HAEMATOLOGICAL STUDIES ON THE KANGAROO ISLAND WALLABY, PROTEMNODON EUGENII (DESMAREST)

by

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TABLE OF CONTENTS

SUMMARY

Paper electrophoresis was used to separate the serum proteins of Kangaroo Island wallabies into 4 broad fractions: albumin, α-, β - and γ-globulin. The concentrations of these 4 fractions, the total protein concentration, haematocrit and haemoglobin concentration increased in young wallabies as they matured. By the time the young were 450 days old, the levels of these haematological measures were similar to those found in healthy adult wallabies.

The sex of healthy adult wallabies had no consistent effect on the levels of the various parameters measured. However, some of the parameters measured in the wallabies were affected by particular seasons.

Healthy adult females sampled in laboratory enclosures from September 1968 to July 1969 had comparatively low red blood cell measures. This may have been partly due to the stress of heavy lactation, or it may have been due to a heavy infection of a blood protozoan found in healthy adult males in the laboratory enclosures. The protozoan resembles Eperythrozoon ovis, a parasite of sheep. It is postulated that the wallabies normally carry a latent infection of the blood parasite without being affected by it, but if they are stressed, e.g. by poor nutrition or some other seasonal aspect, the numbers of the parasite increase and adversely affect the wallabies.

A heavy infection of the parasite may have caused the hypochromic normocytic anaemia observed in male wallabies in the laboratory enclosures in March 1969.

An experiment was conducted in which 2 groups of male wallabies were fed a low nitrogen diet for 2 months. One group had a water intake restricted to about half their ad lib. intake, and these wallabies developed a hyperchromic normocytic anaemia by the end of the experiment. At the end of the study these experimental wallabies were not haemoconcentrated compared with the control group which had an ad lib. water intake. Although the control wallabies did not develop an anaemia during the study, the changes in their blood parameters followed the same trend as in the experimental wallabies. Large numbers of the eperythrozoon-like parasite were found in the blood of both groups of wallabies at the end of the experiment. It is possible that the experimental regime imposed on the wallabies was only indirectly responsible for their anaemia and that it was directly caused by a heavy infection of the parasite. If this was so, the parasite could not have caused the different type of anaemia observed in male wallabies in the laboratory enclosures in March 1969.

Wallabies in the field and in the laboratory enclosures generally showed the same fluctuations in their blood parameters. The red blood cell measures of wallabies in the field were low in March 1969 and this may have been caused by a heavy infection of the eperythrozoon-like parasite.

Wallabies in the field in May 1968 had high concentrations of γ-globulin and low concentrations of albumin in their serum. This indicated that they had a chronic infection of an organism other than the eperythrozoon-like parasite. The patterns of their nitrogen and water excretion indicated that the wallabies were drinking little in late summer and were short of nitrogen in early winter, and this, coupled with a latent infection of the eperythrozoon-like parasite, may have stressed the wallabies and made them more susceptible to other diseases during the winter.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

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I. INTRODUCTION

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This investigation forms part of a broader study on the ecology of the Kangaroo Island wallaby (Protemnodon eugenii Desmarest) living in Flinders Chase, Kangaroo Island.

The wallaby is a relatively small one, an adult male weighing 7 - 9 kg and an adult female 5 - 7 kg. The female is extremely fecund. She commences to breed in her first year and thereafter may produce a young once a year during the breeding season (January - August) (Andrewartha and Barker, 1969) until she is at least 8 years old. The wallaby has few predators on Kangaroo Island and probably because of this, and the females' high fecundity, it is now extremely numerous on the island. Because of its abundance, the wallaby can be readily studied, especially in Flinders Chase, a flora and fauna reserve occupying the western end of the island.

The physiography, climate and general vegetation of Flinders Chase have been described by Bauer (1960) in a thesis on the regional geography of Kangaroo Island. Although the annual rainfall in Flinders Chase is about 22" - 30", he reports that all the streams in the Chase, except Breakneck River, dry up in summer, and even those running in spring may be too saline for human use. Several artificial watering points are always available near the Ranger's establishment, but the sectors of the wallaby population in the Chase appear to be

isolated from each other, and so in summer, some wallabies apparently do not drink.

Despite the high annual rainfall, the vegetation in the area may best be described as dry schlerophyll woodland (Andrewartha and Barker, 1969). Green food is abundant in the cleared areas in early winter but it soon disappears, presumably due to the heavy grazing pressures exerted on it, and to the onset of heavy winter rains. In summer there is little green food available and the wallabies are known to forage for the available seed crop of Acacia retinodes (Andrewartha and Barker, 1969).

These and other observations in the field suggest that the wallabies may, at certain seasons of the year, experience a serious shortage of food and water; the quality of food is poor and water is scarce. This conclusion is reinforced by certain adaptations in the wallaby. Laboratory experiments have shown that wallabies can survive on a diet low in protein (Lintern and Barker, 1969), and that wallabies restricted to a small intake of water conserve water by reducing its excretion in urine and faeces (Barker, Lintern and Murphy, 1970).

The present study was designed to test the hypothesis that in nature, wallabies experience a shortage of protein and water during summer; haematological measurements were used to indicate the condition of the animals.

The relation of haematological measures to an animal's state of nutrition

Although there are few reports in the literature on the effects of an inadequate protein intake on marsupials (Shield, 1958; Ealey and Main, 1967; Main, 1968), this aspect of nutrition has been well-documented for eutherians (Zeldis, Alling, McCoord and Kulka, 1945; Garrow, 1959; Holt, Halac and Kajdi, 1962; Wannemacher, Russell and Allison, 1963; Meacham, Warnick, Cunha, Hentges and Shirley, 1964; Rothschild, Oratz and Schreiber, 1967; Chandler, McCarthy and Kesler, 1968; Pond, van Vleck, Walker, Eisenhard and O'Connor, 1969; Shoemaker and Elwyn, 1969). The general conclusion from these investigations is that when a mammal is fed a diet inadequate in protein its body proteins have to be catabolized to provide the amino acids necessary for the synthesis of the more essential proteins such as enzymes and hormones.

It is thought that the body proteins of a mammal are lost in a definite order of priority, the more essential ones being spared to the last (Garrow, 1959; Holt *et al.*, 1962; Schultze and Heremans, 1966; Rothschild *et al.*, 1967). The tissue proteins, such as muscle proteins, are generally broken down first while blood proteins, such as haemoglobin and the serum proteins, are not utilized until much later.

This order of priority is not surprising since haemoglobin is the respiratory pigment in the blood of mammals, while serum proteins have important functions in the transport of carbohydrates, lipids, ions and antibodies in the blood. The serum proteins are also involved in the maintenance of the pH and osmotic pressure of the blood (Harper, 1959; Putnam, 1960; MacFarlane and Robb-Smith, 1961; Hoffman, 1964; Roman, 1966).

In severe starvation, there is a drastic reduction in the concentration of circulating haemoglobin and in the number of red blood cells, giving rise to the anaemia typical of many starving animals (Bethard, Wissler, Thompson, Schroeder and Robson, 1958; Shield, 1958; Meacham et al., 1964; Ealey and Main, 1967; Casperson, 1968; Nasser and Platt, 1968).

The decreased concentration of total serum protein found in starving animals is due to the overall catabolic rate of the serum proteins being in excess of their overall rate of synthesis. However, the rates of turnover of the various protein fractions in the serum are not the same (Schultze and Heremans, 1966; Morgan, 1968, 1969; Ryoo and Tarver, 1968) and the turnover of the fractions is not altered in the same way by protein depletion (Wannemacher et al., 1963; Rothschild et al., 1967). Hence, although the absolute concentration of the various fractions may decrease during protein depletion the relative concentrations of some fractions may increase.

The serum proteins of most eutherians can be separated by the technique of paper electrophoresis into 5 fractions: albumin and 4 globulins, the α_1-, α_2-, β-, and γ- globulins (Ribeiro, Mitidieri and Affonso, 1961). In the protein-depleted animal, it has been found that the relative concentration of albumin is generally decreased, but this is usually compensated by an increase in the relative concentration of the α-globulins (Chow, Allison, Cole and Seeley, 1945; Zeldis et al., 1945; Cartwright, Smith, Brown and Wintrobe, 1948; Scrimshaw and Behar, 1961; Masters and Horgan, 1962; Pond, Barnes, Bradfield, Kwong and Krook, 1965). The relative concentration of the β-globulins generally remains unchanged during protein depletion.

The α-and β-globulins are the proteins chiefly involved in the transport of lipids in the blood (Putnam, 1960; MacFarlane and Robb-Smith, 1961; Ribeiro et al., 1961). During protein depletion, the fat stored in the body is usually mobilized and is carried in combination with the serum globulins to the liver and peripheral tissues where it is oxidized to provide energy for essential body processes (Robinson, 1964). Some free fatty acids are transported by albumin, but the bulk of the serum lipids are combined with the α-and β-globulins as the α-and β- lipoproteins (Ribeiro et al., 1961; Searcy and Bergquist, 1962). This is probably why the concentrations of these serum globulins are usually maintained during protein depletion while the concentration of serum albumin declines markedly.

The concentration of γ-globulin may either decrease or increase in the protein-depleted animal (Chow et al., 1945; Cartwright et al., 1948; Scrimshaw and Behar, 1961; Pond et al., 1965).

The γ-globulin fraction contains mainly antibodies, some of which may also be found in the β-globulin fraction of some animals, especially ungulates (Humphrey and White, 1964). In these animals the β-globulin fraction, like the γ-globulins may either increase or decrease during protein depletion (Cartwright et al., 1948; Scrimshaw and Behar, 1961; Masters and Horgan, 1962). The antibodies are produced extra-hepatically by the body's reticulo-endothelial system in response to an exogenous (antigenic) stimulus (Miller, Bly and Bale, 1954; Ortega and Mellors, 1957; Humphrey and White, 1964; Schultze and Heremans, 1966). In these ways they differ from all other serum proteins, which are synthesized in the liver in response to an endogenous stimulus (Miller and Bale, 1954; Gordon and Humphrey, 1961; Campbell, 1963).

In the protein-depleted animal, in the absence of infection, the anti-bodies are usually catabolized to provide amino acids for the synthesis of more essential proteins. But a protein-depleted animal is more susceptible to infection than a well-fed animal (Scrimshaw, Taylor and Gordon, 1959), and a marked increase in the concentration of γ-globulin in the serum

of such an animal is invariably associated with the establishment of an infection. An increase in the β-globulin fraction in a protein-depleted animal may either be associated with the establishment of an infection, or with an increase in the β- lipoprotein components, or else with both these factors.

An animal's state of hydration can also be indicated by the relative and absolute concentrations of the serum protein fractions, and by the erythrocyte concentration and red cell volume of the blood (Wehmeyer, 1954; Wolf, 1958; MacFarlane, Morris, Howard, McDonald and Budtz-Olsen, 1961; Rosenblum and Asofsky, 1967; Rosenmann and Morrison, 1967).

During dehydration the plasma volume decreases in many eutherian mammals (MacFarlane et al., 1961; Schmidt-Nielsen, 1964). If these animals are simply dehydrated the relative percentages of the protein fractions in their serum do not change, while the absolute concentrations of all the protein fractions increase by the same proportion. During simple dehydration the erythrocyte concentration and red cell volume of the blood also increase.

But it has been found that when an animal is experimentally deprived of water, it often voluntarily restricts its food intake (Wolf, 1958; MacFarlane et al., 1961; Rosenmann and Morrison, 1967). Similarly, an animal fed an inadequate diet often voluntarily restricts its water intake (Wolf, 1958).

The haematological picture in these animals reflects both dehydration and protein depletion (Wolf, 1958). In this situation the concentrations of the protein fractions generally increase, but because of protein depletion, they do not increase by the same proportion. The red cell volume and the erythrocyte concentration may also be elevated in this situation, but the average concentration of haemoglobin in the erythrocytes will usually be decreased.

The Present Study

To investigate the general state of health of wallabies in the field, blood samples were taken from wallabies captured in the field at various times of the year. The haematological measurements made on these samples included an estimation of the concentration of total serum protein and the concentration of the various serum protein fractions, haemoglobin concentration, haematocrit, and the number of red blood cells.

These parameters can also be affected by other factors, such as geographic variation, season of the year, and the sex and age of an animal, and some of them may also be affected by the genotype of the animal (Dessauer and Fox, 1956; Shield, 1958; Alwynelle, 1968; Casperson, 1968; Keating, Jones, Elveback and Randall, 1969).

The methods used in the present study were not precise enough to allow the detection of different genotypes for the

serum protein groups which may exist among the wallabies in Flinders Chase. Controlled experiments in the laboratory have, however, allowed the effects on the blood parameters due to the season of the year and the sex and age of the animal to be taken into account.

To test the effect of age on most of these parameters, changes in their concentration have been measured during the growth and development of young Kangaroo Island wallabies, both in the field and in the laboratory. The concentration of most blood parameters has been found to increase during the growth of young animals (Moore, Shen and Alexander, 1945; Dobson, 1966; Schultze and Heremans, 1966; Jordon and Morgan, 1968; Brooks and Davis, 1969; McEwan and Whitehead, 1969) and so the parameters were measured until they had reached the levels found in healthy adult wallabies.

The effect of the sex of a wallaby on the blood parameters was measured by taking separate blood samples from a number of male and female wallabies kept in apparently optimum conditions in a domestic colony of wallabies. To test the effects of season on the blood parameters, blood samples were taken from these males and females at regular sampling periods throughout the year.

From these procedures, allowances could be made for variations in the concentrations of the blood parameters due to

the age and sex of an animal and to the season of the year. When the allowances had been made, since genetic variation was not detectable in this study, variations observed in the blood parameters of wallabies in the field could be reasonably assumed to be due to the animal's state of health and nutrition.

To investigate the effect that a restricted nitrogen and water intake had on the blood parameters of the wallabies, an experiment was carried out in which wallabies were fed a low nitrogen diet and were given either a restricted water intake or water ad lib. The results from this experiment were then related to findings on wallabies in the field to see if the wallabies did ever appear to be short of nitrogen, or of nitrogen and water, in the field.

II. METHODS

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1. Collection of blood samples

Blood samples were collected by heart puncture from all wallabies in the field. In the laboratory, blood samples were drawn from the lateral tail vein of adult wallabies, and were obtained by heart puncture from young wallabies.

All juvenile (8 - 12 months old), yearling (1 - 2 years old) and adult wallabies were manually restrained in jute sacks while blood samples were being taken. Pouch young were restrained manually during blood sampling.

A heparinised 2 ml sample of blood was taken from all adult wallabies, together with a non-heparinised sample of 5 ml. The size of the sample taken from young wallabies varied according to the age of the young, and ranged from 0.25 ml of heparinised blood and 1 ml of non-heparinised blood, to 1 ml of heparinised blood and 4 ml of non-heparinised blood.

The heparinised sample in each case was used for the estimation of the haemoglobin content and haematocrit of the blood. In addition, red blood cell counts and plasma urea estimations were made on the heparinised sample taken from yearling and adult wallabies.

The non-heparinised samples were allowed to stand overnight at room temperature in covered centrifuge tubes to let the blood clot and to allow the clot to retract from the sides of the tube.

These samples were then centrifuged and the serum was removed from the sample and stored at either -5°C or -20°C until analysis. The samples were analysed within 4 months of their being obtained and it was found that storage at either temperature for this time did not significantly alter the serum protein contents or profiles of the samples.

2. Haematological methods

(a) Haematocrit

For all wallabies in the laboratory, haematocrit readings were made after heparinised blood had been spun in Yankee micro-haematocrit tubes in a Hawksley micro-haematocrit centrifuge at 12,000 r.p.m. for 6 minutes. For pouch young and juvenile wallabies in the field, micro-haematocrit tubes were used, but since the micro-haematocrit centrifuge was not available in the field, the tubes were centrifuged at 3,000 r.p.m. for 30 minutes. Haematocrit readings for adult wallabies in the field were made after centrifuging heparinised blood in standard Wintrobe tubes at 3,000 r.p.m. for 30 minutes. The latter readings were made by Dr. S. Barker.

(b) Haemoglobin

All haemoglobin estimations were carried out by the oxy-haemoglobin method (Dacie, 1956). The solutions were read at 540 mμ in a Bausch and Lomb Spectronic 20. This machine had

been calibrated using dilutions of a blood sample of known haemoglobin content obtained from the Haematology Department of the Institute of Medical and Veterinary Science (I.M.V.S.), Adelaide.

Haemoglobin estimations for adult wallabies in the field were made by Dr. S. Barker.

(c) Red Blood Cells

In the field, red cell counts were made using a 1 : 251 dilution of blood in the formalin-trisodium citrate diluting fluid of Dacie (1956).

Dr. S. Barker carried out these red cell counts using the photographic recording method of Barker (1960).

In the laboratory, all red cell counts were made by the Haematology Department of the I.M.V.S., Adelaide, using a Coulter Model A cell counter at a threshold setting of 10 and a discriminator setting of 6.

(d) Mean Cell Volume (M.C.V.)

The mean cell volume is calculated by dividing the volume of red cells in 1 cu.mm. of blood by the number of red cells in 1 cu.mm. of blood.

$$\text{i.e. } M.C.V. = \frac{Ht}{100} \times \frac{10^9}{Rbc} \text{ cubic micra } (c\mu)$$

(e) Mean Cell Haemoglobin Concentration (M.C.H.C.)

This is calculated by dividing the haemoglobin concentration per 100 ml blood by the red cell volume.

i.e. $M.C.H.C. = \frac{Hb}{Ht} \times 100\%$

(f) Total Protein Concentration

For all adult wallabies, the concentration of total serum protein was measured gravimetrically using the copper sulphate method described by Phillips, Van Slyke, Hamilton, Dole, Emerson and Archibald, 1950.

A stock copper sulphate solution of specific gravity 1.1000 was made up and this was used to prepare a graded series of copper sulphate solutions covering a range of specific gravities from 1.0175 to 1.0320. The specific gravity of these solutions was tested with a specific gravity bottle.

A detailed description of the principle of the copper sulphate method is given by Van Slyke, Hiller, Phillips, Hamilton, Dole, Archibald and Eder, 1950.

The figure obtained for the specific gravity of a serum was corrected for the non-protein nitrogen present in the serum as urea (Van Slyke et al., 1950). The error caused in the estimation of total protein concentration by the presence of excess non-protein nitrogen has been taken as 360 (0.00621 x mg urea in excess of 30 mg/100 ml serum) = '2.24 x excess urea'. (Van Slyke et al., 1950; Varley, 1962).

Using the corrected specific gravity, the total protein content of the serum was read from a previously prepared table. As the table was prepared from an equation calculated for human serum (Varley, 1962) the concentrations obtained for the wallaby sera are relative and not absolute values.

The copper sulphate method was initially chosen because it was simple and quick and required less serum than alternative methods available for estimating protein concentration. It was thought desirable, however, to check that the protein concentration of sera estimated by the copper sulphate method agreed with the protein concentration estimated by an alternative method, such as the biuret method of Reinhold (1953); cited by Varley (1962). Accordingly, the protein concentration of sera from some 80 wallabies was estimated by both methods. The correlation co-efficient between the 2 estimates was $r = 0.926$, which was significantly different from 0 at the 0.1% level of significance. It was therefore considered that the copper sulphate method gave a reliable indication of the protein concentration of adult wallabies' sera.

Since the copper sulphate method is not applicable to solutions containing less than 2 - 3 g protein/100 ml, the concentration of total protein in sera of pouch young and juvenile wallabies was estimated by the biuret method (Reinhold, 1953; cited by Varley, 1962).

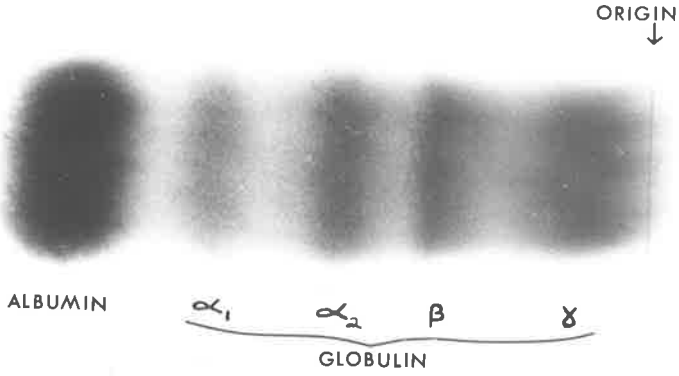
The protein standard used in biuret estimations was a solution of crystalline bovine serum albumin containing 10.2 mg protein nitrogen per ml, obtained from Armour Pharmaceutical Company, Chicago. The nitrogen content of the standard was converted to protein content using the conversion factor of 6.25. Since this conversion factor applies to human serum, the protein concentrations estimated by the biuret method are also relative and not absolute values.

The solutions were read at a wavelength of 555 mμ in a Beckman/Spinco 151 Spectrocolorimeter.

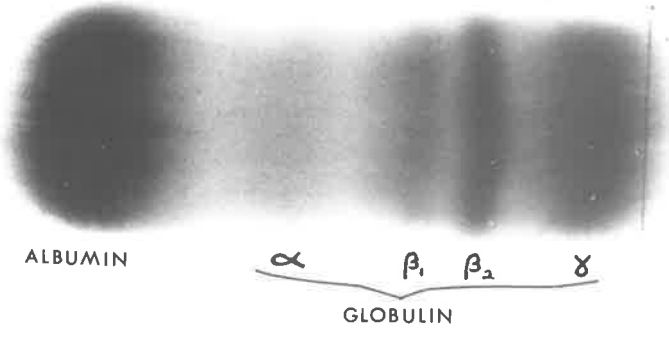
(g) Relative Percentages of the Protein Fractions in the Sera

Paper electrophoresis (Wolstenholme and Millar, 1956; Smith, 1960) was used to separate the serum proteins into fractions. Five discernable fractions were usually obtained. By comparison with the fractions obtained when human serum was separated under the same conditions, the 5 protein fractions in wallaby serum were designated albumin, α-globulin, β_1-globulin, β_2-globulin and γ-globulin (Figure 1). However, it is not implied that the fractions with similar mobility in wallaby and human sera necessarily represent the same proteins.

In some sera examined towards the end of the study, only 1 broad β-globulin band was evident instead of the 2 β-globulin bands usually obtained. The cause of this altered pattern was



A. NORMAL HUMAN SERUM



B. SERUM FROM A KANGAROO ISLAND WALLABY

not found; it does not appear to be a fault of the technique used, since normal patterns were obtained for other sera run under the same conditions as the sera which had the altered pattern. Because of the anomolous pattern, the separate β_1- and β_2-globulin fractions normally obtained in wallaby sera have been combined for the presentation of results into a single β-globulin fraction.

Electrophoresis was performed on 34 cm long x 4 cm wide strips of Whatman No. 1 chromatography paper; 0.015 ml of serum was applied to each paper along a straight line drawn at 11.5 cm from 1 end of the paper. Duplicate papers were run for each serum sample. A veronal buffer (Appendix 1) was used. The electrophoresis was carried out at a constant voltage of 150 volts for 16 hours at a temperature of 20°C. After electrophoresis, the papers were air dried for 25 minutes. They were then stained with Amido Black dye (Appendix 1) for 10 minutes. Next the papers were washed 4 times in first wash solution (Appendix 1) and then twice in second wash solution (Appendix 1) and lastly they were rinsed in pure methanol. Each first wash was left on for 5 minutes while both lots of second wash, and the pure methanol, were each left on for 10 minutes. The papers were again air dried. They were later scanned in a Carl Zeiss automatic scanner at the I.M.V.S., Adelaide. Before being scanned, the papers were soaked in oil (Appendix 1) in a vacuum desiccator to remove the air from the paper.

The relative percentages of the serum protein fractions were readily established from the integrated scans obtained.

(h) Absolute Concentrations of the Protein Fractions in the Sera

These are the product of the total protein content of a serum and the relative percentage of each protein fraction in the serum.

(i) Measurement of Plasma Urea

Urea was estimated by the microdiffusion technique of Conway (1962) using Dunning urease tablets and the buffer of Wu and Wu (1951). S. Lintern estimated plasma urea concentrations for wallabies used in Section III, 3. Plasma urea concentrations for adult wallabies in the field were estimated by Dr. S. Barker.

(j) Measurement of Plasma Volume

In Section III, 3, plasma volume was estimated by the dye dilution method using Evans Blue (T-1824). The wallabies were anaesthetised with veterinary nembutal injected into a marginal ear vein. The femoral vein was then cannulated percutaneously with a G22 bore needle filled with heparinised saline and attached to thin bore polythene cannulation tubing. Each animal was injected with 1.5 ml of the standard dye solution (12.5 mg/ml) through another marginal ear vein.

A dye-disappearance curve was then constructed for each animal by collecting blood samples from the patent cannula at 10, 20 and 30 minutes after injection of the dye.

3. Animal husbandry

A domestic colony of Kangaroo Island wallabies has been kept in this Department throughout the present study. These wallabies were used in Section III, 1, 2 and in the study reported in Appendix 2. Throughout the study period the wallabies were housed in groups of 8 or 9 in 20 feet x 20 feet cement-floored yards which were cleaned twice weekly. Shelter and water were provided in the yards and the animals were fed a diet of kangaroo pellets (15% protein) supplemented with vegetables, green grass and lucerne hay. The animals were given 3- weekly courses of vitamin E tablets at irregular intervals throughout the study (Kakulas, 1961, 1963).

A different method of animal husbandry was used in Section III, 3 and the method adopted is described with the other experimental procedures used in that study.

4. Procedures

(a) Catching Wallabies

(i) In the field

The field work in this study was carried out in Flinders Chase, Kangaroo Island, in a cleared area of land surrounding the Ranger's establishment.

The Zoology Department has a field station in this area and members of the Department have erected several fence traps around the south-eastern corner of the cleared area. The construction and use of the fence traps have been described in detail by Andrewartha and Barker (1969). These authors also describe the procedure for catching the wallabies and the routine for examining them once they are caught.

(ii) In laboratory enclosures

Juvenile, yearling and adult wallabies in laboratory enclosures were sometimes captured with hand nets similar to those used in the field. More often, however, they were cornered and caught by hand. Both methods of capture were quick and efficient. On being caught, the animals were placed individually into jute sacks in which they remained for the whole blood sampling procedure.

(b) Sampling Procedures

This study consisted of 4 investigations:

(1) The effect of the age of a wallaby on some of its blood parameters,

(2) The effect of the season of the year and the sex of a wallaby on some blood parameters in healthy adult wallabies,

(3) The effect of a low nitrogen diet and a restricted water intake on various blood parameters in adult wallabies,

(4) The levels of some blood parameters in adult wallabies captured in the field at various times of the year.

Sampling procedures varied in the different investigations, and so the procedures adopted are described with the results of each investigation.

III. RESULTS

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LABORATORY AND FIELD WORK: PROCEDURES AND RESULTS

1. A study of the effect of the age of a wallaby on some of its blood parameters

(a) Procedures

In order to age young accurately in the field, growth curves prepared for laboratory-reared young of known age were used. The method for construction of these growth curves is outlined in Appendix 2. The validity of using these curves to age young wallabies in the field is also assessed in Appendix 2.

Blood samples were taken from the pouch young of 82 females captured in Flinders Chase, Kangaroo Island during July 1968, and March, May and September 1969. The ages of these young, estimated from head, leg and foot lengths, were from 34 to 225 days. Blood samples were also taken from 18 juveniles (aged 250 - 320 days), captured in the field in December 1968. Of these juveniles, 3 had been bled in July 1968, but all other young taken in the field were bled only once. After being bled, pouch young were replaced in their mother's pouch before she was released; juveniles were released near the place of their capture.

Appendices 2B - 2D show that the growth curves are too inaccurate for estimating the age of young older than 320 days.

