 10½	1.09	44.5	48.51	10.3	11.23	14.17	5.45
10½ - 11	1.22	46.5	56.73	6.1	7.44	17.08	7.32
11 - 11½	1.06	46	48.76	6.8	7.21	14.84	6.36
11½ - 12	.74	39	28.86	6.7	4.96	11.84	5.18
12 - 12½	.82	43	35.26	9.8	8.36	20.50	6.56
12½ - 13	.99	44	43.36	8.8	8.66	11.88	4.95
13 - 13½	1.04	39	40.56	7.8	8.11	13.52	5.20
13½ - 14	1.10	44	48.40	8.2	9.02	20.90	7.70

APPENDIX TABLE **78**

FOETUS 223 (121 DAYS) ACTH INFUSION (17 µg/kg/hr; 8 hr)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH
0 - 1	.22	30.5	6.71	.66	13.64	3.30	6.80
1 - 2	.31	32.0	9.92	.93	39.06	5.27	6.90
2 - 3	.24	38.5	9.24	.72	32.64	3.60	6.95
3 - 4	.23	31.5	7.25	.23	34.04	3.68	6.90
4 - 5	.41	41.5	16.81	.41	51.25	2.87	6.80
5 - 6	.54	48.1	25.92	1.62	30.78	3.24	7.10
6 - 7	.47	46.5	21.86	1.88	22.09	4.23	7.15
7 - 8	.40	47.5	19.0	1.20	29.20	3.20	7.00
8 - 9	.43	48.0	20.64	1.29	20.21	3.01	7.10
9 - 10	.46	47.0	21.62	.46	20.24	3.68	7.45
10 - 11	.66	59.0	38.94	3.30	33.00	2.64	7.20
11 - 12	.69	31.5	21.39	1.38	11.73	3.45	7.45
12 - 13	.65	38.1	24.7	1.30	15.60	5.20	7.60
13 - 14	.36	37.0	13.32	1.44	10.80	3.96	7.60

APPENDIX TABLE **79**

FOETUS 219 (113 DAYS) ACTH INFUSION (10 µg/kg/hr; 10 hr)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	KER (µEq/min)	CrER (µg/min)	UAER (µg/min)	PH
0 - 1	.72	36	25.92	.72	15.12	2.16	7.3
1 - 2	.91	22.5	20.48	.91	10.92	4.55	7.55
2 - 3	.44	54	23.76	1.76	13.20	2.20	7.4
3 - 4	.44	54	23.76	1.32	14.08	2.20	7.7
4 - 5	.83	53	43.99	2.49	28.22	3.32	7.5
5 - 6	.51	24	12.24	1.53	12.75	1.53	7.6
6 - 7	.48	19.5	9.36	2.40	8.16	3.36	7.6
7 - 8	.36	20.0	7.20	1.80	11.52	2.16	7.5
8 - 9	.26	28.5	7.41	1.82	9.10	4.68	7.35
9 - 10	.05	31.0	1.55	.20	4.50	2.60	6.95
10 - 11	.08	32.0	2.56	.24	18.00	1.44	6.55
11 - 12	.17	25.0	4.25	.17	19.21	2.55	6.8
12 - 13	.15	22.5	3.38	.60	12.45	1.05	6.9

APPENDIX TABLE 80

FOETUS 233 (124 DAYS) ACTH INFUSION (10 ug/kg/hr; 12 hr)