Since no alternative method is yet available for accurately aging yearling Kangaroo Island wallabies, the blood samples from yearling wallabies in this study have come from laboratory-reared yearlings of known age. These wallabies and their mothers were part of the domestic colony kept in the Zoology Department.

Blood samples were first taken from the yearlings when they were from 364 - 464 days old, and thereafter they were bled at 2-monthly intervals for 6 months. Both of the females sampled at the start of the study had pouch young. Of the 6 wallabies initially sampled, 3 males and 1 female survived to the end of the sampling period.

(b) Results

The results of this study are presented in Figures 2 - 4. In any 1 age group there was no sex difference in the blood parameters measured, and so the values from male and female wallabies have been combined.

As Figures 2 - 4 show, the levels of the blood parameters change with increasing age of the young. Accompanying these changes are changes in the morphology of the young, and some of the body characters which develop, and the age groups in which they occur are presented in Figure 5.

FIGURE 2.

Mean haematocrits and haemoglobin concentrations of different age groups of Kangaroo Island wallabies.

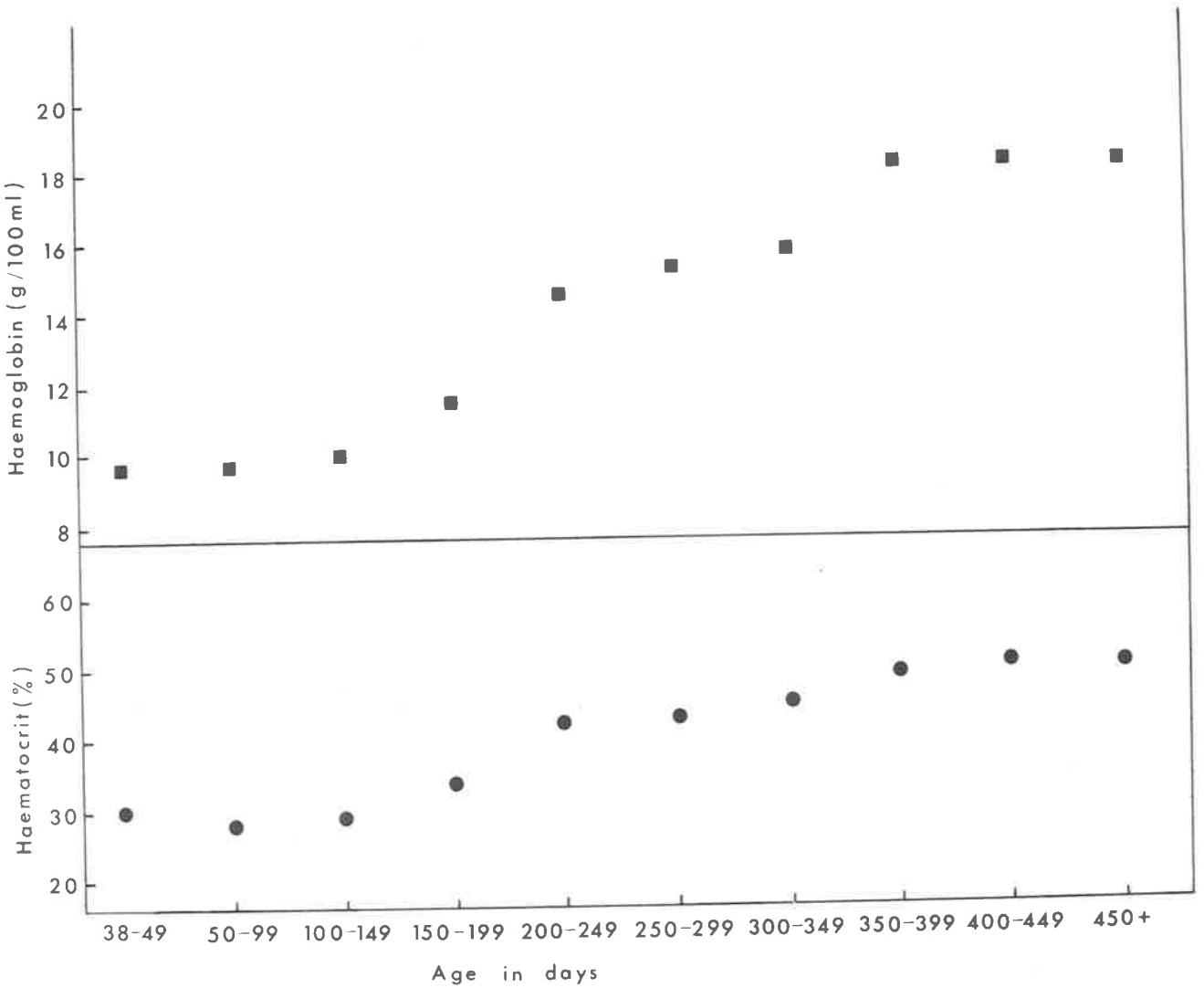

Haemoglobin (g/100ml)
20
18
16
14
12
10
8
Haematocrit (%)
60
50
40
30
20
38-49
50-99
100-149
150-199
200-249
250-299
300-349
350-399
400-449
450+
Age in days

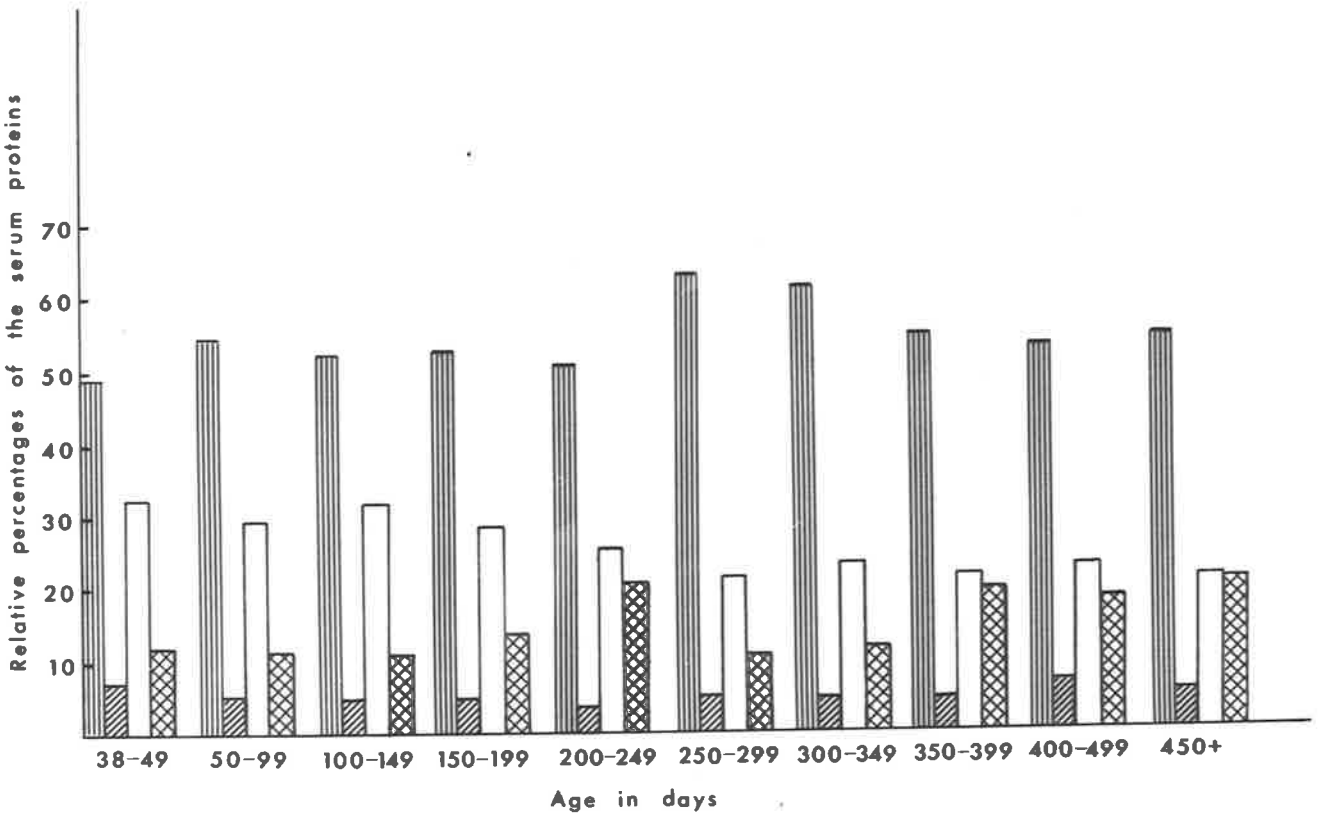
Figure 2 shows that both the haemoglobin concentration and the haemotocrit were maintained almost constant until the young were 100 - 149 days old. Both these parameters increased markedly in young aged from 150 - 399 days, and in older young they were again constant, this time at levels nearly twice those found in young aged less than 150 days.

The relative concentrations of the various serum protein fractions did not alter in the same way during maturation of the young (Figure 3). The relative concentration of albumin was maintained nearly constant except for an increase in the juveniles. There was little change in the relative concentration of α-globulin during the development of the young, while the relative concentration of β-globulin gradually decreased until the young were 250 - 299 days old, after which it was maintained constant. The estimate of the relative concentration of γ-globulin in young aged less than 150 days is an overestimate; electrophoresis of sera from these young performed on cellulose acetate shows a faint γ-globulin region (M. Coles, pers. comm.). Although paper electrophoresis of these sera does not show a band of γ-globulin, it clearly shows protein in the γ-globulin region; this may be due to the streaking of the faster migrating proteins as they move along the paper. A definite γ-globulin band is evident when cellulose acetate electrophoresis is performed on sera of young older than 150 days (M. Coles,

FIGURE 3.

Mean relative percentages of the serum proteins of different age groups of Kangaroo Island wallabies.

Legend: vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.


Relative percentages of the serum proteins
70
60
50
40
30
20
10
38-49
50-99
100-149
150-199
200-249
250-299
300-349
350-399
400-499
450+
Age in days

pers. comm.). Hence the relative concentration of γ-globulin in fact increases in young aged 38 - 199 days. Young aged 200 - 249 days showed a sharp increase in the relative concentration of γ-globulin but this level was not maintained in young aged 250 - 349 days. The relative concentration of γ-globulin rose again in yearlings and was maintained at nearly twice its level in young aged 250 - 349 days.

Figure 4 shows that the absolute concentration of total serum protein increased until the young were 200 - 249 days old. Until they were 349 days old, this parameter was maintained constant, but it increased again in yearling wallabies until they were about 450 days old, when it again became constant.

From Figure 4 it can be seen that increases in all serum protein fractions were responsible for the initial increase in the absolute concentration of total serum protein. The absolute concentration of serum albumin continued to increase until the young were 250 - 299 days old, after which it was maintained constant. There was a gradual increase in the absolute concentration of α-globulin as the young matured, while the absolute concentration of β-globulin increased until the young were 200 - 249 days old, after which its level was maintained almost constant. The concentration of γ-globulin in young aged 38 - 149 days was in fact less than is shown in Figure 4. However its concentration increased slowly in young aged

FIGURE 4.

Mean absolute concentrations of the serum proteins of different age groups of Kangaroo Island wallabies.

Legend: black, total protein; vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.

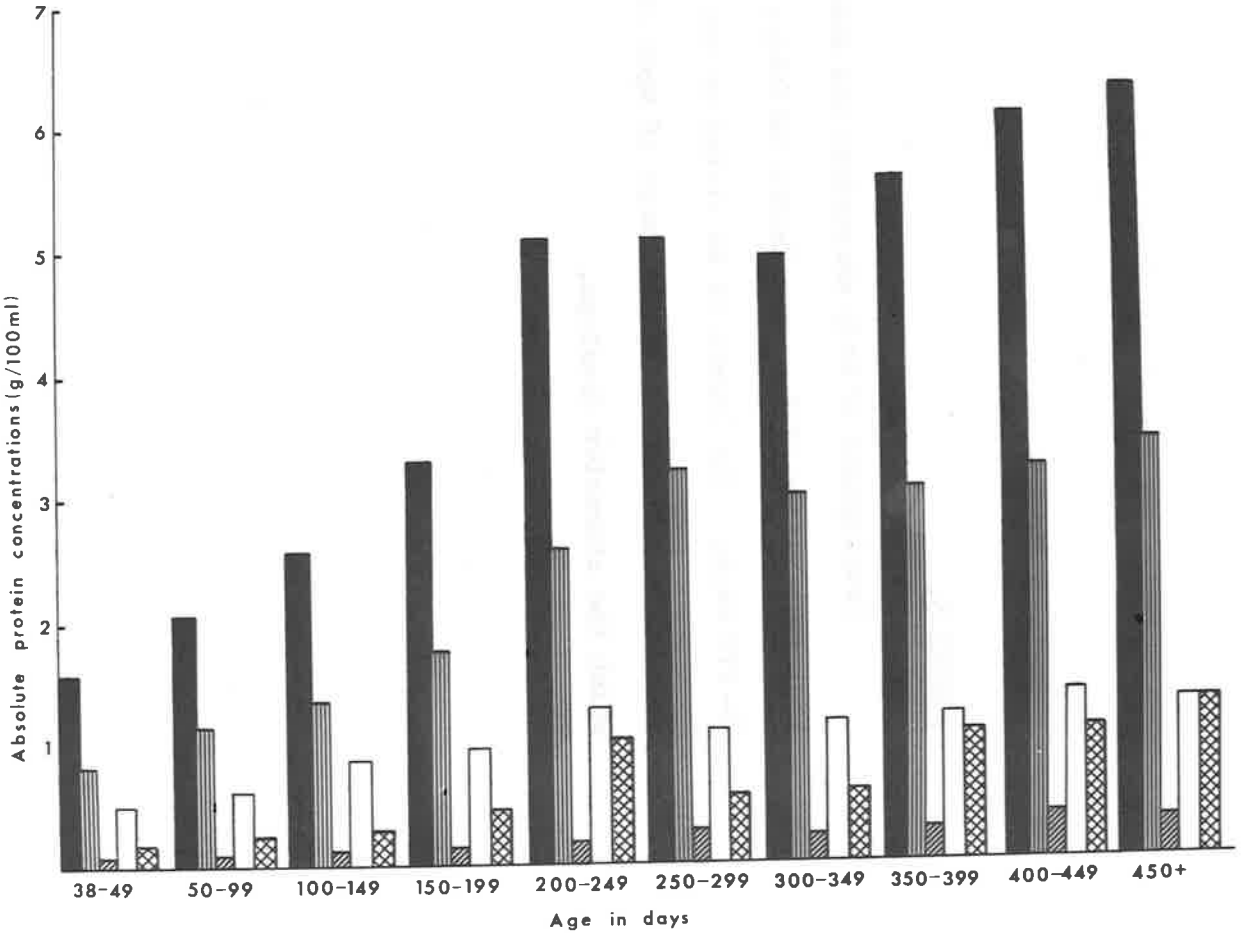
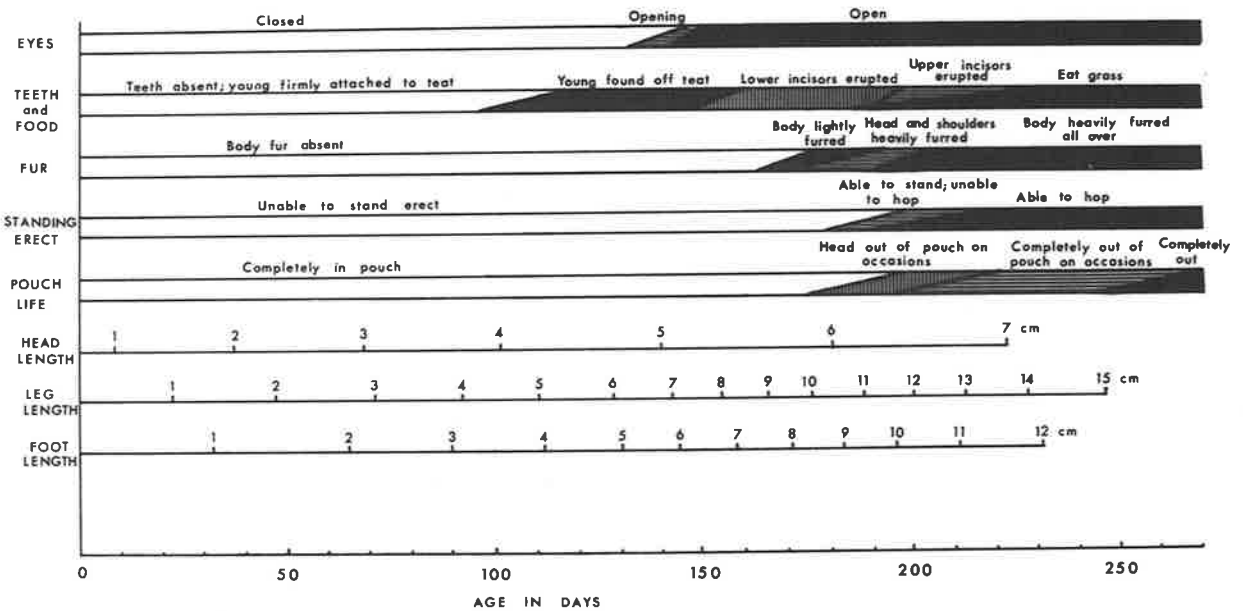

Absolute protein concentrations (g/100ml)
7
6
5
4
3
2
1
38-49
50-99
100-149
150-199
200-249
250-299
300-349
350-399
400-449
450+
Age in days

FIGURE 5.

Development of body characters and changes in body proportions of Kangaroo Island wallabies aged 0 - 270 days. The legend is as stated on the figure. The sloped lines indicate the range of ages over which the character develops.


EYES
Closed
Opening
Open
TEETH and FOOD
Teeth absent; young firmly attached to teat
Young found off teat
Lower incisors erupted
Upper incisors erupted
Eat grass
FUR
Body fur absent
Body lightly furred
Head and shoulders heavily furred
Body heavily furred all over
STANDING ERECT
Unable to stand erect
Able to stand; unable to hop
Able to hop
POUCH LIFE
Completely in pouch
Head out of pouch on occasions
Completely out of pouch on occasions
Completely out
HEAD LENGTH
1 2 3 4 5 6 7 cm
LEG LENGTH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 cm
FOOT LENGTH
1 2 3 4 5 6 7 8 9 10 11 12 cm
0 50 100 150 200 250
AGE IN DAYS

150 - 349 days, apart from a sharp, transitory increase in young aged 200 - 249 days. Its level increased markedly in yearling wallabies and this increased level was maintained at least until the young were 600 days of age.

2. A study of the effects of the season of a year and the sex of a wallaby on some blood parameters

(a) Procedures

For this study, healthy adult wallabies from the domestic colony were used.

Blood samples were drawn from 6 - 7 males and 6 - 7 females at 2-monthly intervals from May 1968 until July 1969. A shortage of animals prevented a larger sample from being used and so where it was possible the same animals were sampled each time. Of the females sampled, only 1 failed to breed successfully during the study period.

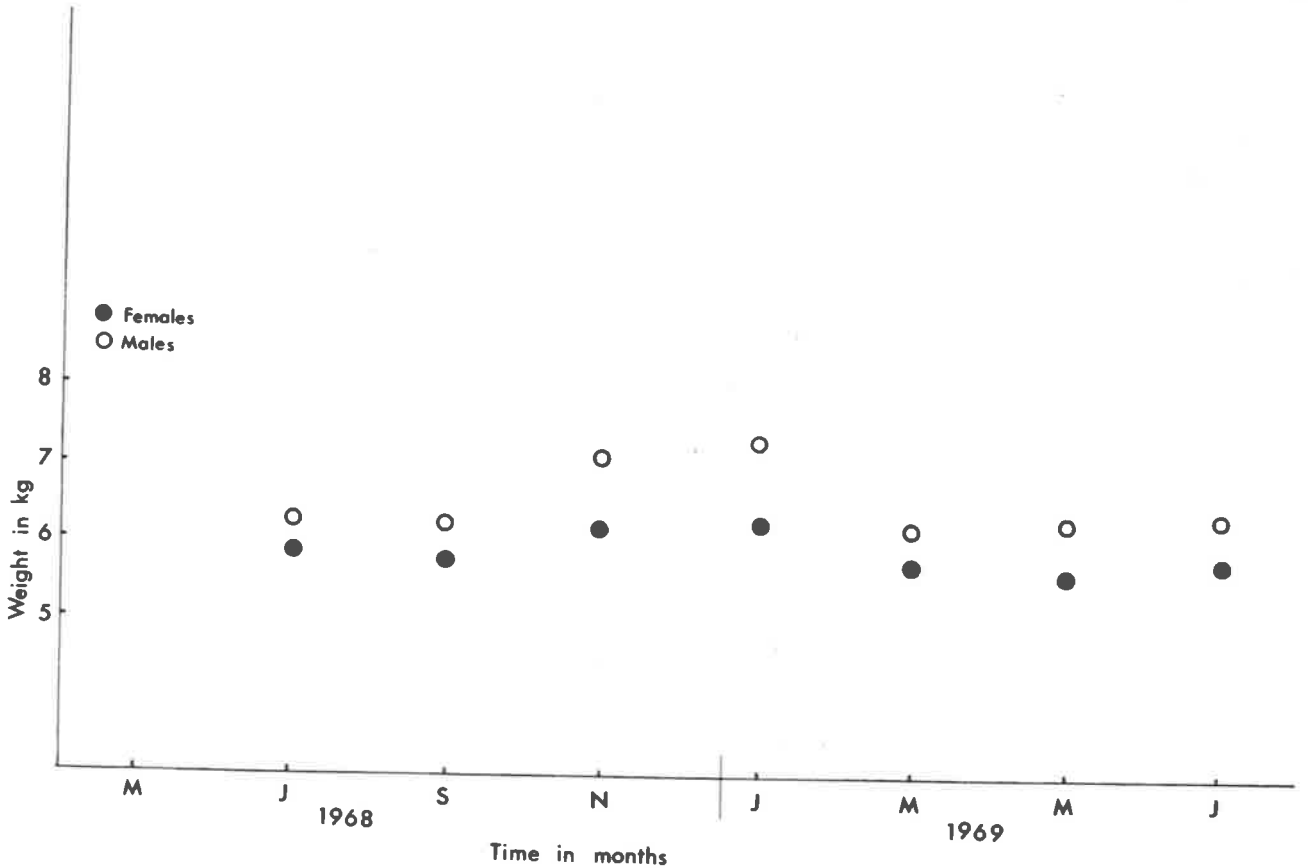
(b) Results

Figures 6 - 10, Table 1 and Appendices 3 - 5 present the results of this study.

As Appendix 3 shows, the body weight, haematocrit, haemoglobin concentration, red blood cell count and relative percentage of β-globulin differed significantly between males and females during the study. The haematocrit, haemoglobin

FIGURE 6.

Seasonal changes in the mean body weights of healthy adult male and female Kangaroo Island wallabies given water *ad lib.* and fed a high protein diet.


● Females
○ Males
8
7
6
5
Weight in kg
M
J
S
N
J
M
M
J
1968
1969
Time in months

concentration and red blood cell count of the males were significantly greater compared with the females in July and November 1968 and in July 1969. The red blood cell count of the males was also significantly higher than that of the females in May 1968 and March 1969. The body weight of the males was greater than that of the females in November 1968 and January and May 1969 and the males had a higher relative percentage of β-globulin than the females in November 1968.

For the males, Appendix 4 shows that the body weight, haematocrit, haemoglobin concentration, M.C.H.C. and the relative percentage of β-globulin differed significantly between the 8 sampling periods. Appendix 5 shows that for the females, the haematocrit, haemoglobin concentration and red blood cell count, and the concentrations of total protein and γ-globulin differed significantly between the 8 samplings.

To detect any pattern in the way these variates differed between the sampling periods, males and females were considered separately and for each variate which differed significantly during the study, the sampling periods were ranked in order of increasing size of the sample mean (Table 1).

FIGURE 7.

Seasonal changes in the mean haematocrits, haemoglobin concentrations, and red blood cell counts of healthy adult male and female Kangaroo Island wallabies given water *ad lib.* and fed a high protein diet.

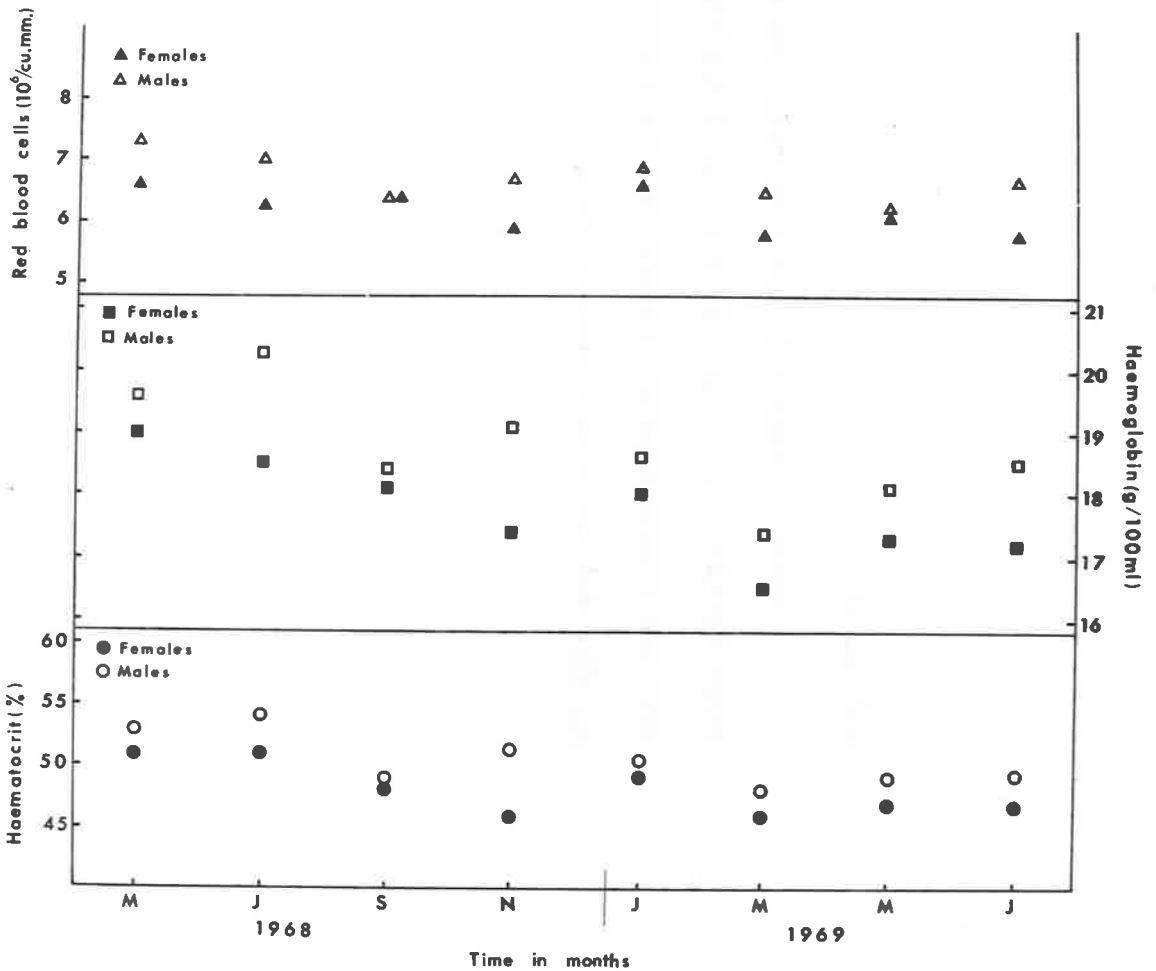

Red blood cells (10^6/cu.mm.)
▲ Females
△ Males
8
7
6
5
■ Females
□ Males
21
20
19
18
17
16
Haemoglobin (g/100ml)
● Females
○ Males
60
55
50
45
Haematocrit (%)
M
J
S
N
J
M
M
J
1968
1969
Time in months

FIGURE 8.