TIME (Hrs)	FLOW (ml/min)	URINE (Na+) (mEq/1)	NaER (uEq/min)	KER (uEq/min)	CrER (ug/min)	UAER (ug/min)	PH
0 - 1	.27	38.5	10.40	.88	18.50	1.11	7.1
1 - 2	.28	37.5	10.50	.84	12.88	.84	7.2
2 - 3	.28	39.0	10.92	.68	16.88	1.08	7.15
3 - 4	.29	35.0	8.40	.80	16.32	1.68	7.2
4 - 5	.32	42.5	13.60	.96	19.29	1.44	7.15
5 - 6	.30	35.0	10.50	.60	12.60	1.20	7.3
6 - 7	.43	41.5	17.85	.86	18.92	2.15	7.2
7 - 8	.51	65.5	33.41	1.53	20.05	3.57	7.3
8 - 9	.61	38.0	23.29	.61	23.42	1.83	7.4
9 - 10	.62	44.0	27.28	1.86	26.74	3.10	7.4
10 - 11	.65	60.1	39.0	4.55	25.30	5.20	7.25
11 - 12	.61	46.1	28.12	3.81	24.61	4.81	7.4
12 - 13	.62	40.0	24.80	1.98	20.18	4.68	7.45
13 - 14	.55	38.1	20.95	1.22	21.01	3.11	7.4

APPENDIX TABLE **81**

FOETUS 69-444 (114 DAYS) ADH INFUSION (96 mu/kg/hr; 2 hr)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	NaER (µEq/min)	[K+] (mEq/1)	KER (µEq/min)	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	1.04	76	79.04	.8	.83		
½ - 1	1.11	84	93.24	1.5	1.67		
1 - 1½	1.21	77	93.17	1.1	1.33	142.5	5.2
1½ - 2	1.5	80	120.00	.9	1.35		
2 - 2½	1.83	78	142.74	.8	1.46		
2½ - 3	1.4	61	85.40	.8	1.12		
3 - 3½	1.76	71	124.96	.8	1.41	157.0	6.1
3½ - 4	1.04	74	76.96	1.0	1.04		
4 - 4½	.94	84	78.96	1.5	1.41	144.4	5.9
4½ - 5	.37	94	34.78	1.1	.41		
5 - 5½	.33	98	32.34	1.1	.36	140.6	5.8
5½ - 6	.37	102	37.74	1.1	.41		
6 - 6½	.55	97	53.35	.9	.50	140.6	5.7
6½ - 7	.43	94	40.42	.9	.39		
7 - 7½	.59	95	56.05	1.0	.59	143.5	5.5
7½ - 8	.48	97	46.56	1.0	.48		
8 - 8½	.51	96	48.96	.9	.46	148.4	5.8

APPENDIX TABLE **82**

FOETUS 230 (124 DAYS) ADH INFUSION (110 mu/kg/hr; 5½ hr)

	URINE					PLASMA	
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/1)	[K+] (mEq/1)	[Cr] (mg/100ml)	pH	[Na+] (mEq/1)	[K+] (mEq/1)
0 - ½	.46	44.5	3	7.5	6.6	149.4	4.9
½ - 1	.45	21.5	2	4.4	6.5		
1 - 1½	.49	21	2	4.2	6.6	145.5	4.7
1½ - 2	.50	31	2	5.4	6.6		
2 - 2½	.28	49	5	8.9	6.6		
2½ - 3	.28	86	14	10.7	6.9	142.3	4.9
3 - 3½	.41	123	14	12.6	7.2		
3½ - 4	.61	58	3	3.6	7.6		
4 - 4½	.80	81	3	4.7	7.5	138.7	5.1
4½ - 5	1.02	28	1	1.6	7.2		
5 - 5½	.57	53.5	2	4.7	7.1		
5½ - 6	.53	75.5	4	8.6	7.1	137.2	4.6
6 - 6½	.28	64.5	5	7.9	7.2		
6½ - 7	.47	35.5	4	5.5	6.4	140.1	4.8
7 - 7½	.41	27	4	4.8	6.8		

APPENDIX TABLE **83**

FOETUS 219 (116 DAYS) ANGIOTENSIN II INFUSION (22 μg/kg/hr; 5 hr)

	URINE						
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (μEq/min)	[K+] (mEq/l)	KER (μEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml)
0 - ½	.99	33.5	33.17	3.0	2.97	2.2	.3
½ - 1	.82	37	30.34	4.0	3.28	3.5	.4
1 - 1½	.82	33.5	27.47	4.0	3.28	3.2	.3
1½ - 2	.81	30.5	24.71	4.1	3.24	3.3	.4
2 - 2½	.72	40.5	29.16	6.1	4.32	4.3	.4
2½ - 3	.44	33.5	14.74	5.0	2.20	4.0	.6
3 - 3½	.69	41	28.29	6.2	4.14	4.4	.5
3½ - 4	.92	40.5	37.26	4.0	3.68	3.6	.4
4 - 4½	.82	22.5	18.45	2.0	1.64	1.8	.2
4½ - 5	.72	22	15.84	2.0	1.44	1.7	.3
5 - 5½	.85	26	22.10	3.3	2.55	2.6	.3
5½ - 6	.92	27.5	25.30	2.0	1.84	2.3	.6
6 - 6½	.91	62.5	56.88	5.0	4.55	4.4	.3
6½ - 7	.72	30	21.6	3.0	2.16	2.7	.5
7 - 7½	.53	35	18.55	4.1	2.12	2.7	.7
7½ - 8	.47	28	13.16	11.0	5.17	6.7	.3
8 - 8½	.62	27.5	17.05	3.3	1.86	3.2	.7
8½ - 9	.75	38	28.5	3.4	2.25	3.7	.5
9 - 9½	.56	27.5	15.4	3.7	1.68	2.2	.4

APPENDIX TABLE **84**

FOETUS 69-495 (140 DAYS) ANGIOTENSIN II INFUSION (14 µg/kg/hr; 4 hr)

	URINE						
TIME (Hrs)	FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	[K+] (mEq/l)	KER (µEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml)
0 - ½	1.32	54	71.28	3.8	5.02	2.7	4.3
½ - 1	1.12	53	59.36	3.5	3.92	2.6	4.3
1 - 1½	1.33	48	63.84	3.1	4.12	14.5	2.8
1½ - 2	1.27	52	66.04	2.0	2.54	10.6	2.2
2 - 2½	1.33	57	75.81	1.4	1.86	6.9	1.6
2½ - 3	.98	96	94.08	2.5	2.45	10.2	2.4
3 - 3½	1.63	68	74.79	2.1	3.42	4.5	1.6
3½ - 4	1.45	54	81.80	1.5	2.18	4.4	1.3
4 - 4½	.60	58	34.80	2.9	1.74	12.6	3.1
4½ - 5	.83	47	39.01	2.6	2.16	11.8	2.9
5 - 5½	1.22	57	69.54	1.8	2.20	4.2	1.6
5½ - 6	1.63	75	122.25	1.7	2.77	3.3	.6
6 - 6½	1.91	86	164.26	1.7	3.25	3.1	.5
6½ - 7	1.67	77	128.59	1.6	2.67	5.9	2.1
7 - 7½	2.38	80	190.40	1.2	2.86	6.1	1.2
7½ - 8	1.66	76	126.16	1.7	2.82	7.2	1.4
8 - 8½	1.50	84	126.00	2.2	3.30	-	-
8½ - 9	1.47	100	147	2.4	3.53	8.1	2.2
9 - 9½	1.53	111	169.83	1.8	2.75	7.9	2.5
9½ - 10	1.90	113	214.70	1.6	3.04	8.1	2.8
10 - 10½	1.90	113	214.70	1.6	3.04	6.5	2.1

APPENDIX TABLE **85**

FOETUS 223 (129 DAYS) ANGIOTENSIN II INFUSION (15 µg/kg/hr; 5½ hr)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (µEq/min)	[K+] (mEq/l)	KER (µEq/min)	[Cr] (mg/100ml)	[UA] (mg/100ml
0 - ½	1.10	82	90.20	16.0	17.6	1.15	.19
½ - 1	1.32	54	71.28	7.0	9.24	.32	.07
1 - 1½	1.63	46.5	75.80	5.0	8.15	.22	.05
1½ - 2	1.03	52.5	54.08	5.0	5.15	.24	.06
2 - 2½	.75	53	39.75	5.0	3.75	.25	.04
2½ - 3	.72	57	41.04	6.0	4.32	.27	.06
3 - 3½	.70	116.5	81.55	10.0	7.00	.64	.11
3½ - 4	.51	68	34.68	6.0	3.06	.42	.07
4 - 4½	.53	58.5	31.01	5.0	2.65	.40	.07
4½ - 5	.38	28	10.64	3.0	1.14	.22	.05
5 - 5½	.32	104	33.28	11.0	3.52	.82	.14
5½ - 6	.45	50	22.5	5.0	2.25	.40	.07
6 - 6½	.29	45.5	13.2	5.0	1.45	.44	.08
6½ - 7	.29	87	25.23	11.0	3.19	1.03	.12