Seasonal changes in the mean cell haemoglobin concentrations and mean cell volumes of healthy adult male and female Kangaroo Island wallabies given water *ad lib.* and fed a high protein diet.

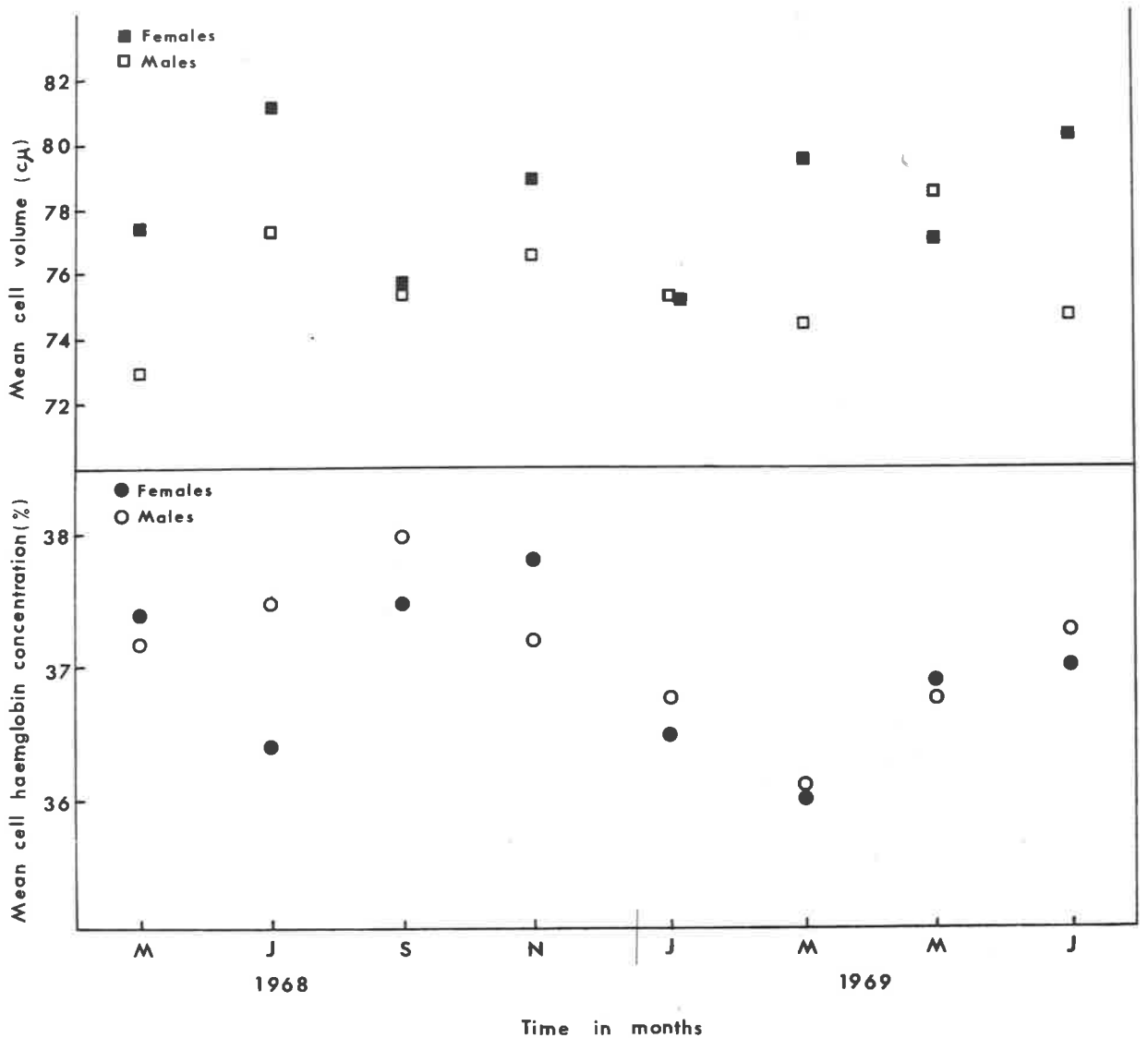

Females
Males
Mean cell volume (cμ)
82
80
78
76
74
72
Females
Males
Mean cell haemglobin concentration(%)
38
37
36
M
J
S
N
J
M
M
J
1968
1969
Time in months

TABLE 1

Sampling periods ranked in order of increasing size of the sample mean for each of the variates which differed significantly in males or females during the study

Variate	Ranked Sampling Periods							
MALES	Lowest sample mean							Highest sample mean
Body weight		6	3	2	7	8	4	5
Haematocrit	6	3	7	8	5	4	1	2
Haemoglobin concentration	6	7	3	8	5	4	1	2
Mean cell haemoglobin concentration	6	7	5	1	4	8	2	3
Relative percentage of β-globulin	8	5	2	6	7	3	4	1
FEMALES								
Haematocrit	6	4	8	7	3	5	1	2
Haemoglobin concentration	6	8	7	4	5	3	2	1
Red blood cell count	6	8	4	7	2	3	1	5
Total protein concentration	8	7	4	1	3	5	2	6
Absolute concentration of γ-globulin	7	8	1	4	3	2	6	5

Table 1 and Figure 7 show that the haematocrit, haemoglobin concentration and red blood cell count of the females all fluctuated in a similar way throughout the study. All 3 parameters were at their lowest levels in March 1969, in contrast to May 1968 when they were at almost their highest levels.

In the females, fluctuations in the haemotocrit, haemoglobin concentration and red blood cell count were generally paralleled by fluctuations in the concentrations of total protein and γ-globulin, except in March 1969 when the concentrations of total protein and γ-globulin were high while the haematocrit, haemoglobin concentration and red blood cell count were low, and in May 1968 when these 3 parameters had high levels while the concentrations of total protein and γ-globulin were low (Table 1 and Figures 7 and 10).

The haematocrit and haemoglobin concentration of the males generally fluctuated in a similar way to the females. As in the females, these 2 parameters were at their lowest levels in March 1969 and were at their highest levels in May and July 1968 (Table 1 and Figure 7). However, compared with the other sampling periods, unlike the females the males had a low haematocrit and haemoglobin concentration in September 1968, and a high haematocrit and haemoglobin concentration in November 1968 (Table 1 and Figure 7).

The fluctuations in the haematocrit and haemoglobin

FIGURE 9.

Seasonal changes in the mean relative percentages of the serum proteins of healthy adult male and female Kangaroo Island wallabies given water *ad lib.* and fed a high protein diet.

Legend: vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.

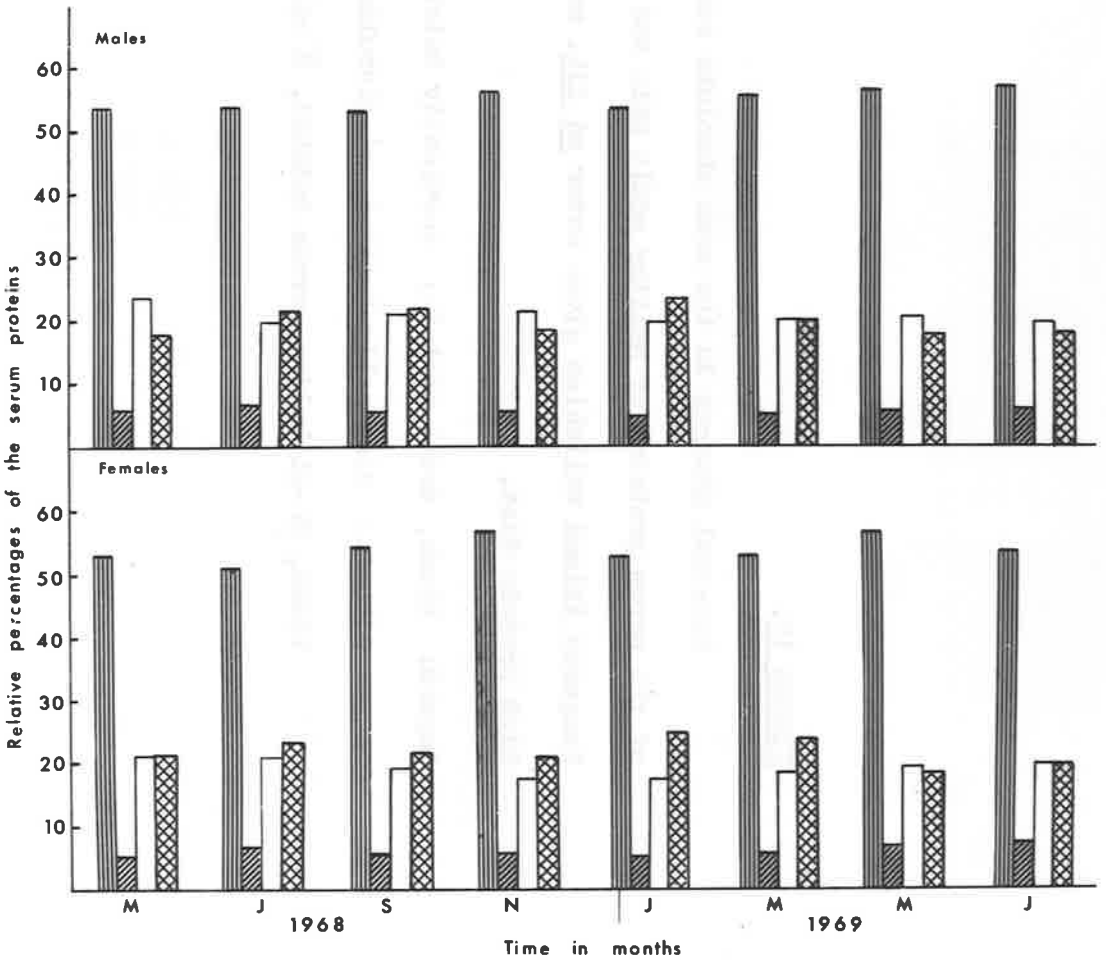
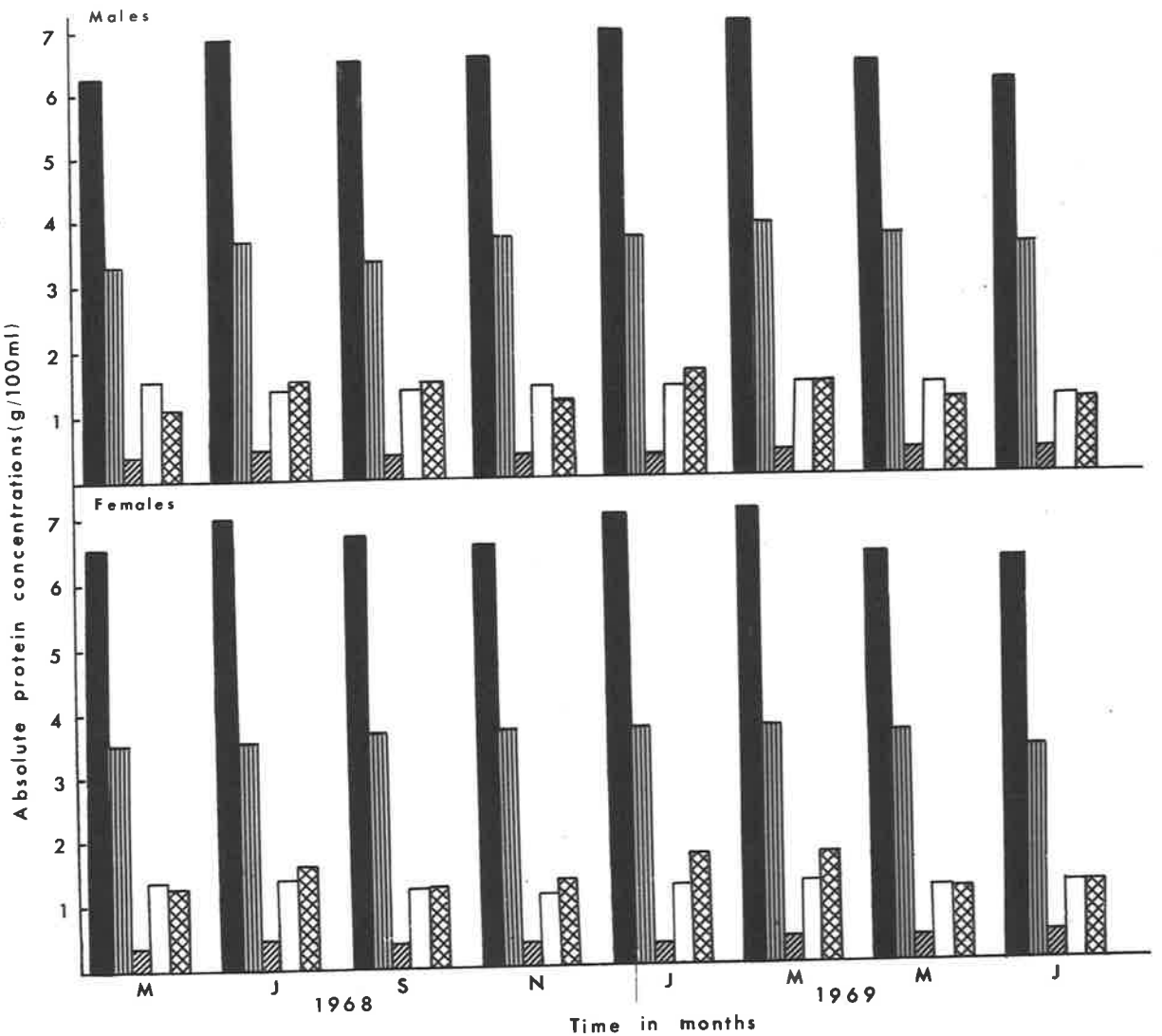

Males
Females
60
50
40
30
20
10
Relative percentages of the serum proteins
M
J
S
N
J
M
M
J
1968
1969
Time in months

FIGURE 10.

Seasonal changes in the mean absolute concentrations of the serum proteins of healthy adult male and female Kangaroo Island wallabies given water *ad lib.* and fed a high protein diet.

Legend: black, total protein; vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.


Males
Females
Absolute protein concentrations(g/100ml)
1
2
3
4
5
6
7
M
J
S
N
J
M
M
J
1968
1969
Time in months

concentration of the males were generally paralleled by fluctuations in their body weight, relative percentage of β-globulin and M.C.H.C. (Table 1 and Figures 6 - 9). The exceptions to this trend were that, compared with the other variates, the relative percentage of β-globulin and the body weight were low in July 1968 and the M.C.H.C. and the relative percentage of β-globulin were high in September 1968.

3. A study of the effect of a low nitrogen diet and a restricted water intake on various blood parameters in adult wallabies

(a) Procedures

Animals. For this experiment a number of sexually mature male wallabies was obtained from Kangaroo Island in March 1968. The summer of 1967 - 68 was particularly severe on the island and so most of these animals were in very poor condition.

Twelve of the wallabies were placed in individual 3' x 12' pens with sawdust covered floors in a water-proofed animal house. These animals were fed a maintenance diet containing 1.3gN/100g dry weight, supplemented with bread and vegetables, and were given water ad lib. The remaining wallabies were placed in the domestic colony. Several of the original 12 wallabies

died and were replaced with the animals kept in the domestic colony. By the time the experiment began on July 18, 1968, there were only 11 of the original group remaining, and so 1 male wallaby which had been kept in the domestic colony since its arrival from Kangaroo Island in January 1967, was used to make up the number.

The dietary supplements were discontinued from July 18. For the next 18 days, the daily food and water intakes were measured for each wallaby.

Diets. The experimental diets used were modified from the mixture used by McDonald and Hall (1957). The composition of the diets is given by Barker (1968). The low nitrogen diet was the basic diet and contained 0.3gN/100g dry weight. The maintenance diet had added ground casein and contained 1.3 gN/100g dry weight.

Experimental design. The 12 animals were divided into 2 groups of 6. Both groups were fed the low nitrogen diet. The control group was allowed an unrestricted water intake while animals in the experimental group were given 170 ml each per day, which was calculated to be approximately 50% of their unrestricted water intake.

Collections. On August 6 the animals were placed in individual metabolism cages (described by Barker, 1968) in an airconditioned animal house. The wallabies were fed maintenance diet and were given water ad lib. Urine and faeces were collected for 24 hours. At the end of this period a blood sample was drawn from each animal and they were then returned to their pens. The experimental regimen was commenced. The daily food and water intakes of each wallaby were measured, and the animals were weighed at weekly intervals. After 4 weeks a second 24 hour collection was made and a blood sample was taken from each animal. Three weeks later the wallabies were placed in metabolism cages for 10 days for the estimation of nitrogen balance. At the end of this period a 24 hour collection was made and blood samples were taken. Following this the wallabies were returned to their pens for another week, at the end of which their plasma volumes were estimated. The experiment was then terminated.

(b) Results

Between the first and second sampling periods, 1 of the control animals died and another was removed from the experiment since it proved an unsuitable laboratory animal. Hence from

the second sampling period, all results reported for the controls were based on results from 4 wallabies.

The analyses made on the wallabies' urine and faeces, and the results of the nitrogen balance trial are to be presented elsewhere (Barker et al., 1970).

Figure 11 shows the mean body weights of the animals at the 3 sampling times. Figures 12 - 15, Table 2 and Appendices 6 - 10 present the results of the haematological estimations made in this study.

Inspection of Appendices 6 and 7 shows that the levels of most of the parameters measured underwent little change in either group of animals during the study period. In the controls, the absolute concentration of albumin was significantly less at the end of the study than at the beginning. In the experimentals the haematocrit was significantly lower at the third collection period than at the other 2 collections. The body weight of the experimentals at the second sampling period was significantly less than at the first and at the third sampling it was significantly less than at the second, while the M.C.H.C. of the experimentals was significantly higher at the second and third sampling periods than at the first.

Appendices 8 - 10 show that at any one of the sampling periods, the controls had significantly different values from the experimentals for only a few of the parameters measured.

FIGURE 11.

Mean body weights of 2 groups of male Kangaroo Island wallabies fed a low nitrogen diet. The controls were given water *ad lib.* and the experimentals had a restricted water intake.

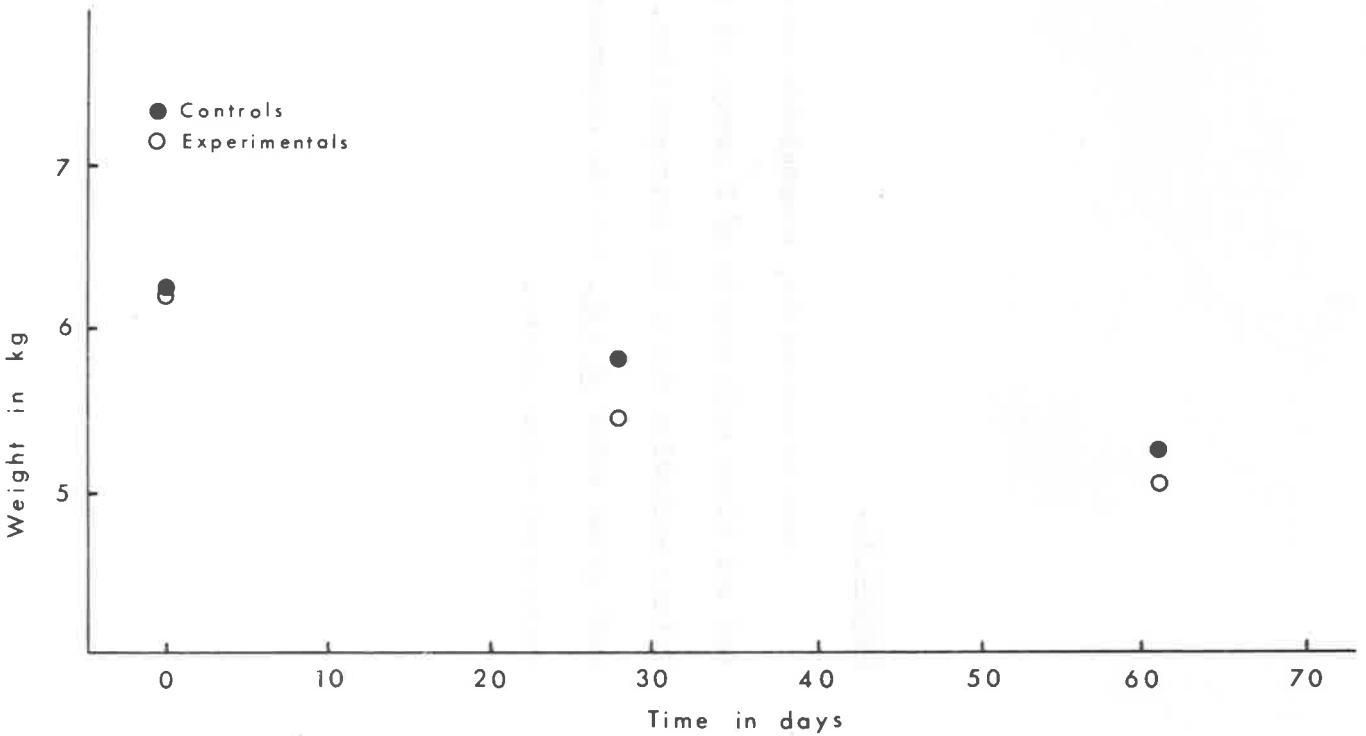

● Controls
○ Experimentals
Weight in kg
7
6
5
0
10
20
30
40
50
60
70
Time in days

FIGURE 12.

Mean haematocrits, haemoglobin concentrations and red blood cell counts of 2 groups of male Kangaroo Island wallabies fed a low nitrogen diet. The controls were given water *ad lib.* and the experimentals had a restricted water intake.

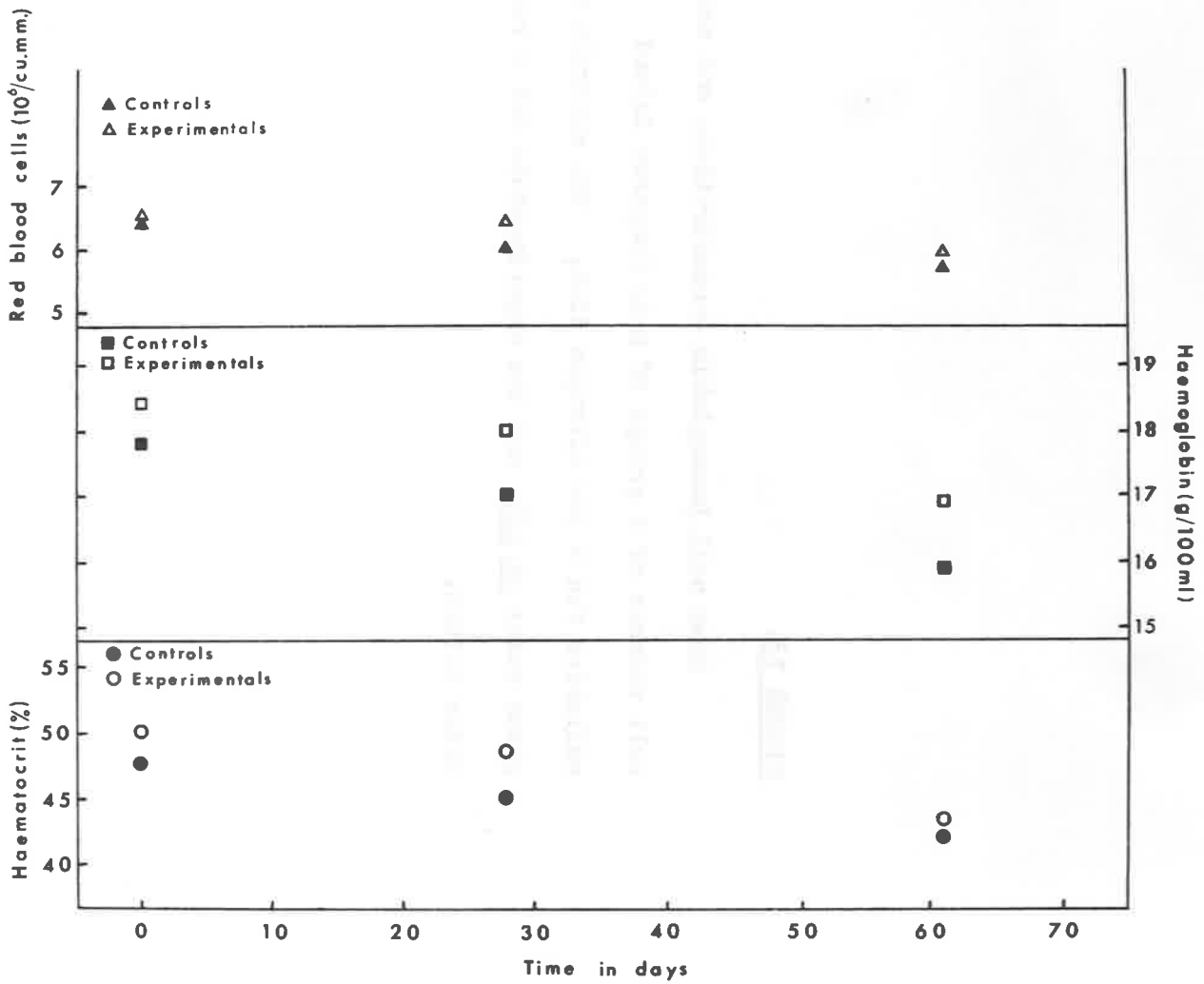
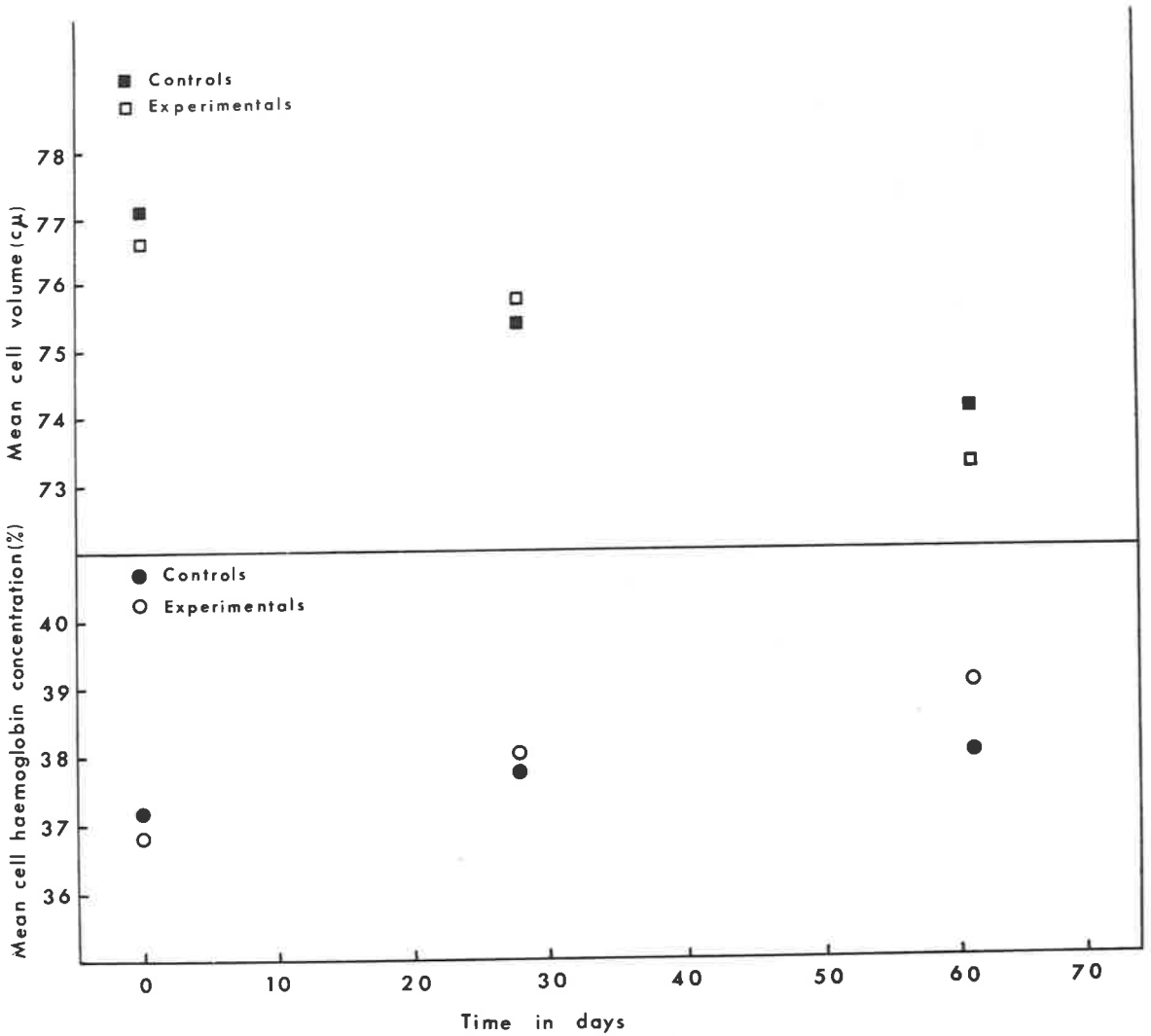

Red blood cells (10^6/cu.mm.)
▲ Controls
△ Experimentals
7
6
5
■ Controls
□ Experimentals
Haemoglobin (g/100ml)
19
18
17
16
15
● Controls
○ Experimentals
Haematocrit (%)
55
50
45
40
0
10
20
30
40
50
60
70
Time in days

FIGURE 13.