APPENDIX TABLE **85**

FOETUS 223 (129 DAYS) ANGIOTENSIN II INFUSION (15 μg/kg/hr; 5½ hr)

TIME (Hrs)	URINE FLOW (ml/min)	[Na+] (mEq/l)	NaER (μEq/min)	[K+] (mEq/l)	KER (μEq/min)	[Cr] (mg/100ml)	[UA] (mg/100r
0 - ½	1.10	82	90.20	16.0	17.6	1.15	.19
½ - 1	1.32	54	71.28	7.0	9.24	.32	.07
1 - 1½	1.63	46.5	75.80	5.0	8.15	.22	.05
1½ - 2	1.03	52.5	54.08	5.0	5.15	.24	.06
2 - 2½	.75	53	39.75	5.0	3.75	.25	.04
2½ - 3	.72	57	41.04	6.0	4.32	.27	.06
3 - 3½	.70	116.5	81.55	10.0	7.00	.64	.11
3½ - 4	.51	68	34.68	6.0	3.06	.42	.07
4 - 4½	.53	58.5	31.01	5.0	2.65	.40	.07
4½ - 5	.38	28	10.64	3.0	1.14	.22	.05
5 - 5½	.32	104	33.28	11.0	3.52	.82	.14
5½ - 6	.45	50	22.5	5.0	2.25	.40	.07
6 - 6½	.29	45.5	13.2	5.0	1.45	.44	.08
6½ - 7	.29	87	25.23	11.0	3.19	1.03	.12

PROGESTIN ASSAY

1. APPARATUS

ALUMINA COLUMN CHROMATOGRAPHY

Preparation of alumina -

(a) Reflux the aluminium oxide over ethanol for 24 - 48 hours to remove impurities. Dry in air oven at 110 deg. for 15 hours. Cool in a desiccator over phosphorous pentoxide.

(b) For chromatography, add 2.7% water (2.7 ml/100g.). Mix by tumbling in a sealed container for 12 hours. Store in wax-sealed stoppered bottles.

A. Preparation of columns -

(a) Columns of alumina (3 x 45mm) are prepared in 2ml. graduated pipettes which have centrifuge tubes fused to their tops to act as solvent reservoirs.

(b) Place a glass bead and a plug of acid-washed sand in the bottom of each column to retain the alumina. Fill the column with water-saturated 100% cyclohexane (5 ml.), tap out air bubbles and then stopper the bottom.

(c) Put alumina in column, shake down.

(d) Place another plug of acid-washed sand on the surface of the alumina. Drain the column before loading sample.

B. Sephadex columns -

1. The columns are made in the outer protective covers in which sterile 2 ml. disposable plastic syringes are supplied. A hole was made in the tip and a disc of scintered polythene heat-sealed into the lower end.

2. 0.5g. Sephadex G-25 (fine) is added to the column, water added until it has swelled and then the column is washed with borate buffer and another scintered disc added to the top of the column. The columns are suspended in a Perspex rack and are prepared for use by passing 0.1 ml. of 0.1% BSA through each.

11. SOLUTIONS

A. INTERNAL RECOVERY STANDARDS

Radioactive solutions of each steroid to be assayed are made up so that 0.1 ml. contains approximately 2000 cpm.

B. STEROID STANDARDS

1. Standards included from the beginning of each assay.

STOCK SOLUTION - 1 mg/ml of each steroid

IMMEDIATE STOCK - 10 µg/ml

WORKING STANDARD - 250 ng/ml; use 0.1-0.2 ml. (25 - 50 ng.

2. Standard solutions for competitive protein binding assay

STOCK	-	1 mg/ml
INTERMEDIATE STOCK	-	1 µg/ml
WORKING STANDARD	-	Prepare fresh for each assay from intermediate stock - 10 ng/ml

11. PREPARATION OF CONVERSION ENZYMES

20∝ HP has to be converted to progesterone (P) before being assayed in the competitive protein-binding assay.

A. OXIDATION OF 20∝HP to 20∝-OXO-STEROID WITH DEHYDROGENASE FROM FOETAL BLOOD

1. Preparation of enzyme -

(a) Separate the red cells from foetal ovine blood by centrifugation. Wash twice with 2 vol. 0.9% saline

(b) Lyse 22 ml. packed cells with 50 ml. of 0.005M phosphate buffer, pH7.4

(c) Centrifuge 20,000g. for 30 min. decant supernatant (70 ml.)

(d) Add 14g. ammonium sulphate to supernatant, allow to stand at 4 deg. for 2 hours. Centrifuge 10,000 for 10 mins.

(e) Decant supernatant (74 ml) and add 11.1g. ammonium sulphate, allow to stand overnight at 4 - 10 deg. Centrifuge 20,000g. for 30 min.

(f) Discard supernatant, resuspend precipitate in 70ml. buffer. Freeze in 5 ml. aliquots to store.

2. Conversion procedure -

(a) Remove solvent from the fraction from the alumina column containing 20∝ HP by evaporation at 45 deg. under a stream of air.

(b) Add 1 ml. of a mixture containing -

8 mg. NADP+
28 mg. nicotinamide
0.8 ml. enzyme from foetal blood
20 ml. hydrogen carbonate buffer (see solutions)

C. SCINTILLANT

(a) 40g. PPO

1g. POPOP

10L toluene

Use for internal recovery samples and standards (in non-aqueous samples)

(b) Take 1 L of the scintillant and add 400 ml. Triton X-100 detergent. 10 ml. of this will solubilize in 1.2 ml. aqueous solution.

D. BORATE BUFFER

190.5g. di-sodium tetraborate (AR)

10_L water

Brought to pH7.6 with about 80 ml. conc. HCL. Final strength of buffer, 0.05 M.

E. SOLUTIONS FOR ENZYME CONVERSIONS

Bicarbonate buffer

Stock X :

Anhydrous sodium carbonate, 0.1 M + 10.6 g/1. Use 2.11g. in 200ml. water.

Stock Y:

Sodium hydrogen carbonate, 0.1M = 8.4 g/1. Use 1.68g. in 200 ml. water. Mix 1 ml. stock X + 9 ml. Stock Y to give a solution of pH 9.1

Vortex, incubate 15 mins. at 37 deg.

Extract with 4 ml. diethyl ether.

IV. PREPARATION OF BINDING PROTEIN

1. Strip by gel filtration on Sephadex G-25 (coarse) at 45 deg. in borate buffer.

2. Heat Sephadex to 60 deg. in borate buffer before use and decant fines.

3. Void volume = 22 ml. collect 20 ml. (=5 ml. CBG)

4. Add 0.6 tritiated cortisol (25 μCi/ml) to a 50 ml. volumetric flask and dry off. Add the 20 ml. CBG/borate and make up with borate to give about 10% CBG.

5. Dilute to 1.5% with borate buffer prior to use in the assay.

V. ASSAY PROCEDURE

A. EXTRACTION OF STEROIDS FROM SAMPLE

1. Set up 2 (no. of samples + 2) screw-top test tubes. The extra 2 tubes in each set are for a blank and a standard to be assayed with the samples.

2. Aliquot 0.1 ml. of the 5 progestin internal recovery standard (IRS) solutions into every tube. Add 0.1 ml. of each of the IRS solutions to separate scintillation vials as well, add 5 ml. scintillant, cap and label.

3. Standard - Aliquot 0.1 ml. (25 ng) or 0.2 ml. (50 ng) of each of the standard steroid solutions into both standard tubes.