Mean cell haemoglobin concentrations and mean cell volumes of 2 groups of male Kangaroo Island wallabies fed a low nitrogen diet. The controls were given water *ad lib.* and the experimentals had a restricted water intake.


Controls
Experimentals
Mean cell volume (cμ)
78
77
76
75
74
73
Controls
Experimentals
Mean cell haemoglobin concentration (%)
40
39
38
37
36
0
10
20
30
40
50
60
70
Time in days

At the second and third sampling periods, the absolute concentration of serum albumin was significantly higher in the experimentals than in the controls, and the concentration of total serum protein of the experimentals was greater than that of the controls at the third sampling period. By the time of the final sampling, the M.C.H.C. was also significantly higher in the experimentals than in the controls.

Since the plasma volumes of the controls and the experimentals were not significantly different (Table 2), the experimentals were not haemoconcentrated compared with the controls. Hence the differences between the 2 groups for the concentrations of total serum protein and serum albumin and for the M.C.H.C. were true differences.

TABLE 2

Plasma volumes of control and experimental wallabies at the end of Experiment 3

Group	Animal Number	Plasma Volume (ml/kgW)
Control	4	43.5
	7	47.6
Experimental	1	46.5
	2	44.7
	5	38.7
	10	44.3
		t = 0.709 n.s.

FIGURE 14.

Mean relative percentages of the serum proteins of 2 groups of male Kangaroo Island wallabies fed a low nitrogen diet. The controls were given water ad lib. and the experimentals had a restricted water intake.

Legend: vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.

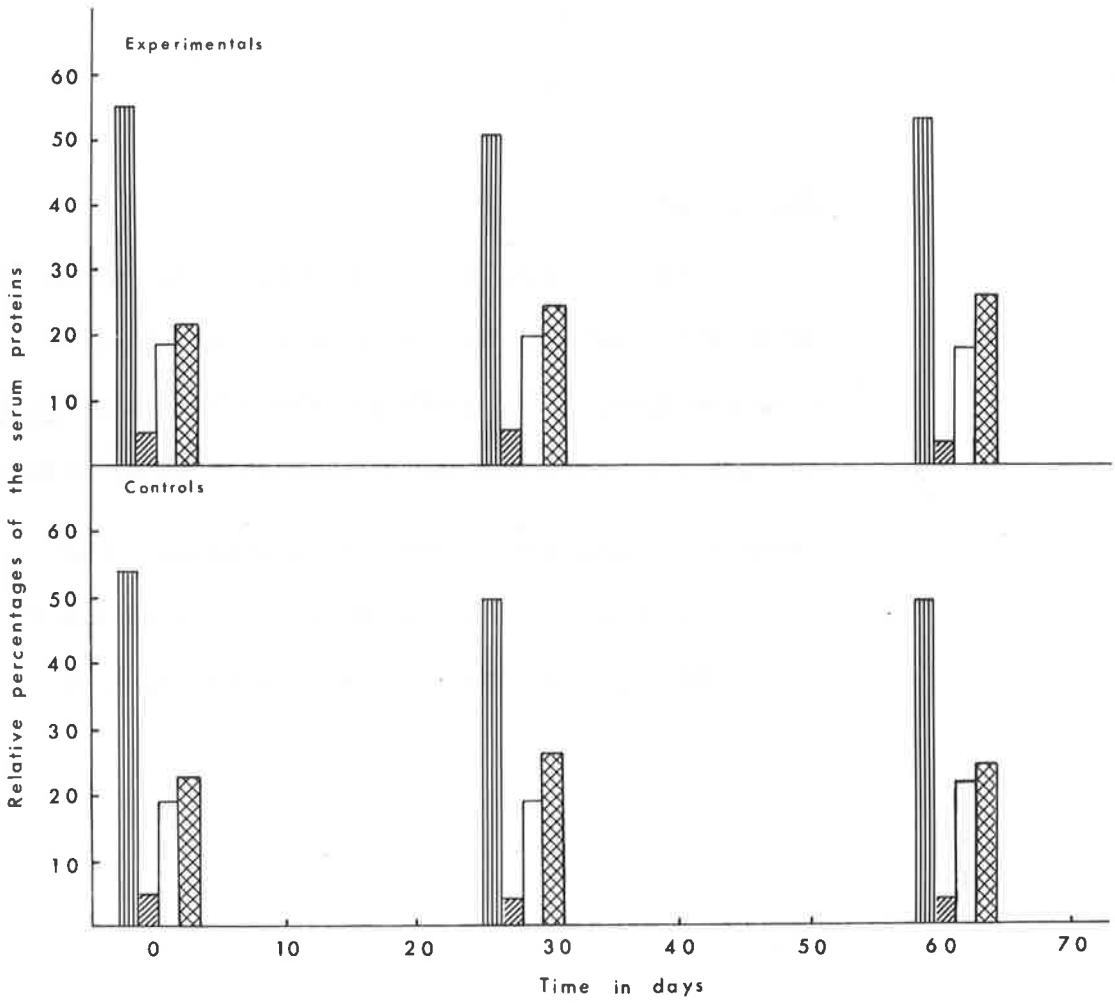
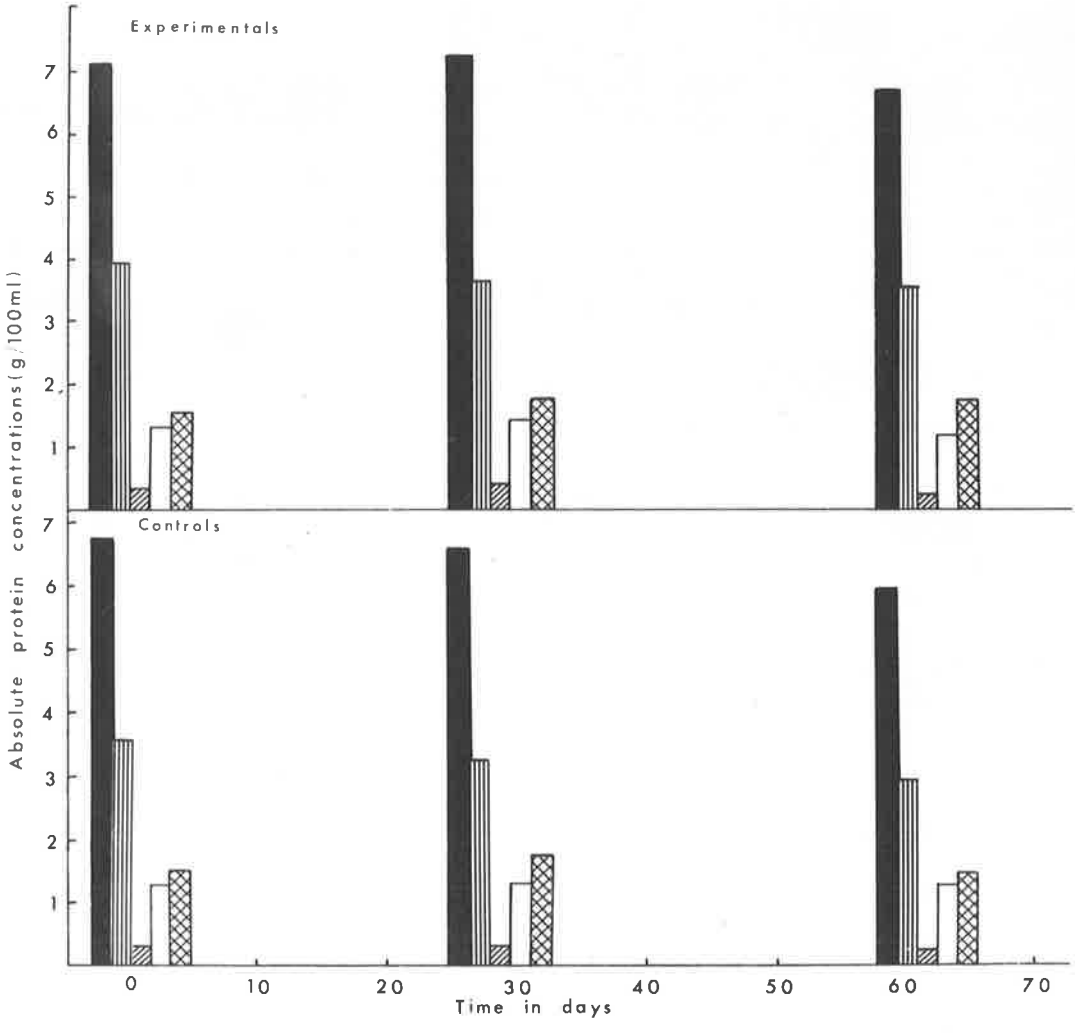

Experimentals
Controls
Relative percentages of the serum proteins
60
50
40
30
20
10
0
10
20
30
40
50
60
70
Time in days

FIGURE 15.

Mean absolute concentrations of the serum proteins of 2 groups of male Kangaroo Island wallabies fed a low nitrogen diet. The controls were given water ad lib. and the experimentals had a restricted water intake.

Legend: black, total protein; vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.


Experimentals
Controls
Absolute protein concentrations(g/100ml)
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
Time in days

4. A study of some blood parameters in adult wallabies captured in the field at various times of the year

(a) Procedures

Field trips were made to the study area in November 1967, February, May, July and December 1968, and March and May 1969. On each of these trips blood samples were taken from 30 adult females and 10 adult males. After they had been sampled, the wallabies were released near the place of their capture.

Throughout the study period, of the females captured which were capable of having young, only about 5% did not have young.

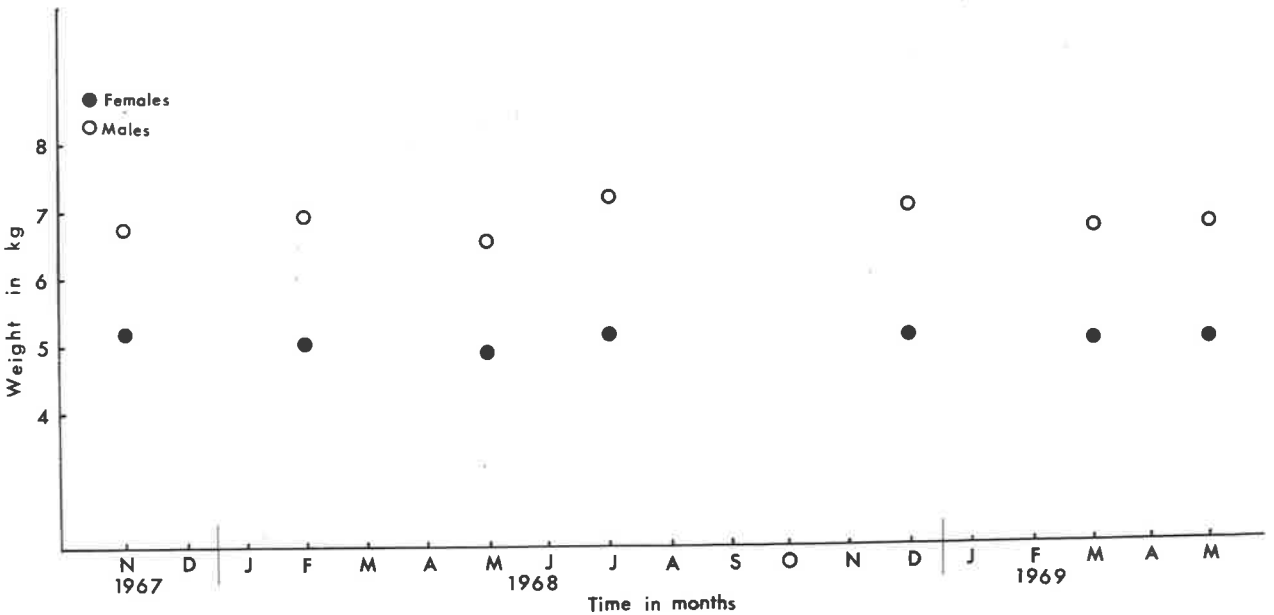
(b) Results

Figures 16 - 20, Tables 4 - 6 and Appendices 11 - 13 present the results of this study. The collection dates on each field trip are given in Table 3.

As Appendix 11 shows, only the haematocrit, haemoglobin concentration, relative percentage of γ-globulin and the body weight differed significantly between males and females during the study. The body weight of the males was significantly greater on all trips while the haematocrit and haemoglobin concentration were significantly greater in the males in November 1967 and July 1968, and the relative percentage of γ-globulin was significantly greater in the

FIGURE 16.

Seasonal changes in the mean body weights of adult male and female Kangaroo Island wallabies captured in Flinders Chase.


● Females
○ Males
Weight in kg
8
7
6
5
4
N
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
1967
1968
1969
Time in months

males in May 1968.

Inspection of Appendix 12 shows that for the female wallabies, only the body weight and the M.C.H.C. did not differ significantly between the 7 trips. Appendix 13 shows that for the males, there was no significant difference in body weight, red blood cell count, M.C.V. and the relative percentages of α- and β-globulin between the 7 trips.

To elucidate any relationships between the parameters measured, correlation matrices between the 15 variates were computed for males and females (Tables 4 and 5).

Inspection of these tables shows that statistically significant relationships existed between many of the variates in males and females. The correlations between the relative percentage of a protein fraction and its absolute concentration, and between the absolute concentration of a fraction and the total protein concentration were expected because of the way the absolute concentrations of the protein fractions are calculated. Correlations between M.C.V. and the haematocrit and red blood cell count, and between M.C.H.C. and the haematocrit and haemoglobin concentration are also implied by the methods through which M.C.V. and M.C.H.C. are calculated.

Tables 4 and 5 show that there was a strong positive correlation between haematocrit, haemoglobin concentration and red blood cell count in both males and females.

FIGURE 17.

Seasonal changes in the mean haematocrits, haemoglobin concentrations and red blood cell counts of adult male and female Kangaroo Island wallabies captured in Flinders Chase.

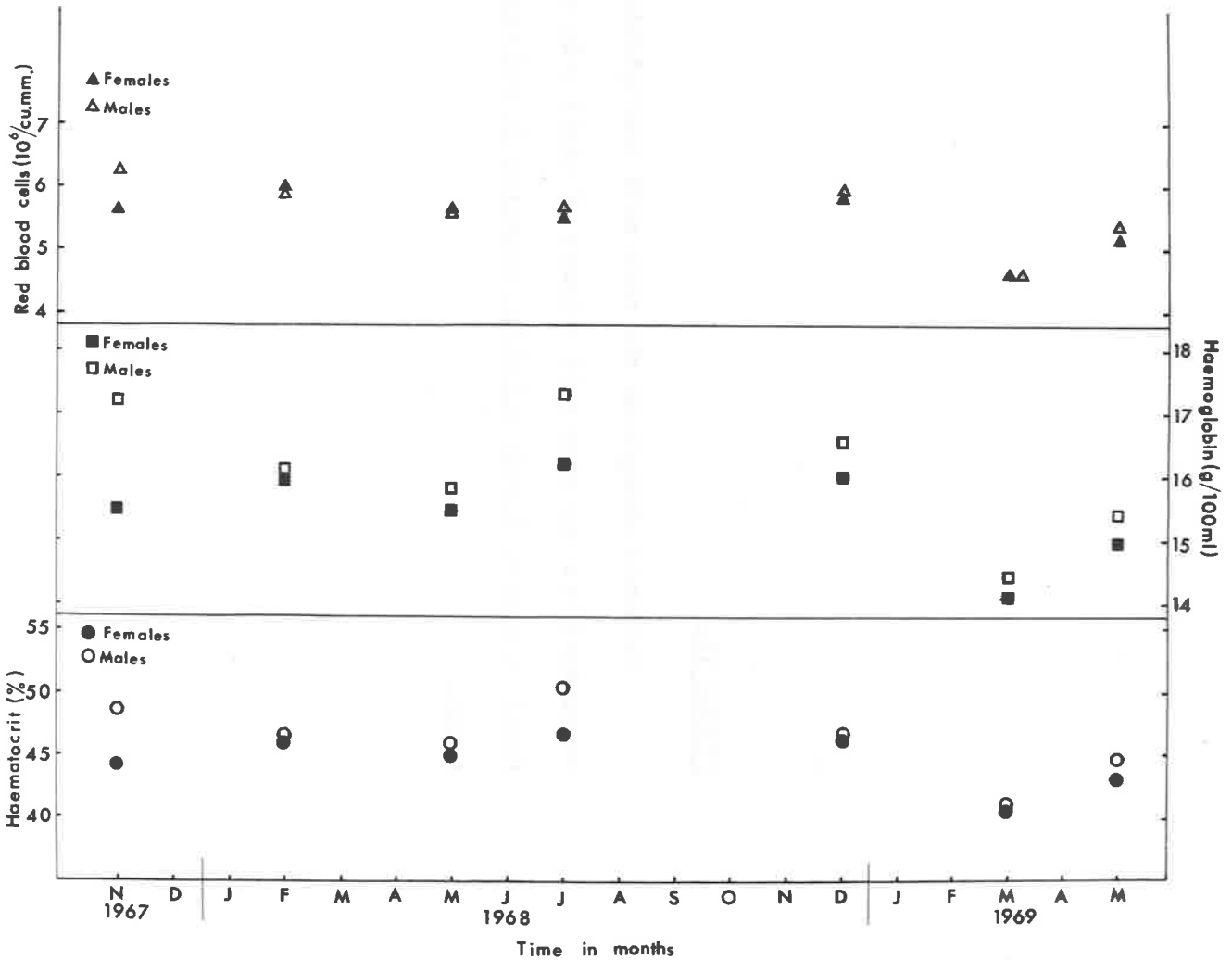
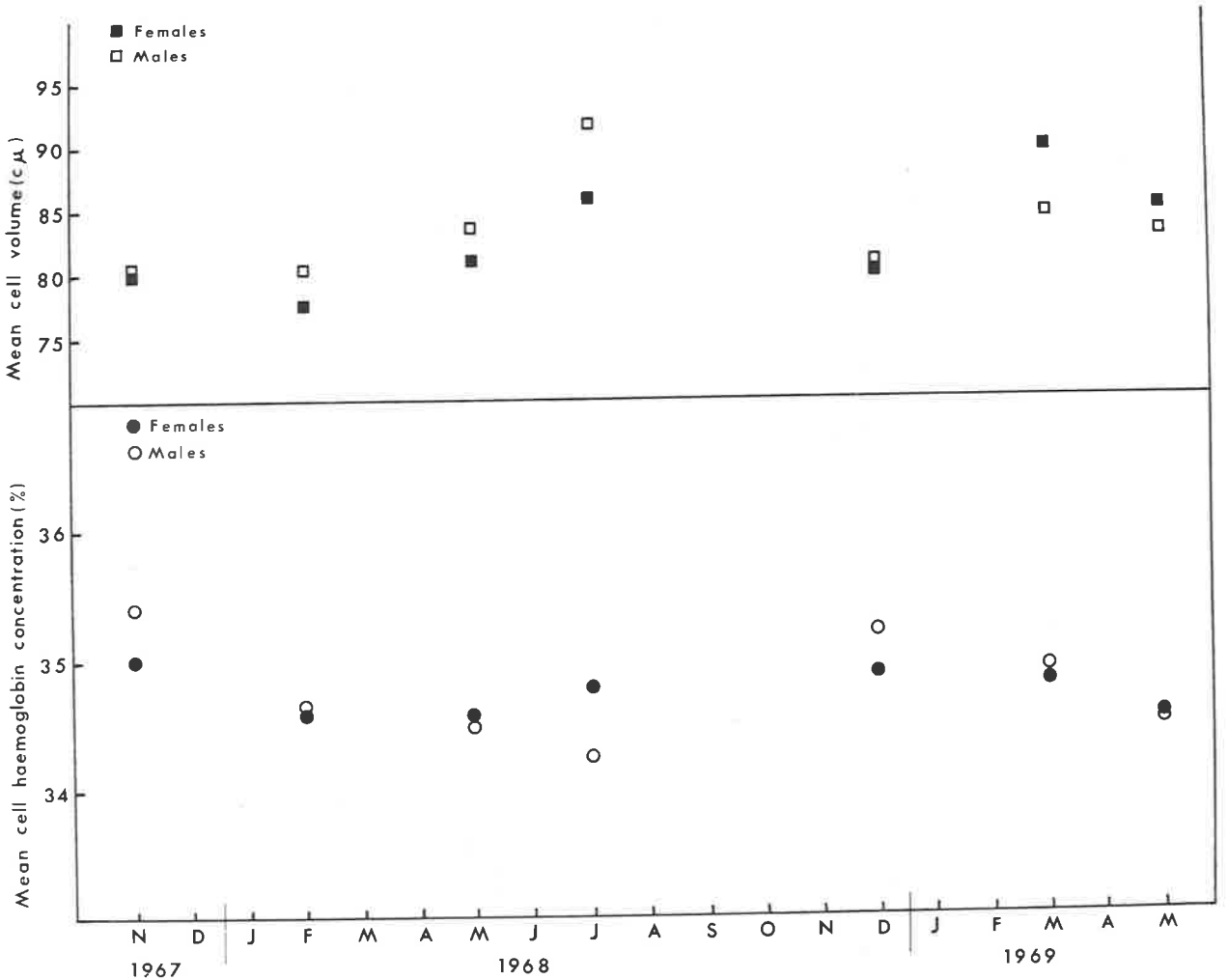

Red blood cells (10^6/cu.mm.)
▲ Females
△ Males
7
6
5
4
■ Females
□ Males
Haemoglobin (g/100ml)
18
17
16
15
14
Haematocrit (%)
● Females
○ Males
55
50
45
40
N
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
1967
1968
1969
Time in months

FIGURE 18.

Seasonal changes in the mean cell haemoglobin concentrations and mean cell volumes of adult male and female Kangaroo Island wallabies captured in Flinders Chase.


Females
Males
Mean cell volume (cμ)
95
90
85
80
75
Females
Males
Mean cell haemoglobin concentration (%)
36
35
34
N D J F M A M J J A S O N D J F M A M
1967
1968
1969

These tables also show that in males and females the relative percentage of β-globulin was negatively correlated with the relative percentage of albumin, and the relative percentage of γ-globulin was negatively correlated with the relative percentages of β-globulin and albumin.

In females the absolute concentration of albumin was negatively correlated with the absolute concentration of β-globulin and in males it was negatively correlated with the concentration of α-globulin (Tables 4 and 5).

Body weight in the females was correlated, either positively or negatively, with the relative and absolute concentrations of all the serum protein components except α-globulin; in the males, it was positively correlated with the concentration of total protein and albumin and negatively correlated with the relative percentage of α- and β-globulin (Tables 4 and 5).

In Table 6, males and females have been considered separately and for each variate that differed significantly between the field trips, the trips have been ranked in order of increasing size of the sample mean. Although changes in many of the variates were positively correlated (Tables 4 and 5), Table 6 and Figures 16-20 show that the times of the maximum values for these related variates often did not coincide; neither did the times of the minimum values. Table 6 and

FIGURE 19.

Seasonal changes in the mean relative percentages of the serum proteins of adult male and female Kangaroo Island wallabies captured in Flinders Chase.

Legend: vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched, γ-globulin.