4. Evaporate solvent at 45 deg. under a stream of air.

5. Add samples to each sample tube; blank solutions to blank and standard tubes.

6. Vortex, leave 10 mins. at 45 deg., shake

7. Add diethyl ether (4 ml.) vortex, stand 5 minutes, vortex, allow to settle.

8. Freeze at 80 deg. pour off upper layer into similarly numbered tubes.

9. Evaporate ether at 45 deg.

B. SEPARATION OF STEROIDS BY ALUMINA COLUMN CHROMATOGRAPHY

1. Dissolve residue from extraction in 3 ml. 0.4% ethanol in cyclohexane (water-saturated). Load each sample on to a column.

2. Solutions containing progressively increasing concentrations of ethanol in cyclohexane are then passed through the columns to elute each steroid as shown in the table below. An aliquot is taken from each fraction (1/10 of fraction volume) to be counted for determination of internal recovery (IR). Each fraction is collected into a screw-top test tube.

3. 20∝HP fractions are then converted to progesterone.

   (a) After extracting the converted product with diethyl ether, freeze and pour off the upper layer as before.

Eluting Solution (% ethanol in cyclohexane)	Steroid Eluted	Treatment of Fraction Eluted
Sample loaded in 3 ml. of 0.4%		Discard
PROGESTINS		
2 ml. of 0.6%	Prog.	Take 0.2 ml. IR cap & label (4^{0})
3 ml. of 0.9%	20∝HP	Label & dry down ready for conversion
3 ml. of 1.4%	17∝HP	Take 0.3 ml. IR, cap & label

(b) Evaporate ether at 45 deg.

(c) Dissolve residue in 3 ml. of 0.4% ethanol in cyclohexane and re-chromatograph each sample on alumina. Collect the Prog. fraction from the column (=20∝HP fraction)

(d) Take 0.2 ml. for IR and assay remainder by competitive protein-binding assay.

C. MEASUREMENT OF STEROID CONCENTRATIONS BY COMPETITIVE PROTEIN-BINDING ASSAY (CPB)

Four CPB assays are then set up to measure the concentration of steroid in each sample. The table over the page shows the binding protein standard solutions and the steroids measured in each assay.

Steroids Measured	Binding Protein	Standards
Prog. & 20∝HP	CBG	.5, 1, 2, 4ng Prog.
17∝HP	CBG	.5, 1, 2, 4ng 17 HP

2. Aliquot the standards into test tubes in duplicate, leaving three tubes as blanks. Add 0.5 ml. of cyclohexane to each tube. Aliquot duplicate, dissimilar volumes of each sample into similar tubes.

3. Evaporate cyclohexane at 45 deg.

4. Add 0.4 ml. of respective binding protein solution to each tube.

5. Briefly vortex, leave 20 min. at 45 deg.

6. Leave at 4 deg. overnight.

7. Separation of bound from free hormone on Sephadex columns:

   (a) Add borate buffer to columns and stir the Sephadex. Allow to run through.

   (b) Pipette 0.3 ml. of each assay solution onto columns and let run through.

   (c) Position scintillation vials under each column and elute bound fraction with 1.2 ml. borate buffer.

   (d) Remove vials and wash columns well with buffer.

   (e) Add 10 ml. Triton X-100 mixture to vials and count 5 mins. (10,000 counts).

8. Prepare standard curve by plotting (mean Bo-bkg) / (mean std. count-bkg) against amount of standard as check before calculating results using program CALC.

EVALUATION OF PROCEDURE -

Recoveries % Progesterone (98, 103, 102, 108, 104) mean

17∝HP (74, 76, 85, 79, 79, 79, 78, 82, 70, 49, 66, 75, 78) mean

20∝HP (48, 59, 46, 31, 26, 23, 29, 20, 38, 33, 18) mean

Results corrected from recovery -

Sensitivity - 0.1 ng/ml Prog.
1.0 ng/ml 20∝HP
0.3 ng/ml 17∝HP

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