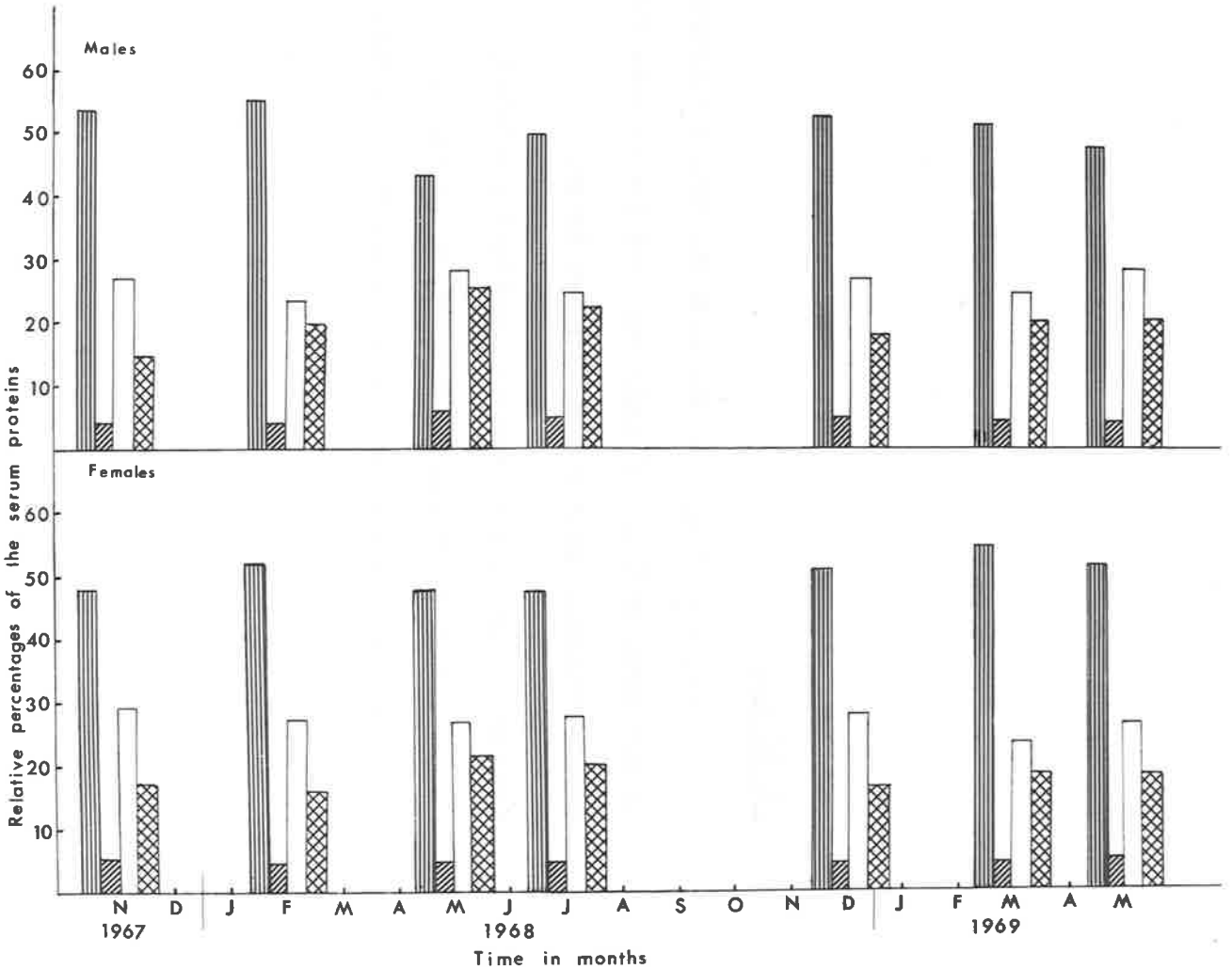
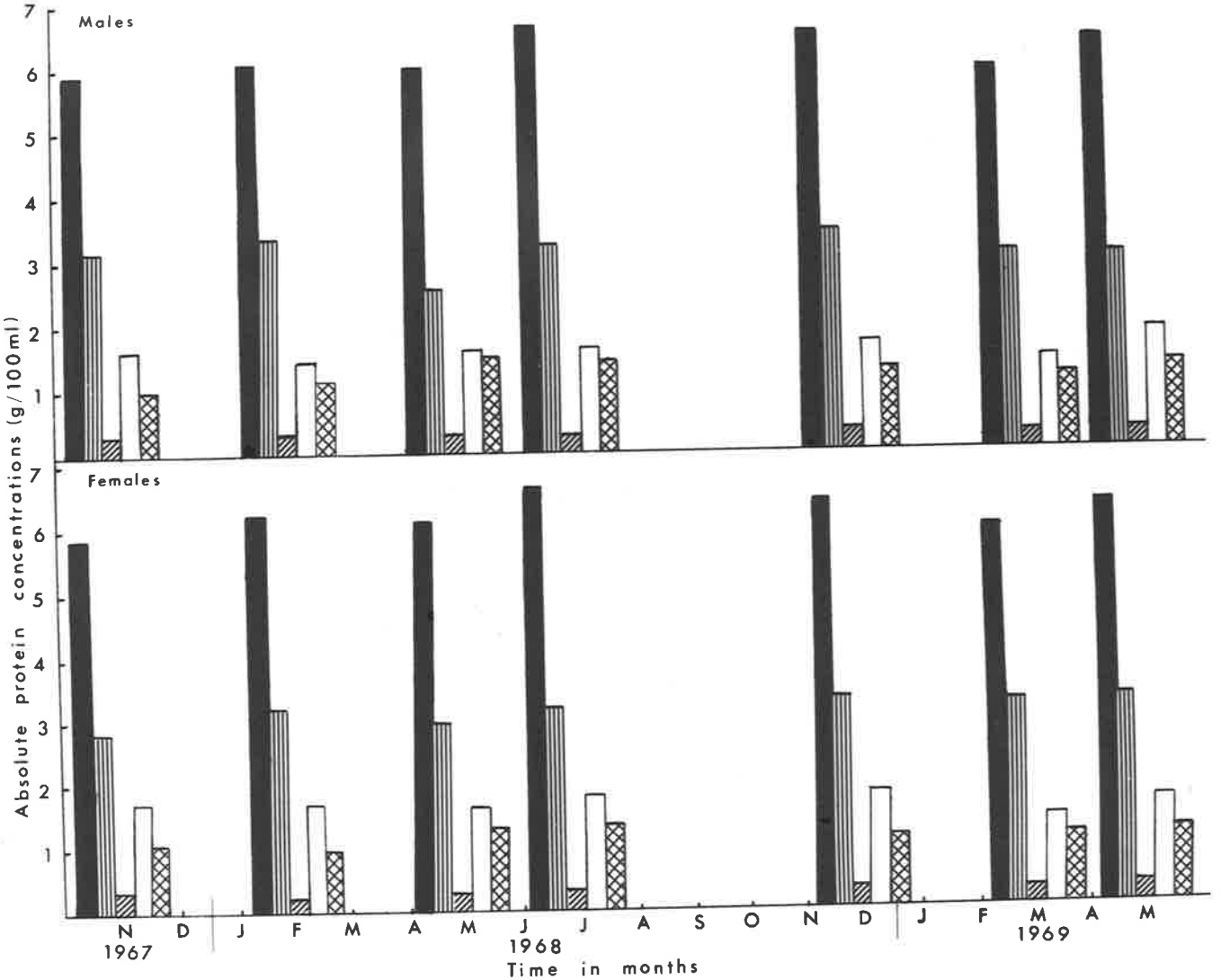

Males
Females
Relative percentages of the serum proteins
60
50
40
30
20
10
N D J F M A M J J A S O N D J F M A M
1967
1968
1969
Time in months

FIGURE 20.

Seasonal changes in the mean absolute concentrations of the serum proteins of adult male and female Kangaroo Island wallabies captured in Flinders Chase.

Legend: black, total protein; vertically hatched, albumin; diagonally hatched, α-globulin; blank, β-globulin; cross hatched; γ-globulin.


Males
Females
Absolute protein concentrations (g/100ml)
1
2
3
4
5
6
7
N
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
1967
1968
1969
Time in months

Figures 16 - 20 also show that for a particular variate, males often had maximum or minimum values at different times from the females. There may be an annual cycle in the way M.C.H.C. varied (Figure 18) but no such cycle is evident for any other variates; in fact, inspection of Table 6 and Figures 16 - 20 shows for example that many of the variates that had high levels in February 1968 had low levels in March 1969. However, Table 6 shows that animals in July 1968 had the greatest number of maxima for the different parameters while those in March 1969 had the greatest number of minima.

TABLE 3

The collection dates on each field trip

Trip No.	Collection Dates
1	22/11/67 - 6/12/67
2	1/ 2/68 - 14/ 2/68
3	11/ 5/68 - 21/ 5/68
4	19/ 7/68 - 29/ 7/68
5	7/12/68 - 17/12/68
6	6/ 3/69 - 15/ 3/69
7	20/ 5/69 - 28/ 5/69

TABLE 4

Correlation matrix of 15 variates from male wallabies captured on all field trips

	‡Ht	Hb	Rbc	TP	Alb	α-G	β-G	γ-G	Ralb	Rα	Rβ	Rγ	Wt	MCV	MCHC
Ht		.961	.422	.411	.455	.027	.129	-.018	.228	-.128	-.074	-.175	.197	.103	-.156
Hb	***		.450	.419	.507	-.003	.104	-.039	.281	-.157	-.104	-.203	.202	.062	.121
Rbc	***	***		.135	.166	-.038	-.082	.084	.110	-.093	-.154	-.024	.046	-.707	.083
TP	***	***	n.s.		.476	.039	.396	.573	-.151	-.360	-.092	.321	.375	.160	.036
Alb	***	***	n.s.	***		-.307	-.214	-.194	.793	-.490	-.494	-.394	.459	.061	.181
α-G	n.s.	n.s.	n.s.	n.s.	**		.033	.212	-.381	.874	.028	.253	-.168	.120	-.107
β-G	n.s.	n.s.	n.s.	***	n.s.	n.s.		-.017	-.516	-.112	.873	-.143	-.154	.192	-.090
γ-G	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.		-.600	-.018	-.299	.956	.182	-.006	-.057
Ralb	n.s.	*	n.s.	n.s.	***	**	***	***		-.328	-.505	-.654	.230	-.043	.179
Rα	n.s.	n.s.	n.s.	**	***	***	n.s.	n.s.	**		.096	.109	-.276	-.001	-.110
Rβ	n.s.	n.s.	n.s.	n.s.	***	n.s.	***	*	***	n.s.		-.305	-.359	.100	-.114
Rγ	n.s.	n.s.	n.s.	**	***	*	n.s.	***	***	n.s.	*		.099	-.036	-.085
Wt	n.s.	n.s.	n.s.	**	***	n.s.	n.s.	n.s.	n.s.	*	**	n.s.		-.010	.012
MCV	n.s	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		-.126
MCHC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

‡ Haematocrit (Ht), haemoglobin concentration (Hb), red blood cell count (Rbc), total protein (TP), absolute concentration of albumin (Alb), α-globulin (α-G), β-globulin (β-G), γ-globulin (γ-G), relative percentage of albumin (Ralb), α-globulin (Rα), β-globulin (Rβ), γ-globulin (Rγ), body weight (Wt), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC).

TABLE 5

Correlation matrix of 15 variates from female wallabies captured on all field trips

	‡Ht	Hb	Rbc	TP	Alb	α-G	β-G	γ-G	Ralb	Rα	Rβ	Rγ	Wt	MCV	MCHC
Ht		.960	.665	.138	.135	.040	.108	-.015	.008	.003	.066	-.088	-.072	-.175	-.187
Hb	***		.672	.115	.126	.026	.094	-.026	.020	-.003	.060	-.092	-.044	-.208	.094
Rbc	***	***		.020	-.031	-.078	.142	-.064	-.062	-.068	.165	-.097	-.072	-.699	-.015
TP	*	n.s.	n.s.		.511	.308	.440	.624	-.385	-.104	.006	.394	.185	.007	-.081
Alb	n.s.	n.s.	n.s.	***		.081	-.204	.097	.592	-.147	-.482	-.034	.334	.094	-.024
α-G	n.s.	n.s.	n.s.	***	n.s.		.139	.093	-.203	.894	.006	.021	.070	.063	-.057
β-G	n.s.	n.s.	*	***	**	*		-.133	-.640	-.039	.895	-.318	-.265	-.143	-.055
γ-G	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.		-.464	-.156	-.440	.951	.240	.054	-.040
Ralb	n.s.	n.s.	n.s.	***	***	**	***	***		-.071	-.539	-.390	.201	.102	.052
Rα	n.s.	n.s.	n.s.	n.s.	*	***	n.s.	*	n.s.		.016	-.139	-.012	.073	-.030
Rβ	n.s.	n.s.	*	n.s.	***	n.s.	***	***	***	n.s.		-.539	-.392	-.175	-.027
Rγ	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	***	***	*	***		.235	.062	-.015
Wt	n.s.	n.s.	n.s.	**	***	n.s.	***	***	**	n.s.	***	***		.012	.107
MCV	*	**	***	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.		-.095
MCHC	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

‡ Haematocrit (Ht), haemoglobin concentration (Hb), red blood cell count (Rbc), total protein (TP), absolute concentration of albumin (Alb), α-globulin (α-G), β-globulin (β-G), γ-globulin (γ-G), relative percentage of albumin (Ralb), α-globulin (Rα), β-globulin (Rβ), γ-globulin (Rγ), body weight (Wt), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC).

TABLE 6

Field trips ranked in order of increasing size of the sample mean for each of the variates which differed significantly in males or females over the 7 trips

Variate	Ranked Sampling Periods						
MALES	Lowest sample mean						Highest sample mean
Haematocrit	6	7	3	2	5	1	4
Haemoglobin concentration	6	7	3	2	5	1	4
Mean cell haemoglobin concentration	4	3	7	2	6	5	1
Total protein concentration	1	6	3	2	7	5	4
Absolute concentration of albumin	3	7	6	1	4	2	5
Absolute concentration of α-globulin	2	1	7	6	5	4	3
Absolute concentration of β-globulin	2	6	1	3	4	5	7
Absolute concentration of γ-globulin	1	2	6	5	7	4	3
Relative percentage of albumin	3	7	4	6	5	1	2
Relative percentage of γ-globulin	1	2	5	6	7	4	3

...../cont.

TABLE 6 (cont.)

Variate	Ranked Sampling Periods						
FEMALES	Lowest sample mean						Highest sample mean
Haematocrit	6	7	1	3	5	2	4
Haemoglobin concentration	6	7	3	1	2	5	4
Red blood cell cell count	6	7	4	3	1	5	2
Mean cell volume	2	5	1	3	7	4	6
Total protein concentration	1	6	3	2	7	5	4
Absolute concentration of albumin	1	3	4	6	7	5	2
Absolute concentration of α-globulin	6	3	2	1	5	4	7
Absolute concentration of β-globulin	6	3	7	2	1	5	4
Absolute concentration of γ-globulin	2	1	5	6	7	3	4
Relative percentage of albumin	4	3	1	5	7	2	6
Relative percentage of α-globulin	6	3	2	5	4	7	1
Relative percentage of β-globulin	6	7	3	2	4	5	1
Relative percentage of γ-globulin	2	5	1	7	6	4	3

IV. DISCUSSION

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1. The effect of age on various blood parameters in Kangaroo Island wallabies

Maximum growth was found to occur in pouch young aged 130 - 230 days. During this period the haematocrit and haemoglobin concentration of the young were found to increase markedly; this must have been largely due to the greater oxygen requirements of the animals as they increased in size.

In young aged 250 - 299 days there was a marked increase in the relative percentage of serum albumin; this coincided with the time when the young permanently vacated their mother's pouch.

Once out of the pouch permanently, the young suckle infrequently and in captivity they eat the same food as adults. The milk from several species of marsupials is known to have a high protein content (6% - 9% of whole milk), (Bolliger and Pascoe, 1953; Gross and Bolliger, 1959; Lemon and Barker, 1967), and if the milk composition of the Kangaroo Island wallaby is similar to that of other marsupials, it is unlikely that grasses and other plants on which the young feed contain more protein than their mothers' milk. Hence the increased concentration of serum albumin in these young is unlikely to be associated with an increased nitrogen content of their food.

The relative concentration of γ-globulin decreased in young aged 250 - 299 days and since the serum proteins, particularly albumin, help to maintain the colloid osmotic pressure of the blood (Harper, 1959; Putnam, 1960; MacFarlane and Robb-Smith, 1961; Hoffman, 1964), it is possible that the increased concentration of albumin compensated for the lower γ-globulin concentration in these young.

Although there was little change in the relative percentage of α-globulin while the young developed, it is interesting to note that young aged 38 - 49 days had a slightly higher concentration of α-globulin than older pouch young. This may be due to the presence of an α-fetoprotein similar to that found in many eutherian foetuses until just prior to birth (Bergstrand and Czar, 1956; Gitlin and Boesman, 1966, 1967; Schultze and Heremans, 1966; Seal and Erickson, 1969).

An α-fetoprotein persists into early pouch life in opossums, (Gitlin and Boesman, 1967) and possibly also in quokkas (Jordan and Morgan, 1968). To see whether an α-fetoprotein is present in young Kangaroo Island wallabies, a more refined technique than paper electrophoresis will have to be used.

The gradual increase in the absolute concentration of α-globulin in the young as they developed may be associated with an increase in the α-lipoprotein fraction. Paper

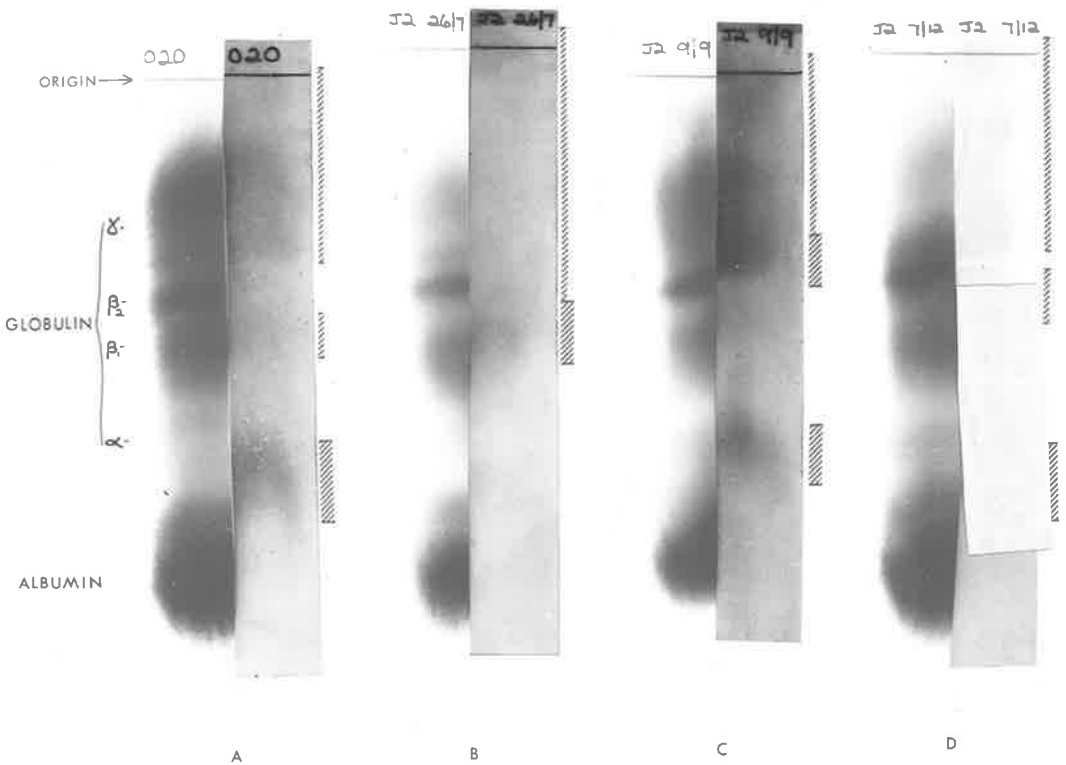
electrophoresis of sera from several young older than 195 days showed a high concentration of lipoprotein in the α-globulin region when the papers were stained with Sudan III according to the method of Ribeiro et al. (1961) (Figure 21).

The absolute concentration of β-globulin gradually increased until the young were 200 - 249 days old. This may be associated with an increase in the β-lipoprotein fraction. Electrophoresis of sera from young older than 170 days showed lipoprotein in the β-globulin region although the concentration of β-lipoprotein was greater in young aged 170 - 222 days than in an older young aged 324 days (Figure 21).

A high concentration of transferrin, similar to that found in human infants at birth (Schultze and Heremans, 1966) may also be responsible for the rise in the β-globulin fraction of the pouch young. Paper electrophoresis of quokka serum showed that transferrin moved with the β-globulin fraction (Ezekial, Lai and Kaldor, 1963). However, as the present study shows, lipoproteins may also be found in the β-globulin fraction. A more refined technique than paper electrophoresis, such as starch gel electrophoresis and autoradiography with Fe^{59}, would be necessary to isolate transferrin, and so show whether increases in the transferrin concentration were responsible for increases in the β-globulin concentration of the young.

FIGURE 21.

Paper electrophoresis of adult serum (A) and of serum from young aged 174 (B), 222 (C) and 324 (D) days. The paper for each animal was cut in 2 and 1 half stained for protein and the other for lipoprotein. Because of technical difficulties in photography, the lipoprotein zones had to be printed on different paper from the protein zones and so this figure is a composite of 2 photographs. The protein zones are on the left of the lipoprotein zones for each serum. The diagonally hatched areas beside the papers indicate the position and the relative intensity of the lipoprotein bands in each serum.


ORIGIN
GLOBULIN
ALBUMIN
A
B
C
D

The concentration of serum γ-globulin increased slowly until the young reached 200-249 days of age when both the relative and absolute concentrations of γ-globulin increased sharply. This coincided with the time when the young began to emerge from the pouch and eat grass.

The relatively low γ-globulin content of sera from Kangaroo Island wallabies less than 200 days old means that the young were either not exposed to many antigens or else that they showed poor immunological development during pouch life. A similar conclusion was drawn by Jordan and Morgan (1968) for the quokka. The reason for the fall in the relative and absolute concentrations of γ-globulin in young aged 250-349 days is not known.

The study shows that by the time the wallabies were 450 days old, their haematocrit, haemoglobin concentration, serum protein profile and concentration of total serum protein were similar to those found in healthy adult wallabies.

2. The effects of the season of a year and the sex of a wallaby on some blood parameters

A number of studies have been made on the effects of the season of the year on various physical and haematological parameters in mammals.

van Tets and Cowan (1966) found that adult female deer sampled in winter had a higher relative concentration of albumin than female deer sampled in the fall. Shield (1958) reported that adult quokkas on Rottnest Island showed seasonal fluctuations in body weight and in some blood parameters, and Ealey and Main (1967) found seasonal changes in the haematocrit, haemoglobin concentration and red blood cell count of euros in the Pilbara region of north-western Australia. All of these authors proposed the availability of food as the cause of the seasonal variations in the parameters measured.

McEwan and Whitehead (1969) found seasonal fluctuations in the haematocrit, haemoglobin concentration and plasma protein concentration of male and female caribou but they related this to reproductive activity.

There have been reports that environmental temperatures can affect the haematocrit and haemoglobin concentration of humans but Wintrobe (1967) doubts that there is sufficient evidence to support this hypothesis.

In the present study, any seasonal fluctuations in the levels of the parameters measured are unlikely to be caused by a shortage of food since an excess of high protein food was available to the wallabies in the laboratory enclosures at all times. However, reproduction and lactation, and possibly ambient temperature, may affect the levels of the various

parameters measured in the wallabies.

The evidence indicates that there may be an annual cycle in the body weight of the wallabies, the maximum body weights being recorded in November 1968 and January 1969.

There is no clear evidence of cyclical fluctuations in any of the haematological measures. Nevertheless particular seasons did seem to affect the levels of some of the blood parameters in both male and female wallabies.

Although many of the parameters measured did not vary significantly in the female wallabies during the study period, the serum concentration of γ-globulin and hence of total protein was high in the females in July 1968, and January and March 1969. High concentrations of γ-globulin are usually indicative of an infection although white blood cell counts performed on the females at these times were normal, except in March 1969 when the count was slightly above normal. None of the females appeared ill during these months and their γ-globulin concentrations had fallen in the samples taken in September 1968 and May 1969.

The females had high haematocrits and haemoglobin concentrations in May and July of 1968 compared with the rest of the study period. High levels of these parameters are usually associated with a large body mass but the females did not weigh more in July 1968 than during the rest of the study.

The females sampled in 1968 had had their young of the previous year removed. By July 1968 the females had pouch young 50 - 150 days old but the demands made on them for milk by these young would have been much less than later in the year when the young were older and required more milk with a higher protein content (Gross and Bolliger, 1959; Lemon and Barker, 1967). Even when these young were permanently out of the pouch, they still suckled occasionally from the teat they had used during pouch life, and so females in March 1969 were feeding a new pouch young from a newly developed mammary gland, as well as their young of the previous year. These females thus had 2 functional mammary glands at different stages of lactation. This may partly explain why females at the beginning of the study period had higher haematocrits and haemoglobin concentrations than those subsequently sampled.

It is interesting to note that lactation apparently had little effect on the relative and absolute concentrations of the serum proteins. Jordan and Morgan (1968) found that lactation in the quokka was associated with only minor changes in the concentrations of the serum proteins, except for the concentration of β-globulin which increased significantly as lactation progressed. There was apparently no increase in the concentration of β-globulin in lactating wallabies in the present study, although a closer analysis of the data may show

some change in this fraction.

Male wallabies sampled in May and July 1968 had high haematocrits and haemoglobin concentrations. The body weight of the males in July 1968 was significantly less than their weight in November 1968 and January 1969, yet the haematocrit and haemoglobin concentration of males sampled in November 1968 and January 1969, were less than in July 1968.

This apparent anomaly may be partly due to a moderate infection of parasitic protozoa found in the blood of males sampled in November 1968. The parasite has not been positively identified but it resembles the eperythrozoon parasite found in sheep (Sheriff, Clapp and Reid, 1966; Macaulay pers. comm.).

There are indications that the parasite may occur normally in low numbers in wallabies in the field. It is postulated that the wallabies usually carry a latent parasitic infection without being affected by it, but if they are stressed, for example by poor nutrition or some other seasonal aspect, the numbers of the parasite increase and adversely affect the hosts. The actual effects of the parasite on the wallabies are not known. However infections of related parasites are reported to cause anaemia in sheep and humans (Weinman, 1944; Sheriff et al., 1966). These anaemias, as with those recorded in this study, were characterised by reductions in the haematocrit, haemoglobin concentrations or the number of red

corpuscles per cu. mm. (Wintrobe, 1967).

It is possible that the anaemia of male wallabies bled in March 1969 was caused by the blood protozoan. The M.C.H.C. of these wallabies was significantly less than that of males sampled during 1968 and in July 1969. The M.C.V. of the males did not change significantly during the study period and so male wallabies sampled in March 1969 had a hypochromic normocytic anaemia compared with males sampled during 1968 and in July 1969. Specific antibodies are thought to play little part in immunity against protozoal infections (Humphrey and White, 1964, Macaulay, pers. comm.). Since the anaemic male wallabies had normal concentrations of β- and γ-globulin, it is thus quite possible that the anaemia was caused by the eperythrozoon-like parasite.

Although the blood of female wallabies was not examined for the presence of the blood parasite it is likely that it was present and if so, it may have been partly responsible for the fluctuations observed in some of the blood parameters of the female wallabies.

If fluctuations in the numbers of a parasite did cause some of the changes observed in the blood parameters of male and female wallabies in the present study, it is difficult to say what components of a season affected the wallabies and caused the fluctuations in the parasite's numbers. It seems obvious

though that particular seasons did indirectly affect the body weight and some haematological measures in Kangaroo Island wallabies.

It is apparent from the present study, however, that the levels of the parameters measured vary with the sex of a wallaby as well as with particular seasons. The results of the study show that the levels of the parameters were not consistently different between male and female wallabies throughout the study period. For instance, in November 1968, male wallabies had a significantly greater body weight, haematocrit, haemoglobin concentration, red blood cell count and relative percentage of β-globulin than the females, while in September 1968 none of the parameters measured differed significantly between males and females.

This study shows that care is needed when drawing conclusions about the effects of sex on body weight and various haematological measures in mammals; such conclusions apply only to the particular group of animals sampled and cannot even be applied to the same group of animals sampled at a different time of the year.

3. The effects of a low nitrogen diet and a restricted water intake on various blood parameters in adult wallabies

Rothschild et al. (1967), writing on the factors affecting serum protein metabolism, reported that one of the earliest effects of a low nitrogen diet is a decreased concentration of serum albumin. In the present study the albumin concentration of the controls was significantly lower at the end of the experiment than at the beginning and the controls had a lower concentration of serum albumin than the experimentals at the second and third sampling periods. The lower concentration of total serum protein in the controls compared with the experimentals at the end of the study was presumably due to the lower albumin concentration of the control wallabies. During the study there were no significant changes in any of the serum protein fractions in the experimental wallabies, although there was a tendency for the absolute concentration of albumin to decrease, and there were no significant changes in any of the globulin fractions in the controls.

However the haematocrit of the experimental wallabies was significantly less at the end of the study than at the other 2 collection periods. A lowered haematocrit often occurs during protein depletion in mammals but it is usually accompanied by a lowered haemoglobin concentration (Shield, 1958; Meacham et al., 1964; Ealey and Main, 1967). Although the haemoglobin

concentration of the experimental wallabies did fall during the present study, the trend was not significant.

The M.C.H.C. of the experimental wallabies rose during the experiment and by the end of the study their M.C.H.C. was significantly higher than at the beginning of the study. The M.C.V. tended to decrease in the experimental wallabies during the experiment but the trend was not significant. By the end of the study the experimental wallabies thus had a hyperchromic normocytic anaemia compared with their red blood cell measures at the beginning of the study.

The experimental wallabies were not haemococentrated compared with the controls at the end of the experiment, and so it may be expected that the control wallabies would develop an anaemia similar to that found in the experimentals. Although there were no significant changes in the haematocrit and M.C.H.C. of the controls during the study, there was a tendency for their haematocrit to decrease and their M.C.H.C. to increase. The nitrogen intake of the controls was higher than that of the experimentals (Barker et al., 1970); this was probably a reflection of their higher water intake (Barker, 1968). It is possible that if the experiment had been continued for a longer time, the controls would have become more nitrogen depleted and developed a hyperchromic normocytic anaemia compared with their red blood cell measures at the beginning of the study.

It is possible that the experimental regime was not directly responsible for the anaemia of the experimental wallabies. The anaemia may have been caused by the eperythrozoon-like parasite found in the blood of male wallabies in the laboratory enclosures. Large numbers of this parasite were found in the blood of both control and experimental wallabies at the end of the experiment (Macaulay, pers. comm.). Since the anaemia in the male wallabies in the laboratory enclosures in March 1969 was of a different type from that of the wallabies in the present study, the parasite could not have been responsible for both the anaemias - in fact it may not have caused either of them. The parasite seems to be normally present in low numbers in wallabies in the field and the laboratory enclosures. If it did make the wallabies in the present study anaemic, its increase in numbers and consequent effects on the wallabies must have been caused by the dietary regime imposed on them. If the parasite caused the anaemia of the experimental animals, wallabies short of nitrogen in the field and carrying a latent infection of the parasite could be expected to show the same changes in their blood parameters as the experimental wallabies.

Since the changes in the haematological measures of the 2 groups of wallabies generally followed the same trend throughout the study, there was no clear evidence from these measures that a restricted water intake and a low nitrogen diet affected

the wallabies differently from an ad lib. water intake and a low nitrogen diet. However, the pattern of nitrogen excretion in the 2 groups of wallabies was clearly different (Barker et al., 1970).

The experimental wallabies excreted less urea in their urine and had a higher plasma urea concentration than those given water ad lib. The experimental wallabies also excreted less nitrogen in their faeces than the controls. Although there was little difference between the urine volumes of the animals in the 2 groups, the experimental wallabies excreted less water in their faeces than the controls. Measured in terms of nitrogen and water excretion, a low nitrogen diet combined with a restricted water intake did have a different effect on the wallabies from a low nitrogen diet and an ad lib. water intake. If the experimental regime imposed had been more severe, the haematological parameters measured may also have revealed a clear difference between the 2 groups of wallabies.

4. Haematological measures in adult wallabies captured in the field at various times of the year

Section III, 1, showed that by the time the wallabies were 450 days old the levels of their blood parameters were similar to those found in healthy adult wallabies. The dentition of the youngest wallabies bled in the present study

indicated that they were at least 21 months old (unpublished data) and so all wallabies in the present study had adult values for their blood parameters.

The season of a year and the sex of a wallaby may both affect its blood parameters but as Section III, 2, shows, these 2 variables have an irregular effect on the blood parameters of healthy adult wallabies. The present study shows that although the levels of the different parameters were generally less in wallabies sampled in the field, the fluctuations in the parameters of these wallabies often paralleled those observed in healthy adult wallabies.

The patterns of change in the haematocrit, haemoglobin concentration and red blood cell count were similar in wallabies sampled in the field and in the laboratory enclosures. Females in the field and in the laboratory enclosures showed similar fluctuations in the concentration of total protein except in March 1969 when the total protein concentration in field animals was low, and in May 1969 when these animals had a high total protein concentration. The fluctuations in the absolute concentration of γ-globulin were also similar in females in the field and in the laboratory enclosures apart from January and March 1969 when animals in the laboratory enclosures had a comparatively higher γ-globulin concentration than animals sampled in the field.

Although the stress of suckling a new pouch young as well as a young-at-heel may have been partly responsible for the low haematocrit, haemoglobin concentration and red blood cell count of females sampled in the laboratory enclosures in March 1969, it cannot have been responsible for the low levels of these 3 parameters in females in the field in March 1969. Females in the field have a different pattern of lactation from those in the laboratory enclosures. While 70% of females in the laboratory enclosures were still suckling their 1968 young at the beginning of March 1969, none of the females sampled in the field at that time was still suckling a young-at-heel. Hence some factor other than the stress of heavy lactation caused the low haematocrit, haemoglobin concentration and red blood cell count of these females.

Females and males bled in the field in March 1969 had low concentrations of total serum protein and β-globulin and moderate relative and absolute concentrations of γ-globulin. The relative percentage of albumin in these animals was moderate - high, as was their plasma urea (S. Barker, pers. comm.), which makes it unlikely that they were protein depleted.

It is possible that these wallabies had a heavy infection of the eperythrozoon-like parasite found in the blood of male wallabies in the laboratory enclosures. A heavy infection of the parasite was proposed as a possible cause of the anaemia observed in male wallabies in the laboratory enclosures in

March 1969. If animals in the field and the laboratory had abnormally high numbers of the parasite in their blood at that time, they must have all been stressed in some way shortly before. The stress which may have acted on the wallabies is not known. The available evidence suggests that the wallabies in the field were not short of nitrogen or water in March 1969 or December 1968 and wallabies in the laboratory enclosures were never short of food or water.

Male and female wallabies sampled in the field in May 1968 had low relative and absolute concentrations of albumin and high relative and absolute concentrations of γ-globulin. The relative and absolute concentrations of γ-globulin were still high in July 1968 and the females still had low relative and absolute concentrations of albumin and the males had a low relative concentration of albumin. The high concentration of γ-globulin and the low concentration of albumin in these wallabies indicate that they had a chronic infection (Gross, Gitlin and Janeway, 1959; Osserman and Lawlor, 1961; Hoffman, 1964).

A die-off of wallabies in the study area was reported in July 1968. It is thought that disease by itself is rarely fatal to animals (Scrimshaw et al., 1959; Lecce, Matrone and Morgan, 1961). However if the animals in a population are stressed, e.g. if they are poorly nourished or have a superimposed infection, they are more susceptible to disease, which may then

kill a large proportion of the population.

The summer of 1967-68 was a particularly severe one and there was a high rainfall during the following winter. It is quite likely that this weather pattern stressed the wallabies. Wallabies captured in the field in February 1968 had high haematocrits, haemoglobin concentrations and red blood cell counts and these were accompanied by high concentrations of albumin and low concentrations of β- and γ-globulin. These wallabies were apparently not protein depleted and this conclusion is supported by the pattern of their nitrogen excretion (S. Barker, pers. comm.). However the urine volumes of these wallabies were low and so was their faecal water loss (S. Barker, pers. comm.) so it is likely that they were drinking little at that time. In May 1968 the wallabies had high urine volumes and a moderate faecal water loss (S. Barker, pers. comm.). It is thus unlikely that they were short of water. The concentration of urea in the urine of these wallabies was moderate and their plasma urea concentration was moderate - high. The nitrogen content of their faeces was low compared with that of wallabies sampled throughout the rest of the study period (S. Barker, pers. comm.). The wallabies sampled in May 1968 also had very low relative and absolute concentrations of albumin and so it is possible that they were short of nitrogen.

Hence a shortage of water in late summer and a shortage

of nitrogen in early winter, coupled with a latent infection of the eperythrozoon-like parasite, may have severely stressed the wallabies making them susceptible to other diseases during the winter.

5. Suggestions for further work

In the present study the wallabies in the field were apparently never drastically short of nitrogen or water. However, it seems that a moderate shortage of either nitrogen or water may stress the wallabies and render them more susceptible to infectious organisms, which may then kill a substantial proportion of the population. In view of this apparent importance of infective organisms in regulating the population numbers of the wallaby, future work should be directed along the following lines:

1. Documenting the parasites of the wallaby. So far 5 species of nematodes have been reported from the alimentary tract of the wallaby (Johnston and Mawson, 1940a, 1940b; Mawson, 1955). A systematic examination will have to be made of blood samples from a large number of male and female wallabies of different ages captured at different times of the year. Sections of the liver, lung, heart, brain, kidney and muscles of the wallaby will also need to be examined.

2. Recording the incidence of the different parasites in male and female wallabies of different ages captured throughout the year. This will show any seasonal fluctuations in the numbers of the parasites and the susceptibility of different age groups of males and females to infection with a particular parasite.

3. Finding out the conditions under which the various parasites adversely affect the wallabies. For instance a hot, dry summer followed by a very wet winter may lead to heavy parasitic infections in the wallabies. Adult wallabies may survive this infection, while it may kill yearlings.

4. Observing the pathogenicity of the different parasites in the wallabies and the effects of superimposed infections of the parasites on the wallabies.

5. Performing experiments to see if the wallabies are able to become immune to any of the parasites. If they can become immune tests will have to be carried out to see if the immunity is specific or non-specific.

6. Finding out the life cycle of the parasites and possible modes of their transmission. The wallaby tick Ixodes hirsti is common on the wallabies at certain times of the year and this may be a vector for some blood parasites. Mosquitoes and sandflies are 2 other possible vectors. An alternative mode

of transmission may be that the wallabies pass eggs or cysts of the parasite in their faeces and other wallabies could become infected by eating contaminated food.

V. APPENDICES

APPENDIX 1

Reagents for Electrophoresis

Veronal buffer pH 8.6 $\mu = 0.08$

(Modified from Wunderly, 1953; cited by Lederer, 1955).

Sodium barbitone 8.0 g

Sodium acetate (hydrated) 5.2 g

N/10 HCl 51.5 ml

Make volume up to 1 litre with deionized water

Amido Black dye solution

(Recipe from I.M.V.S., Adelaide.)

10 g of Amido Black (Merck) are dissolved in 2 litres of first wash. Stand overnight, then filter through double filter papers.

First wash solution

(Recipe from I.M.V.S., Adelaide.)

To make up 2 litres, 1,800 ml of methanol are mixed with 200 ml of glacial acetic acid.

Second wash solution

(Recipe from I.M.V.S., Adelaide.)

Mix: 1,080 ml methanol

120 ml glacial acetic acid

600 ml deionized water

18 ml 1N HCl

...../cont.

Oil for clearing papers

(Recipe from I.M.V.S., Adelaide.)

Mix: 99 ml liquid paraffin

48 ml 1 - bromo naphthalene

60 ml xylene

3 ml sorbitol mono-oleate (Span 80)

APPENDIX 2

Age Determination of Pouch Young and Juvenile Kangaroo Island Wallabies

Mrs. J. Smith of the Zoology Department, University of Adelaide, collaborated in this work.

The aim of this work was to provide a method for accurately aging young wallabies taken in the field.

This study presents age regressions for 3 characters of young Kangaroo Island wallabies reared in a domestic colony, and assesses the reliability of using these regressions in determining the age of young wallabies. The validity of applying the regressions to aging young Kangaroo Island wallabies in the field is also examined.

Method

Growth was studied on 16 young born in captivity. During the breeding season, the pouch of each female was examined daily until the birth of a young. The young were subsequently measured each week until they were about 1 year old. Yearlings were measured once a fortnight because of their slow growth rate.

The lengths of the head, left foot and left leg were measured (Appendix 2A), and some of the young were also weighed until they were about 1 year old. Vernier callipers were used to measure head and foot lengths of all the animals and the leg

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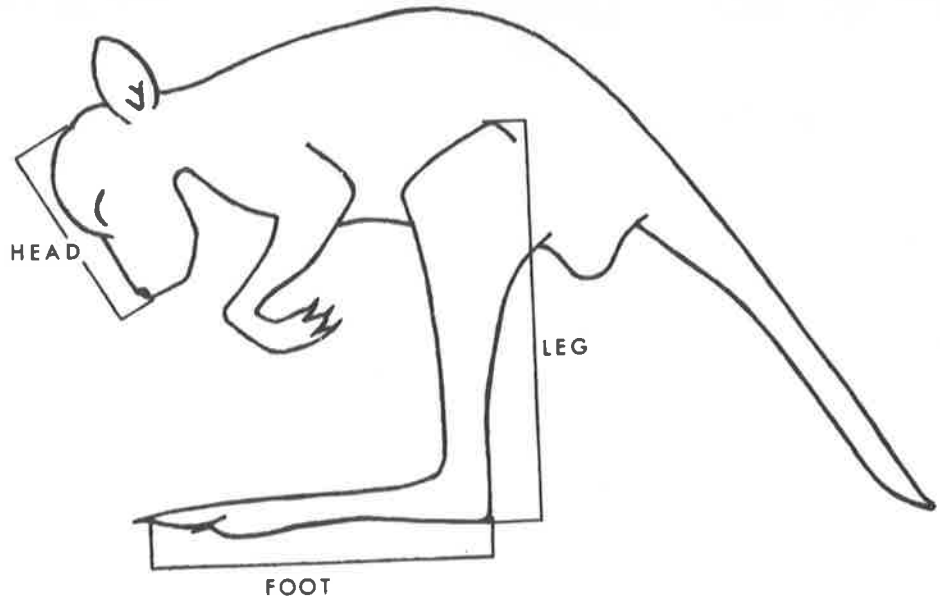
APPENDIX 2A.

Measurements taken on young Kangaroo Island wallabies. (Adapted from Sharman, Frith and Calaby, 1964).


HEAD
LEG
FOOT

length of pouch young, while a steel tape measure was used to measure leg lengths of older animals. The young were weighed on a variety of balances, since no 1 balance covered the range of their weights.

Until they were 100 days old, the young were removed from the pouch and measured while still attached to the teat. Older pouch young were detached from the teat and measured. During measurement of the young, the mothers were restrained in jute bags; no young were lost through the handling of the mothers (Shield and Woolley, 1961). Juveniles and yearlings were restrained in jute sacks while their body parts were being measured.

Both operators measured the young every time and the average of the 2 estimates was taken to the nearest 0.1 mm with the callipers and to the nearest 0.5 mm with the tape measure.

Results

The measurements made on young aged 3 - 450 days are presented as regressions of age versus head length, leg length and foot length (Appendices 2B - 2D).

The regressions show no marked differences in the growth rate of young male and female wallabies up until the time they leave the pouch permanently (at 245 - 270 days of age). From this time the regressions for male and female young begin to

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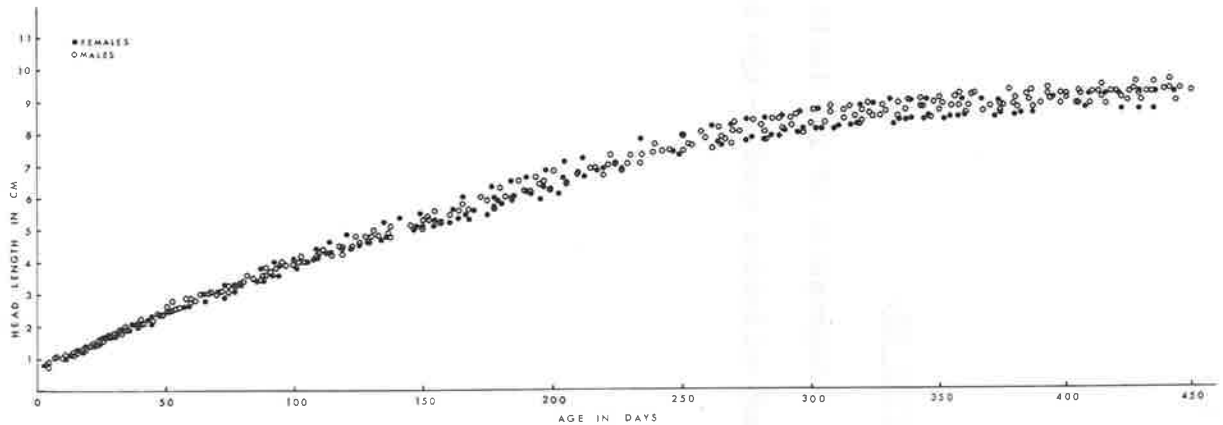
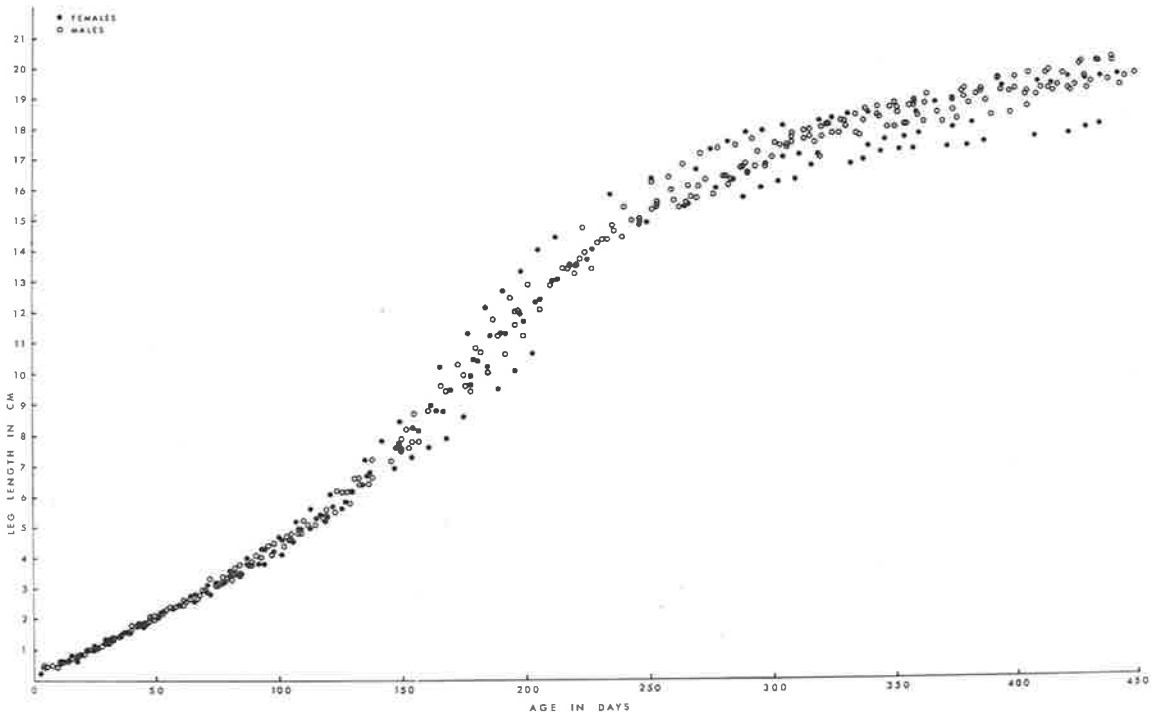
APPENDIX 2B.

Regression of head length on age for Kangaroo Island wallabies aged 3-450 days.


● FEMALES
○ MALES
HEAD LENGTH IN C.M
1
2
3
4
5
6
7
8
9
10
11
0
50
100
150
200
250
300
350
400
450
AGE IN DAYS

APPENDIX 2C.

Regression of leg lenth on age for Kangaroo Island wallabies aged 3-450 days.


● FEMALES
○ MALES
LEG LENGTH IN CM
AGE IN DAYS
0
50
100
150
200
250
300
350
400
450
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

diverge, the males being on average larger than the females.

Inspection of the 3 regressions shows a considerable scatter of points, much of which is due to the difficulty in making accurate measurements on the young. An age estimation based on a single body measurement could therefore be subject to a greater error than an estimation based on all 3 body measurements.

A series of measurements were made on 14 young of known age which were not included in the regressions. Appendix 2E shows the measurements of these young, together with their ages as estimated from the 3 regressions, and their actual ages.

It can be seen that the largest actual error in age estimations for any of the young was 9 days when the estimate was made from all the measurements (young No. 94♀, Appendix 2E). This represents an error of about 5%.

Appendices 2B - 2D show that size can be used for age determination of young aged less than 320 days, but thereafter it has little value for age determination. The leg has the longest period of rapid growth and it appears that in using leg length as a criterion for age, if males and females are considered separately, an error of between 20 and 45 days might occur when aging young 320 days old (Appendix 2C).

The data so far presented deal only with the growth rates of young wallabies born and reared in captivity.

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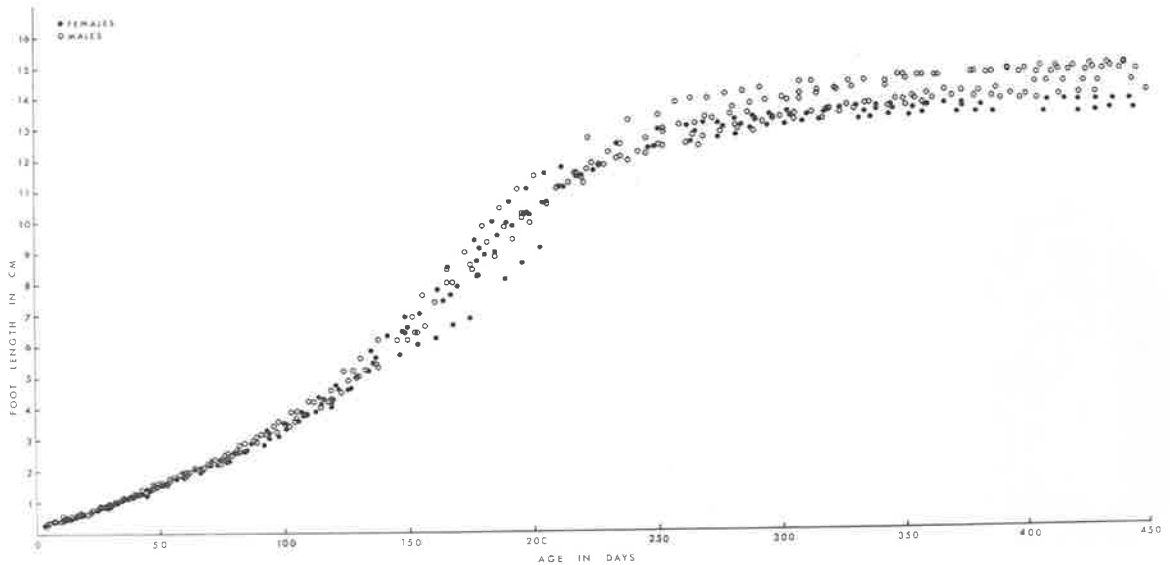
APPENDIX 2D.

Regression of foot length on age for Kangaroo Island wallabies aged 3-450 days.


● FEMALES
○ MALES
FOOT LENGTH IN CM
AGE IN DAYS
0
50
100
150
200
250
300
350
400
450

APPENDIX 2E

Measurements of young Kangaroo Island wallabies of known age, not included in the growth regressions, and the ages of these young estimated from the regressions.

Reference Number and Sex	Lengths (cm)*			Estimated Age (Days)+	Actual Age (Days)
	Head	Leg	Foot		
74(?)	0.87 (4)	0.41 (5)	0.33 (5)	5	3
1 ♂	1.12 (12)	0.61 (12)	0.41 (8)	11	10
2 ♀	1.22 (15)	0.69 (14)	0.55 (14)	14	16
3 ♂(?)	1.29 (17)	0.78 (17)	-	17	17
14 ♂	1.67 (27)	1.15 (26)	0.84 (27)	27	26
67 ♀	2.00 (36)	1.42 (34)	1.08 (35)	35	36
595 ♂	2.78 (58)	2.45 (57)	1.84 (59)	58	59
14P ♂	3.77 (92)	4.03 (91)	3.18 (95)	93	94
84 ♀	4.35 (115)	5.31 (116)	4.15 (114)	115	111
77 ♀	4.42 (118)	5.50 (120)	4.34 (118)	119	123
594 ♀	4.50 (121)	5.83 (124)	4.90 (126)	124	129
76 ♀	5.45 (162)	8.74 (164)	7.55 (166)	164	163
94 ♀	5.79 (174)	9.76 (176)	8.47 (177)	176	185
SE ♂	6.45 (199)	11.80 (197)	10.74 (203)	200	197

* Estimated ages from each measurement are in parentheses

\+ Based on average of ages estimated from each measurement

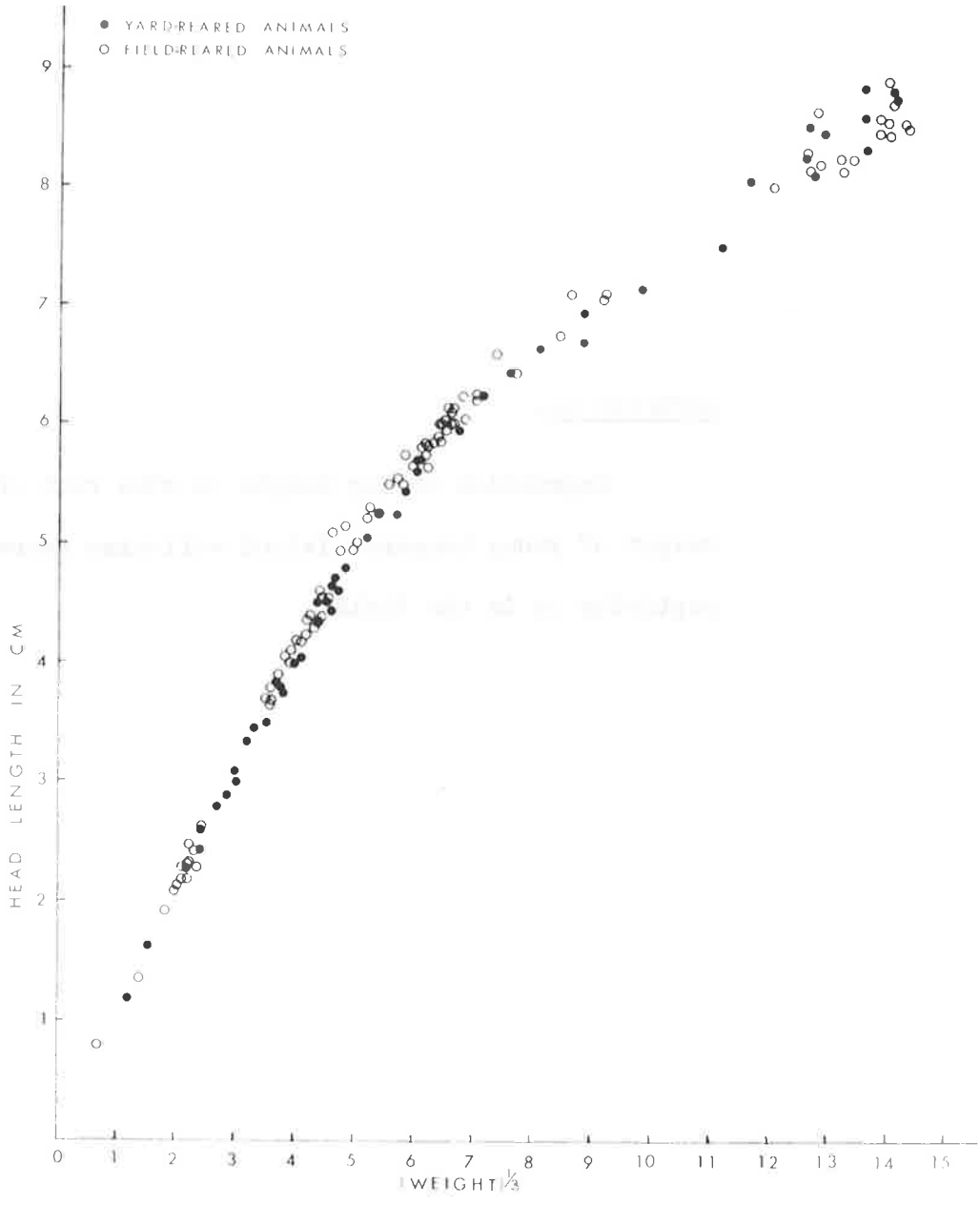
The growth rate of young in the field is extremely difficult to estimate (Shield and Woolley, 1961). Shield and Woolley (1961) found that growth proportions of compound- and field-reared quokkas did not differ significantly, and so considered that the growth rates of field and captive animals were probably also similar.

Young wallabies collected at different times of the year in Flinders Chase, Kangaroo Island have been measured and weighed by the writer in the same way as the laboratory-reared animals. The regressions of cube root of weight (condition) versus the lengths of the foot, leg and head (age) have been drawn for laboratory- and field-reared animals (Appendices 2F - 2H). These regressions show no marked differences in the growth proportions of laboratory- and field-reared young up to an age of about 350 days.

It is therefore considered likely that growth rates of captive and field animals are also similar during this time, and hence that age estimation procedures established on laboratory-reared animals are applicable to field animals until they are about 350 days old.

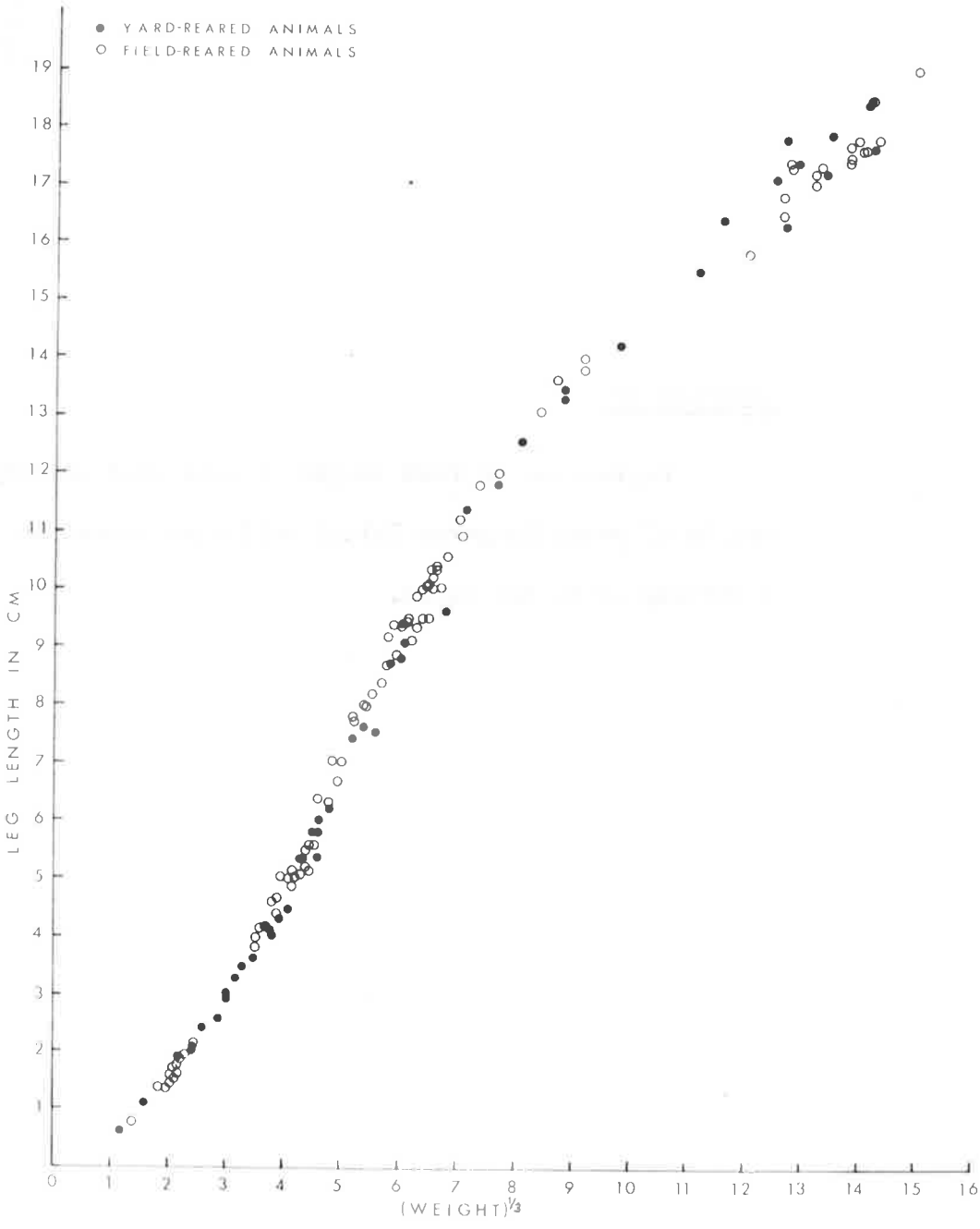
APPENDIX 2F.

Regression of head length on cube root of body weight of young Kangaroo Island wallabies reared in captivity or in the field.


● YARD-REARED ANIMALS
○ FIELD-REARED ANIMALS
HEAD LENGTH IN CM
WEIGHT 1/3
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

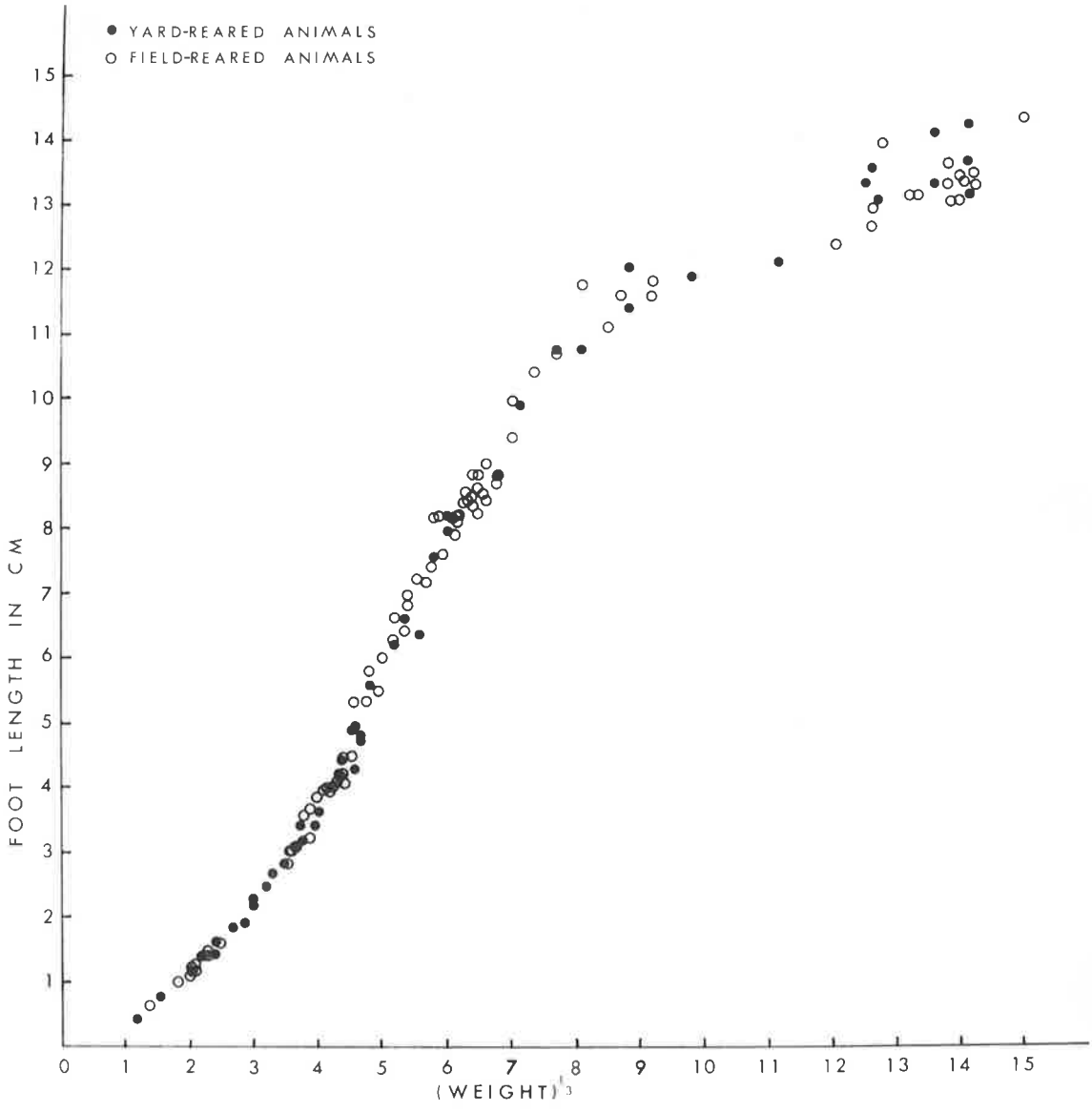
APPENDIX 2G.

Regression of leg length on cube root of body weight of young Kangaroo Island wallabies reared in captivity or in the field.


YARD-REARED ANIMALS
FIELD-REARED ANIMALS
LEG LENGTH IN CM
(WEIGHT)$^{1/3}$
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

APPENDIX 2H.

Regression of foot length on cube root of body weight of young Kangaroo Island wallabies reared in captivity or in the field.


● YARD-REARED ANIMALS
○ FIELD-REARED ANIMALS
FOOT LENGTH IN CM
(WEIGHT)$^{1/3}$
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

APPENDIX 3

Analyses of variance[ø] between healthy adult male and female wallabies sampled at different times of the year for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, absolute concentrations of albumin, α -, β - and γ -globulin, and relative percentages of albumin, α -, β - and γ -globulin. Where the F value is significant, the 8 sample means and sizes for each sex and the Least Significant Difference at the 5% level ($L.S.D._{5\%}$) are given for that variate.

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Body Weight	Sex	7	10.3012	1.4716	3.670
	Residual	75	30.0770	.4010	**

Sampling period	1	2	3	4	5	6	7	8
Mean (♂)	-	6.30	6.28	7.12	7.28	6.26	6.32	6.38
n (♂)	-	6	5	6	6	7	7	6
Mean (♀)	-	5.86	5.77	6.22	6.33	5.75	5.65	5.75
n (♀)	-	6	6	7	6	7	7	7

For sample sizes $n_1 = 6$, $n_2 = 5$ $L.S.D._{5\%} = .76$; for $n_1 = 6$, $n_2 = 6$ $L.S.D._{5\%} = .73$; for $n_1 = 6$, $n_2 = 7$ $L.S.D._{5\%} = .70$; for $n_1 = 7$, $n_2 = 7$ $L.S.D._{5\%} = .67$.

Haematocrit	Sex	8	220.112	27.514	4.128
	Residual	85	566.534	6.665	***

Sampling period	1	2	3	4	5	6	7	8
Mean (♂)	52.73	54.17	48.72	51.47	50.60	48.11	49.33	49.55
n (♂)	6	6	5	6	6	7	7	6
Mean (♀)	50.82	50.85	48.20	46.01	49.33	45.71	46.76	46.44
n (♀)	6	6	6	7	6	7	7	7

For $n_1 = 6$, $n_2 = 5$ $L.S.D._{5\%} = 3.11$; for $n_1 = 6$, $n_2 = 6$ $L.S.D._{5\%} = 2.97$; for $n_1 = 6$, $n_2 = 7$ $L.S.D._{5\%} = 2.86$; for $n_1 = 7$, $n_2 = 7$ $L.S.D._{5\%} = 2.74$

...../cont.

APPENDIX 3 (cont.)

Variate	Source of Variation	Analysis of Variance DF	SS	MS	F
Haemoglobin concentration	Sex	8	32.476	4.0595	4.614
	Residual	85	74.789	.8799	***

Sampling period	1	2	3	4	5	6	7	8
Mean (♂)	19.60	20.28	18.44	19.12	18.60	17.39	18.11	18.47
n (♂)	6	6	5	6	6	7	7	6
Mean (♀)	19.02	18.52	18.07	17.39	17.98	16.46	17.26	17.19
n (♀)	6	6	6	7	6	7	7	7

For $n_1 = 6$, $n_2 = 5$ L.S.D.$_{5\%}$ = 1.13; for $n_1 = 6$, $n_2 = 6$ L.S.D.$_{5\%}$ = 1.08; for $n_1 = 6$, $n_2 = 7$ L.S.D.$_{5\%}$ = 1.04; for $n_1 = 7$, $n_2 = 7$ L.S.D.$_{5\%}$ = 1.00

Red blood cell count	Sex	8	9.784	1.223	4.531
	Residual	85	22.943	.270	***

Sampling period	1	2	3	4	5	6	7	8
Mean (♂)	7.24	7.01	6.48	6.73	6.85	6.49	6.29	6.66
n (♂)	6	6	5	6	6	7	7	6
Mean (♀)	6.59	6.29	6.39	5.85	6.62	5.77	6.09	5.81
n (♀)	6	6	6	7	6	7	7	7

For $n_1 = 6$, $n_2 = 5$ L.S.D.$_{5\%}$ = .63; for $n_1 = 6$, $n_2 = 6$ L.S.D.$_{5\%}$ = .60; for $n_1 = 6$, $n_2 = 7$ L.S.D.$_{5\%}$ = .58; for $n_1 = 7$, $n_2 = 7$ L.S.D.$_{5\%}$ = .55

Mean cell volume	Sex	8	322.501	40.3127	1.456
	Residual	85	2354.22	27.6967	n.s.
Mean cell haemoglobin concentration	Sex	8	5.7455	.71819	.6372
	Residual	85	95.8023	1.12709	n.s.

...../cont.

APPENDIX 3 (cont.)

Variate	Source of Variation	Analysis of Variance DF	SS	MS	F
Total protein concentration	Sex	8	.5217	.06521	.2669
	Residual	85	20.768	.24433	n.s.
Absolute concentration of albumin	Sex	8	.4879	.06099	.5926
	Residual	85	8.7481	.10292	n.s.
Absolute concentration of α-globulin	Sex	8	.05054	.00632	.9503
	Residual	85	.56513	.00649	n.s.
Absolute concentration of β-globulin	Sex	8	.37228	.04654	1.138
	Residual	85	3.4750	.04088	n.s.
Absolute concentration of γ-globulin	Sex	8	.70668	.08834	.8202
	Residual	85	9.1545	.10770	n.s.
Relative percentage of albumin	Sex	8	80.4333	10.0542	.4818
	Residual	85	1773.90	20.8694	n.s.
Relative percentage of α-globulin	Sex	8	9.6057	1.2007	.8784
	Residual	85	116.186	1.3669	n.s.

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APPENDIX 3 (cont.)

Variate	Source of Variation	DF	Analysis of Variance SS	MS	F
Relative percentage of β-globulin	Sex	8	99.3430	12.4179	2.205
	Residual	85	478.798	5.6329	*

Sampling period	1	2	3	4	5	6	7	8
Mean (♂)	23.70	19.80	20.58	21.00	19.62	20.11	20.53	19.38
n (♂)	6	6	5	6	6	7	7	6
Mean (♀)	21.18	20.17	18.63	17.73	17.65	18.17	18.83	19.56
n (♀)	6	6	6	7	6	7	7	7

For $n_1 = 6$, $n_2 = 5$ L.S.D.$_{5\%} = 2.86$; for $n_1 = 6$, $n_2 = 6$ L.S.D.$_{5\%} = 2.73$; for $n_1 = 6$, $n_2 = 7$ L.S.D.$_{5\%} = 2.63$; for $n_1 = 7$, $n_2 = 7$ L.S.D.$_{5\%} = 2.52$

Relative percentage of γ-globulin	Sex	8	121.466	15.1832	1.002
	Residual	85	1287.43	15.1462	n.s.

ø The following convention has been followed in presenting results of statistical analyses, regarding levels of significance.

n.s. means not significant; * means significant at 5% level; ** means significant at 1% level; *** means significant at 0.1% level.

APPENDIX 4

Analyses of variance between healthy, adult male wallabies sampled at different times of the year for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, absolute concentrations of albumin, α-, β- and γ-globulin and relative percentages of albumin, α-, β- and γ-globulin. Where the F value is significant, the 8 sample means and sizes and the L.S.D.5% are given for that variate.

Variate	Source of Variation	Analysis of Variance DF	SS	MS	F
Body Weight	Period	6	7.01169	1.16861	6.491
	Residual	36	6.48174	.18005	***

	Sampling period	1	2	3	4	5	6	7	8
	Mean	-	6.30	6.28	7.12	7.28	6.26	6.32	6.38
	n	-	6	5	6	6	7	7	6

For $n_1 = 5$, $n_2 = 6$ L.S.D.5% = .52; for $n_1 = 5$, $n_2 = 7$ L.S.D.5% = .50; for $n_1 = 6$, $n_2 = 6$ L.S.D.5% = .50; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = .48; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = .46

Haematocrit	Period	7	186.856	26.6937	3.061 *
	Residual	41	357.526	8.7201	

	Sampling period	1	2	3	4	5	6	7	8
	Mean	52.73	54.17	48.72	51.47	50.60	48.11	49.33	49.55
	n	6	6	5	6	6	7	7	6

For $n_1 = 6$, $n_2 = 5$ L.S.D.5% = 3.61; for $n_1 = 7$, $n_2 = 5$ L.S.D.5% = 2.99; for $n_1 = 6$, $n_2 = 6$ L.S.D.5% = 3.45; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = 3.32; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = 3.19

...../cont.

APPENDIX 4 (cont.)

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Haemoglobin concentration	Period	7	36.1478	5.1640	5.898
	Residual	41	35.8991	.8756	***
	Sampling period	1	2	3	4	5	6	7	8
	Mean	19.60	20.28	18.44	19.12	18.60	17.39	18.11	18.47
	n	6	6	5	6	6	7	7	6
	For $n_1 = 5$, $n_2 = 6$ L.S.D.5% = 1.15; for $n_1 = 5$, $n_2 = 7$ L.S.D.5% = 1.11; for $n_1 = 6$, $n_2 = 6$ L.S.D.5% = 1.09; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = 1.05; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = 1.01								
Red blood cell count	Period	7	4.23996	.60571	1.913
	Residual	41	12.9829	.31666	n.s.
Mean cell volume	Period	7	145.305	20.7578	.740
	Residual	41	1150.03	28.0496	n.s.
Mean cell haemoglobin concentration	Period	7	12.8521	1.8360	2.306
	Residual	41	32.6443	.7962	*
	Sampling period	1	2	3	4	5	6	7	8
	Mean	37.17	37.46	37.95	37.19	36.76	36.11	36.75	37.28
	n	6	6	5	6	6	7	7	6
	For $n_1 = 6$, $n_2 = 5$, L.S.D.5% = 1.09; for $n_1 = 5$, $n_2 = 7$ L.S.D.5% = 1.06; for $n_1 = 6$, $n_2 = 6$ L.S.D.5% = 1.04; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = 1.00; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = .96								

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APPENDIX 4 (cont.)

Variate	Source of Variation	DF	Analysis of Variance SS	MS	F
Total protein concentration	Period	7	4.4372	.6339	1.696
	Residual	41	15.3277	.3738	n.s.
Absolute concentration of albumin	Period	7	1.2144	.1735	1.646
	Residual	41	4.3213	.1054	n.s.
Absolute concentration of α-globulin	Period	7	.04978	.007111	.970
	Residual	41	.30059	.007331	n.s.
Absolute concentration of β-globulin	Period	7	.38449	.05493	1.168
	Residual	41	1.92845	.04704	n.s.
Absolute concentration of γ-globulin	Period	7	1.6480	.23542	2.173
	Residual	41	4.4424	.10835	n.s.
Relative percentage of albumin	Period	7	133.826	19.1179	.849
	Residual	41	923.205	22.5172	n.s.
Relative percentage of α-globulin	Period	7	12.4947	1.7850	1.290
	Residual	41	56.7334	1.3837	n.s.

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APPENDIX 4 (cont.)

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Relative percentage of β-globulin	Period	7	78.8121	11.2589	2.734
	Residual	41	168.848	4.11823	*
	Sampling period	1	2	3	4	5	6	7	8
	Mean	23.70	19.80	20.58	21.00	19.62	20.11	20.53	19.38
	n	6	6	5	6	6	7	7	6
	For n_1 = 5, n_2 = 6 L.S.D.5% = 2.48; for n_1 = 5, n_2 = 7 L.S.D.5% = 2.40; for n_1 = 6, n_2 = 6 L.S.D.5% = 2.37; for n_1 = 6, n_2 = 7 L.S.D.5% = 2.28; for n_1 = 7, n_2 = 7 L.S.D.5% = 2.19								
Relative percentage of γ-globulin	Period	7	194.052	27.7217	2.172
	Residual	41	523.270	12.7627	n.s.

APPENDIX 5

Analyses of variance between healthy, adult female wallabies sampled at different times of the year for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, absolute concentrations of albumin, α-, β- and γ-globulin and relative percentages of albumin, α-, β- and γ-globulin. Where the F value is significant, the 8 sample means and sizes and the L.S.D.5% are given for that variate.

Variate	Source of Variation	DF	SS	MS	F
		Analysis of Variance			
Body Weight	Period	6	2.71682	.4528	.748
	Residual	39	23.5952	.6050	n.s.
Haematocrit	Period	7	198.439	28.3484	5.968
	Residual	44	209.008	4.7502	***
	Sampling period	1	2	3	4	5	6	7	8
	Mean	50.82	50.85	48.20	46.01	49.33	45.71	46.76	46.44
	n	6	6	6	7	6	7	7	7
	For $n_1 = 6$, $n_2 = 6$ L.S.D.5% = 2.54; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = 2.45; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = 2.35								
Haemoglobin concentration	Period	7	30.4047	4.3435	4.914
	Residual	44	38.8898	.8839	***
	Sampling period	1	2	3	4	5	6	7	8
	Mean	19.02	18.52	18.07	17.39	17.98	16.46	17.26	17.19
	n	6	6	6	7	6	7	7	7
	For $n_1 = 6$, $n_2 = 6$ L.S.D.5% = 1.10; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = 1.06; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = 1.02								

...../cont.

APPENDIX 5 (cont.)

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Red blood cell count	Period	7	5.4656	.7808	3.449
	Residual	44	9.9603	.2264	**
	Sampling period	1	2	3	4	5	6	7	8
	Mean	6.59	6.29	6.39	5.85	6.62	5.77	6.09	5.81
	n	6	6	6	7	6	7	7	7
	For $n_1 = 6$, $n_2 = 6$ L.S.D.5% = .56; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = .54; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = .51								
Mean cell volume	Period	7	207.119	29.589	1.081
	Residual	44	1204.19	27.368	n.s.
Mean cell haemoglobin concentration	Period	7	17.1949	2.4564	1.711
	Residual	44	63.1580	1.4354	n.s.
Total protein concentration	Period	7	4.27824	.61118	4.943
	Residual	44	5.44065	.12365	***
	Sampling period	1	2	3	4	5	6	7	8
	Mean	6.57	7.02	6.70	6.56	6.98	7.04	6.38	6.24
	n	6	6	6	7	6	7	7	7
	For $n_1 = 6$, $n_2 = 6$, L.S.D.5% = .41; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = .40; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = .38								
Absolute concentration of albumin	Period	7	.62535	.08934	.888
	Residual	44	4.42678	.10061	n.s.

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APPENDIX 5 (cont.)

Variate	Source of Variation	DF	Analysis of Variance SS	MS	F
Absolute concentration of α-globulin	Period	7	.05713	.008162	1.358
	Residual	44	.26454	.006012	n.s.
Absolute concentration of β-globulin	Period	7	.35080	.050115	1.426
	Residual	44	1.54654	.035149	n.s.
Absolute concentration of γ-globulin	Period	7	2.10223	.30032	2.804
	Residual	44	4.71204	.10709	*

Sampling period	1	2	3	4	5	6	7	8
Mean	1.30	1.62	1.44	1.35	1.72	1.69	1.17	1.23
n	6	6	6	7	6	7	7	7

For $n_1 = 6$, $n_2 = 6$ L.S.D.5% = .38; for $n_1 = 6$, $n_2 = 7$ L.S.D.5% = .37; for $n_1 = 7$, $n_2 = 7$ L.S.D.5% = .35

Relative percentage of albumin	Period	7	188.400	26.9143	1.392
	Residual	44	850.695	19 3340	n.s.
Relative percentage of α-globulin	Period	7	17.1216	2.4459	1.810
	Residual	44	59.4529	1.3512	n.s
Relative percentage of β-globulin	Period	7	66.9169	9.5596	1.357
	Residual	44	309.950	7.0443	n.s.
Relative percentage of γ-globulin	Period	7	228.574	32.6535	1.880
	Residual	44	764.160	17.3673	n.s.

APPENDIX 6

Analyses of variance between the 3 sampling periods for control (water ad lib.) wallabies in Section III, 3 for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, the absolute concentrations of albumin, α-, β- and γ-globulin, and the relative percentages of albumin, α-, β- and γ-globulin. Where the F value is significant, the sample means and the L.S.D.5% are given for that variate.

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Body weight	Period	2	2.4076	1.2038	2.571
	Residual	11	5.1515	.4683	n.s.
Haematocrit	Period	2	98.384	49.192	3.202
	Residual	11	168.971	15.361	n.s.
Haemoglobin concentration	Period	2	9.689	4.844	2.497
	Residual	11	21.343	1.940	n.s.
Red blood cell count	Period	2	.6885	.3443	.737
	Residual	11	5.1376	.4671	n.s.
Mean cell volume	Period	2	23.252	11.626	.364
	Residual	11	351.093	31.918	n.s.

...../cont.

APPENDIX 6 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Mean cell haemoglobin concentration	Period	2	1.7840	.8920	1.783
	Residual	11	5.5049	.5004	n.s.
Total protein concentration	Period	2	1.6551	.8276	3.096
	Residual	11	2.9400	.2673	n.s.
Absolute concentration of albumin	Period	2	.8572	.4286	5.139
	Residual	11	.9171	.0834	*
	Sampling Period		1	2	3
	Mean		3.55	3.26	2.96
	For $n_1 = 4$, $n_2 = 6$ L.S.D.5% = .41; for $n_1 = 4$, $n_2 = 4$ L.S.D.5% = .45.				
Absolute concentration of α-globulin	Period	2	.0157	.0079	1.197
	Residual	11	.0722	.0066	n.s.
Absolute concentration of β-globulin	Period	2	.0072	.0036	.050
	Residual	11	.7893	.0718	n.s.
Absolute concentration of γ-globulin	Period	2	.2092	.1046	1.177
	Residual	11	.9778	.0889	n.s.

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APPENDIX 6 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Relative percentage of albumin	Period	2	25.3488	12.674	.751
	Residual	11	185.5833	16.871	n.s.
Relative percentage of α-globulin	Period	2	1.459	.729	.552
	Residual	11	14.536	1.321	n.s.
Relative percentage of β-globulin	Period	2	9.709	4.854	.393
	Residual	11	136.048	12.368	n.s.
Relative percentage of γ-globulin	Period	2	30.440	15.220	1.145
	Residual	11	146.163	13.288	n.s.

APPENDIX 7

Analyses of variance between the 3 sampling periods for experimental (water-restricted) wallabies in Section III, 3 for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, the absolute concentrations of albumin, α-, β - and γ-globulin, and the relative percentages of albumin, α -, β - and γ -globulin. Where the F value is significant, the sample means and the L.S.D.$_{5\%}$ are given for that variate.

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Body weight	Period	2	4.3045	2.1523	25.68
	Residual	15	1.2577	.0838	***
	Sampling Period		1	2	3
	Mean		6.23	5.46	5.05
	L.S.D.$_{5\%}$ = 0.36				
Haematocrit	Period	2	145.990	72.995	7.700
	Residual	15	142.195	9.480	**
	Sampling Period		1	2	3
	Mean		50.07	48.57	43.42
	L.S.D.$_{5\%}$ = 3.79				
Haemoglobin concentration	Period	2	9.205	4.602	3.303
	Residual	15	20.900	1.393	n.s.

...../cont.

APPENDIX 7 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Red blood cell count	Period	2	1.1269	.5635	2.111
	Residual	15	4.0035	.2669	n.s.
Mean cell volume	Period	2	39.091	19.546	1.004
	Residual	15	294.364	19.624	n.s.
Mean cell haemoglobin concentration	Period	2	14.653	7.3266	8.097
	Residual	15	13.572	.9048	**
	Sampling Period		1	2	3
	Mean		36.83	38.01	39.04
	$L.S.D._{5\%} = 1.17$				
Total protein concentration	Period	2	.9615	.4808	1.832
	Residual	15	3.9365	.2624	n.s.
Absolute concentration of albumin	Period	2	.4899	.2450	3.066
	Residual	15	1.1978	.0799	n.s.
Absolute concentration of α-globulin	Period	2	.0965	.0483	3.308
	Residual	15	.2183	.0146	n.s.

...../cont.

APPENDIX 7 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Absolute concentration of β-globulin	Period	2	.1826	.0913	2.249
	Residual	15	.6093	.0406	n.s.
Absolute concentration of γ-globulin	Period	2	.1949	.0975	.775
	Residual	15	1.8875	.1258	n.s.
Relative percentage of albumin	Period	2	79.055	39.527	2.376
	Residual	15	249.582	16.639	n.s.
Relative percentage of α-globulin	Period	2	13.968	6.984	3.105
	Residual	15	33.735	2.249	n.s.
Relative percentage of β-globulin	Period	2	12.1811	6.091	1.502
	Residual	15	60.8283	4.055	n.s.
Relative percentage of γ-globulin	Period	2	61.631	30.816	1.624
	Residual	15	284.707	18.980	n.s.

APPENDIX 8

Analyses of variance between the control (water ad lib.) and experimental (water-restricted) groups of wallabies at the first sampling period in Section III, 3 for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, the absolute concentrations of albumin, α-, β - and γ-globulin, and the relative percentages of albumin, α -, β - and γ - globulin.

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Body weight	Treatment	1	.003	.003	.010
	Residual	10	2.915	.292	n.s.
Haematocrit	Treatment	1	11.21	11.21	.930
	Residual	10	120.53	12.05	n.s.
Haemoglobin concentration	Treatment	1	.907	.907	.607
	Residual	10	14.942	1.494	n.s.
Red blood cell count	Treatment	1	.2241	.2241	1.069
	Residual	10	2.0971	.2097	n.s.
Mean cell volume	Treatment	1	.340	.340	.025
	Residual	10	136.743	13.674	n.s.

...../cont.

APPENDIX 8 (cont.)

Variate	Analysis of Variance				
	Source of variation	DF	SS	MS	F
Mean cell haemoglobin concentration	Treatment	1	.375	.375	.331
	Residual	10	11.332	1.133	n.s.
Total protein concentration	Treatment	1	.3960	.3960	1.128
	Residual	10	3.5109	.3511	n.s.
Absolute concentration of albumin	Treatment	1	.4447	.4447	3.027
	Residual	10	1.4692	.1469	n.s.
Absolute concentration of α-globulin	Treatment	1	.001008	.001008	.169
	Residual	10	.059617	.005962	n.s.
Absolute concentration of β-globulin	Treatment	1	.00067	.00067	.012
	Residual	10	.55155	.05516	n.s.
Absolute concentration of γ-globulin	Treatment	1	.0030	.0030	.027
	Residual	10	1.0959	.1096	n.s.
Relative percentage of albumin	Treatment	1	27.30	27.30	1.459
	Residual	10	187.18	18.72	n.s.

...../cont.

APPENDIX 8 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Relative percentage of α-globulin	Treatment	1	.008	.008	.007
	Residual	10	10.222	1.022	n.s.
Relative percentage of β-globulin	Treatment	1	4.813	4.813	.595
	Residual	10	80.873	8.087	n.s.
Relative percentage of γ-globulin	Treatment	1	8.50	8.50	.572
	Residual	10	148.64	14.86	n.s.

APPENDIX 9

Analyses of variance between the control (water ad lib.) and experimental (water-restricted) groups of wallabies at the second sampling period in Section III, 3 for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, the absolute concentrations of albumin, α-, β - and γ-globulin, and the relative percentages of albumin, α-, β - and γ-globulin.

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Body weight	Treatment	1	.3193	.3193	1.0758
	Residual	8	2.3743	.2968	n.s.
Haematocrit	Treatment	1	34.969	34.969	3.696
	Residual	8	75.683	9.460	n.s.
Haemoglobin concentration	Treatment	1	6.136	6.136	4.145
	Residual	8	11.841	1.480	n.s.
Red blood cell count	Treatment	1	.5759	.5759	3.052
	Residual	8	1.5095	.1887	n.s.
Mean cell volume	Treatment	1	.276	.276	.010
	Residual	8	218.016	27.252	n.s.

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APPENDIX 9 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Mean cell haemoglobin concentration	Treatment	1	.1685	.1685	.399
	Residual	8	3.3781	.4223	n.s.
Total protein concentration	Treatment	1	1.2732	1.2732	4.869
	Residual	8	2.0918	.2615	n.s.
Absolute concentration of albumin	Treatment	1	.44710	.44710	15.234
	Residual	8	.23478	.02935	**
Absolute concentration of α-globulin	Treatment	1	.04613	.04613	1.763
	Residual	8	.20935	.02617	n.s.
Absolute concentration of β-globulin	Treatment	1	.07093	.07093	1.905
	Residual	8	.29790	.03724	n.s.
Absolute concentration of γ-globulin	Treatment	1	.0006	.0006	.005
	Residual	8	1.0124	.1265	n.s.
Relative percentage of albumin	Treatment	1	1.26	1.26	.068
	Residual	8	147.53	18.44	n.s.

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APPENDIX 9 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Relative percentage of α-globulin	Treatment	1	5.649	5.649	1.420
	Residual	8	31.829	3.979	n.s.
Relative percentage of β-globulin	Treatment	1	.140	.140	.036
	Residual	8	30.808	3.851	n.s.
Relative percentage of γ-globulin	Treatment	1	16.19	16.19	1.041
	Residual	8	124.36	15.55	n.s.

APPENDIX 10

Analyses of variance between the control (water ad lib.) and experimental (water-restricted) groups of wallabies at the third sampling period in Section III, 3 for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, the absolute concentrations of albumin, α-, β - and γ-globulin, and the relative percentages of albumin, α -, β - and γ - globulin.

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Body weight	Treatment	1	.1065	.1065	.402
	Residual	8	2.1202	.2650	n.s.
Haematocrit	Treatment	1	8.20	8.20	.571
	Residual	8	114.96	14.37	n.s.
Haemoglobin concentration	Treatment	1	3.307	3.307	1.711
	Residual	8	15.461	1.933	n.s.
Red blood cell count	Treatment	1	.1654	.1654	.239
	Residual	8	5.5347	.6918	n.s.
Mean cell volume	Treatment	1	1.546	1.546	.043
	Residual	8	290.699	36.337	n.s.

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APPENDIX 10 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Mean cell haemoglobin concentration	Treatment	1	2.5502	2.5502	7.124
	Residual	8	2.8636	.3580	*
Total protein concentration	Treatment	1	1.7424	1.7424	11.027
	Residual	8	1.2641	.1580	*
Absolute concentration of albumin	Treatment	1	1.04980	1.04980	20.440
	Residual	8	.41088	.05136	**
Absolute concentration of α-globulin	Treatment	1	.000094	.000094	.035
	Residual	8	.021608	.002701	n.s.
Absolute concentration of β-globulin	Treatment	1	.03051	.03051	.444
	Residual	8	.54923	.06865	n.s.
Absolute concentration of γ-globulin	Treatment	1	.23145	.23145	2.445
	Residual	8	.75726	.09466	n.s.
Relative percentage of albumin	Treatment	1	28.52	28.52	2.271
	Residual	8	100.46	12.56	n.s.

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APPENDIX 10 (cont.)

Variate	Analysis of variance				
	Source of variation	DF	SS	MS	F
Relative percentage of α-globulin	Treatment	1	.8662	.8662	1.114
	Residual	8	6.2209	.7776	n.s.
Relative percentage of β-globulin	Treatment	1	40.598	40.598	3.812
	Residual	8	85.198	10.650	n.s.
Relative percentage of γ-globulin	Treatment	1	4.43	4.43	.224
	Residual	8	157.87	19.73	n.s.

APPENDIX 11

Analyses of variance between male and female wallabies over the 7 field trips for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, absolute concentrations of albumin, α-, β- and γ-globulin and relative percentages of albumin, α-, β- and γ-globulin. Where the F value is significant the 7 means for each sex and the L.S.D.$_{5\%}$ are given for that variate.

Variate	Source of Variation	DF	Analysis of Variance SS	MS	F
Body Weight	Sex	1	168.87	168.87	342.341
	Residual	272	134.17	.49	***
	Trip No.	1	2	3	4	5	6	7
	Mean (♂)	6.78	6.94	6.55	7.18	7.02	6.70	6.73
	Mean (♀)	5.21	5.05	4.91	5.13	5.09	5.03	4.95
	L.S.D.$_{5\%}$ = .50							
Haematocrit	Sex	1	177.8	177.8	14.616			
	Residual	272	3309.5	12.2	***			
	Trip No.	1	2	3	4	5	6	7
	Mean (♂)	48.70	46.55	45.95	50.55	47.05	41.35	44.70
	Mean (♀)	44.32	46.13	44.82	46.65	46.02	40.58	43.45
	L.S.D.$_{5\%}$ = 2.50							
Haemoglobin concentration	Sex	1	23.13	23.13	16.283			
	Residual	272	386.43	1.42	***			
	Trip No.	1	2	3	4	5	6	7
	Mean (♂)	17.24	16.13	15.85	17.30	16.57	14.43	15.43
	Mean (♀)	15.52	15.94	15.48	16.22	16.04	14.13	14.99
	L.S.D.$_{5\%}$ = .85							

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APPENDIX 11 (cont.)

Variate	Source of Variation	DF	Analysis of Variance SS	MS	F
Red blood cell count	Sex	1	2.10	2.10	,3.051
	Residual	271	186.49	.69	n.s.
Mean cell volume	Sex	1	29	29	.262
	Residual	271	30141	111	n.s.
Mean cell haemoglobin concentration	Sex	1	.10	.10	.142
	Residual	272	200.59	.74	n.s.
Total protein concentration	Sex	1	.001	.001	.003
	Residual	271	77.858	.287	n.s.
Absolute concentration of albumin	Sex	1	.018	.018	.171
	Residual	271	29.018	.017	n.s.
Absolute concentration of α-globulin	Sex	1	.0015	.0015	.353
	Residual	271	1.1719	.0043	n.s.
Absolute concentration of β-globulin	Sex	1	.320	.320	2.685
	Residual	271	32.340	.119	n.s.
Absolute concentration of γ-globulin	Sex	1	.506	.506	3.807
	Residual	271	36.001	.133	n.s.
Relative percentage of albumin	Sex	1	10.6	10.6	.443
	Residual	272	6514.9	24.0	n.s.
Relative percentage of α-globulin	Sex	1	.00	.00	.005
	Residual	272	283.64	1.04	n.s.

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APPENDIX 11 (cont.)

Variate	Source of Variation	Ananlysis of Variance DF	SS	MS	F
Relative percentage of β-globulin	Sex	1	67.9	67.9	2.614
	Residual	272	7069.7	26.0	n.s.
Relative percentage of γ-globulin	Sex	1	143.3	143.3	6.214
	Residual	272	6271.0	23.1	*

Trip No.	1	2	3	4	5	6	7
Mean (♂)	14.85	18.17	24.91	22.11	18.43	19.91	20.07
Mean (♀)	17.33	15.98	20.99	19.89	16.43	18.29	17.96

$L.S.D._{5\%} = 3.44$

APPENDIX 12

Analyses of variance between female wallabies captured on all field trips for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, absolute concentrations of albumin, α-, β - and γ -globulin and relative percentages of albumin, α-, β- and γ -globulin. Where the F value is significant, the 7 means and the L.S.D.$_{5\%}$ are given for that variate.

Variate	Source of Variation	Analysis of Variance DF	SS	MS	F
Body Weight	Period	6	1.85919	.30987	.759
	Residual	203	82.8729	.40824	n.s.
Haematocrit	Period	6	784.083	130.681	10.40
	Residual	203	2550.48	12.564	***
	Trip No.	1	2	3	4	5	6	7
	Mean	44.32	46.13	44.82	46.65	46.02	40.58	43.45
	L.S.D.$_{5\%}$ = 1.79							
Haemoglobin concentration	Period	6	94.1199	15.6867	10.72			
	Residual	203	296.964	1.46288	***			
	Trip No.	1	2	3	4	5	6	7
	Mean	15.52	15.94	15.48	16.22	16.04	14.13	14.99
	L.S.D.$_{5\%}$ = .61							
Red blood cell count	Period	6	40.9982	6.83303	10.73			
	Residual	203	129.261	.63675	***			
	Trip No.	1	2	3	4	5	6	7
	Mean	5.65	6.02	5.63	5.52	5.84	4.60	5.15
	L.S.D.$_{5\%}$ = .40							

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APPENDIX 12 (cont.)

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Mean cell volume	Period	6	3279.11	546.519	5.830
	Residual	203	19029.5	93.7412	***
	Trip No.	1	2	3	4	5	6	7
	Mean	79.95	77.53	80.72	85.60	79.84	89.68	85.11
	$L.S.D._{5\%}$ = 4.90							
Mean cell haemoglobin concentration	Period	6	5.96284	.99381	1.288
	Residual	203	156.630	.77158	n.s.
Total protein concentration	Period	6	12.0703	2.01171	6.439
	Residual	202	63.1133	.31244	***
	Trip No.	1	2	3	4	5	6	7
	Mean	5.84	6.21	6.15	6.60	6.41	5.98	6.34
	$L.S.D._{5\%}$ = .28							
Absolute concentration of albumin	Period	6	5.40302	.90050	9.692
	Residual	202	18.7675	.09291	***
	Trip No.	1	2	3	4	5	6	7
	Mean	2.81	3.24	2.95	3.15	3.24	3.22	3.24
	$L.S.D._{5\%}$ = .15							
Absolute concentration of α-globulin	Period	6	.085355	.014226	3.106
	Residual	202	.925304	.004581	**
	Trip No.	1	2	3	4	5	6	7
	Mean	.29	.28	.27	.31	.29	.25	.31
	$L.S.D._{5\%}$ = .03							

...../cont.

APPENDIX 12 (cont.)

Variate	Source of Variation	DF	SS	MS	F
		Analysis of Variance			
Absolute concentration of β-globulin	Period	6	3.52093	.58682	4.571
	Residual	202	25.9300	.12837	***
	Trip No.	1	2	3	4	5	6	7
	Mean	1.72	1.69	1.63	1.83	1.80	1.41	1.65
	$L.S.D._{5\%}$ = .18							
Absolute concentration of γ-globulin	Period	6	2.92380	.487300	3.516
	Residual	202	27.9985	.138606	**
	Trip No.	1	2	3	4	5	6	7
	Mean	1.04	1.00	1.31	1.32	1.08	1.10	1.14
	$L.S.D._{5\%}$ = 1.9							
Relative percentage of albumin	Period	6	1022.71	170.452	8.076
	Residual	203	4284.50	21.1059	***
	Trip No.	1	2	3	4	5	6	7
	Mean	48.56	52.38	48.04	47.85	50.89	54.12	51.19
	$L.S.D._{5\%}$ = 2.33							
Relative percentage of α-globulin	Period	6	13.4243	2.23738	2.170
	Residual	203	209.321	1.03114	*
	Trip No.	1	2	3	4	5	6	7
	Mean	4.93	4.44	4.34	4.65	4.57	4.17	4.85
	$L.S.D._{5\%}$ = .51							

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APPENDIX 12 (cont.)

Variate	Source of Variation	DF	SS	MS	F
		Analysis of Variance			
Relative percentage of β-globulin	Period	6	627.929	104.655	3.844
	Residual	203	5526.59	27.2246	***
	Trip No.	1	2	3	4	5	6	7
	Mean	29.30	27.31	26.42	27.76	28.24	23.50	20.69
	$L.S.D._{5\%}$ = 2.64							
Relative percentage of γ-globulin	Period	6	584.838	97.4730	4.173
	Residual	203	4741.25	23.3559	***
	Trip No.	1	2	3	4	5	6	7
	Mean	17.33	15.98	20.99	19.89	16.43	18.29	17.96
	$L.S.D._{5\%}$ = 2.45							

APPENDIX 13

Analyses of variance between male wallabies captured on all field trips for body weight, haematocrit, haemoglobin concentration, red blood cell count, mean cell volume, mean cell haemoglobin concentration, total protein concentration, absolute concentrations of albumin, α-, β - and γ -globulin and relative percentages of albumin, α-, β - and γ -globulin. Where the F value is significant, the 7 means and the L.S.D.$_{5\%}$ are given for that variate.

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Body Weight	Period	6	2.8105	.46841	.591
	Residual	63	49.917	.79233	n.s.
Haematocrit	Period	6	515.521	85.920	8.410
	Residual	63	643.625	10.216	***
	Trip No.	1	2	3	4	5	6	7
	Mean	48.70	46.55	45.95	50.55	47.05	41.35	44.70
	L.S.D.$_{5\%}$ = 2.86							
Haemoglobin concentration	Period	6	62.5277	10.4213	8.644
	Residual	63	75.9530	1.2056	***
	Trip No.	1	2	3	4	5	6	7
	Mean	17.24	16.13	15.85	17.30	16.57	14.43	15.43
	L.S.D.$_{5\%}$ = .98							
Red blood cell count	Period	6	8.76851	1.4614	1.689
	Residual	63	53.6328	.86505	n.s.
Mean cell volume	Period	6	956.941	159.490	.939
	Residual	63	10532.8	169.884	n.s.

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APPENDIX 13 (cont.)

Variate	Source of Variation	DF	SS	MS	F
		Analysis of Variance			
Mean cell haemoglobin concentration	Period	6	10.5431	1.7572	2.788
	Residual	63	39.7046	.6032	*
	Trip No.	1	2	3	4	5	6	7
	Mean	35.41	34.68	34.50	34.24	35.21	34.93	34.51
	$L.S.D._{5\%}$ = .71							
Total protein concentration	Period	6	5.83361	.97227	4.314
	Residual	63	14.1995	.22538	**
	Trip No.	1	2	3	4	5	6	7
	Mean	5.88	6.05	5.99	6.65	6.54	5.96	6.41
	$L.S.D._{5\%}$ = .43							
Absolute concentration of albumin	Period	6	4.31422	.71904	5.989
	Residual	63	7.56339	.12005	***
	Trip No.	1	2	3	4	5	6	7
	Mean	3.16	3.29	2.56	3.25	3.37	3.06	3.05
	$L.S.D._{5\%}$ = .31							
Absolute concentration of α-globulin	Period	6	.05281	.008802	2.985
	Residual	63	.18578	.002949	*
	Trip No.	1	2	3	4	5	6	7
	Mean	.26	.24	.32	.31	.29	.27	.26
	$L.S.D._{5\%}$ = .05							

...../cont.

APPENDIX 13 (cont.)

Variate	Source of Variation	DF	SS	MS	F
			Analysis of Variance		
Absolute concentration of β-globulin	Period	6	1.16770	.19462	2.297
	Residual	63	5.33716	.08472	*
	Trip No.	1	2	3	4	5	6	7
	Mean	1.59	1.40	1.62	1.64	1.66	1.45	1.82
	L.S.D.$_{5\%}$ = .26							
Absolute concentration of γ-globulin	Period	6	2.71743	.45291	3.869
	Residual	63	7.37452	.11706	**
	Trip No.	1	2	3	4	5	6	7
	Mean	.88	1.13	1.51	1.46	1.23	1.19	1.29
	L.S.D.$_{5\%}$ = .31							
Relative percentage of albumin	Period	6	993.810	165.635	6.469
	Residual	63	1613.14	25.605	***
	Trip No.	1	2	3	4	5	6	7
	Mean	53.81	54.19	42.60	48.80	51.70	51.33	47.46
	L.S.D.$_{5\%}$ = 4.53							
Relative percentage of α-globulin	Period	6	11.0377	1.83962	1.998
	Residual	63	57.9960	.92057	n.s.
Relative percentage of β-globulin	Period	6	193.815	32.3026	1.586
	Residual	63	1282.79	20.3618	n.s.
Relative percentage of γ-globulin	Period	6	605.663	100.944	4.688
	Residual	63	1356.49	21.5317	***
	Trip No.	1	2	3	4	5	6	7
	Mean	14.85	18.17	24.91	22.11	18.43	19.91	20.07
	L.S.D.$_{5\%}$ = 4.15							

VI. BIBLIOGRAPHY

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Alwynelle, S.A. (1968). Electrophoretic examination of hemoglobin and plasma proteins from three altitude groups of Peromyscus maniculatus nebrascensis. Comp. Biochem. Physiol. 24: 427.

Andrewartha, H.G. and Barker, S. (1969). Introduction to a study of the ecology of the Kangaroo Island wallaby, Protemnodon eugenii (Desmarest), within Flinders Chase, Kangaroo Island, South Australia. Trans. R. Soc. S. Aust. 93: 127.

Barker, S. (1960). The role of trace elements in the biology of the quokka (Setonix brachyurus Quoy and Gaimard). Ph.D. thesis, University of Western Australia.

Barker, S. (1968). Nitrogen balance and water intake in the Kangaroo Island wallaby, Protemnodon eugenii (Desmarest). Aust. J. exp. Biol. med. Sci. 46: 17.

Barker, S., Lintern, S.M., and Murphy, C.R. (1970). The effect of water restriction on urea retention and nitrogen excretion in the Kangaroo Island wallaby, Protemnodon eugenii (Desmarest). Comp. Biochem. Physiol. (in press).

Bauer, F.H. (1960). The regional geography of Kangaroo Island, South Australia. Ph.D. thesis, Public Library of South Australia, Adelaide.

Bergstrand, C.G. and Czar, B. (1956). Demonstration of a new protein fraction in serum from the human fetus. Scand. J. clin. Lab. Invest. 8: 174.

Bethard, W.F., Wissler, R.W., Thompson, J.S., Schroeder, M.A. and Robson, M.J. (1958). The effect of acute protein deprivation upon erythropoiesis in rats. Blood 13:216.

Bolliger, A. and Pascoe, J.V. (1953). Composition of kangaroo milk. Aust. J. Sci. 15: 215.

Brooks, C.C. and Davis, J.W. (1969). Changes in hematology of the perinatal pig. J. Anim. Sci. 28: 517.

Campbell, P.N. (1963). The biosynthesis of rat serum albumin in Proceedings of the 5th International Congress of Biochemistry, Moscow. Vol. II. Pergamon Press, Oxford.

Cartwright, G.E., Smith, E.L., Brown, P.M., and Wintrobe, M.M. (1948). Electrophoretic analyses of sera of normal and hypoproteinemic swine. J. biol. Chem. 176: 585.

Casperson, K. (1968). Influence of environment upon some physiological parameters of the rabbit, Oryctolagus cuniculus (L), in natural populations. Proc. ecol. Soc. Aust. 3: 113.

Chandler, P.T., McCarthy, R.D. and Kesler, E.M. (1968). Effect of dietary lipid and protein on serum proteins, lipids and glucose in the blood of dairy calves. J. Nutr. 95: 461.

Chow, B.F., Allison, J.B., Cole, W.H. and Seeley, R.D. (1945). Effect of protein depletion on plasma proteins in the dog measured by electrophoretic analysis. Proc. Soc. exp. Biol. Med. 60: 14.

Conway, E.J. (1962). Microdiffusion analysis and volumetric error, p. 162, 5th ed. Crosby Lockwood and Son Ltd., London.

Dacie, J.V. (1956). Practical haematology, 2nd ed. J. and A. Churchill Ltd., London.

Dessauer, H.C. and Fox, W. (1956). Characteristic electrophoretic patterns of plasma proteins of orders of Amphibia and Reptilia. Science 124: 225.

Dobson, C. (1966). Cellulose acetate electrophoresis of the serum proteins of sheep: a study of developmental changes in young lambs. Aust. J. exp. Biol. med. Sci. 44: 575.

Ealey, E.H.M. and Main, A.R. (1967). Ecology of the euro, Macropus robustus (Gould), in north-western Australia. III. Seasonal changes in nutrition. CSIRO Wildl. Res. 12: 53.

Ezekial, E., Lai, L.Y.C. and Kaldor, I. (1963). Electrophoretic variations in the iron-binding proteins of blood plasma and milk whey in the albino rat and a marsupial. Comp. Biochem. Physiol. 10: 69.

Garrow, J.S. (1959). The effect of protein depletion on the distribution of protein synthesis in the dog. J. clin. Invest. 38: 1241.

Gitlin, D. and Boesman, M. (1966). Serum ∝-fetoprotein, albumin and ɣG-globulin in the human conceptus. J. clin. Invest. 45: 1826.

Gitlin, D. and Boesman, M. (1967). Fetus-specific serum proteins in several mammals and their relation to human ∝-fetoprotein. Comp. Biochem. Physiol 21: 327.

Gordon, A.H. and Humphrey, J.H. (1961). Measurement of intracellular albumin in rat liver. Biochem. J. 78: 551.

Gross, P.A.M., Gitlin, D. and Janeway, C.A. (1959). The gamma globulins and their clinical significance (continued). III. Hypergammaglobulinemia. New Engl. J. Med. 260: 121.

Gross, R. and Bolliger, A. (1959). Composition of milk of the marsupial Trichosurus vulpecula. A.M.A. J. Dis. Child. 98: 768.

Harper, H.A. (1959). Review of physiological chemistry, 7th ed. Lange Medical Publications, Los Altos.

Hoffman, W.S. (1964). The biochemistry of clinical medicine, 3rd ed. Year Book Medical Publishers, Inc., Chicago.

Holt, L.E., Halac, E. (Jr.) and Kajdi, C.N. (1962). The concept of protein stores and its implications in diet. J. Am. med. Ass. 181: 699.

Humphrey, J.H. and White, R.G. (1964). Immunology for students of medicine, 2nd ed. Blackwell Scientific Publications, Oxford.

Johnston, T.H. and Mawson, P.M. (1940a). On a collection of nematodes from Australian marsupials. Rec. Aust. Mus. 20: 360.

Johnston, T.H. and Mawson, P.M. (1940b). Nematodes from South Australian marsupials. Trans. R. Soc. S. Aust. 64: 95.

Jordan, S.M. and Morgan, E.H. (1968). The changes in the serum and milk whey proteins during lactation and suckling in the quokka (Setonix brachyurus). Comp. Biochem. Physiol. 25: 271.

Kakulas, B.A. (1961). Myopathy affecting the Rottnest quokka (Setonix brachyurus) reversed by ∝-tocopherol. Nature 191: 402.

Kakulas, B.A. (1963). Influence of the size of enclosure on the development of myopathy in the captive Rottnest quokka. Nature 198: 673.

Keating, F.R. (Jr.), Jones, J.D., Elveback, L.R. and Randall, R.V. (1969). The relation of age and sex to distribution of values in healthy adults of serum calcium, inorganic phosphorus, magnesium, alkaline phosphatase, total proteins, albumin, and blood urea. J. Lab. clin. Med. 73: 825.

Lecce, J.G., Matrone, G. and Morgan, D.O. (1961). The effect of diet on the maturation of the neonatal piglet's serum protein profile and resistance to disease in Plasma proteins in health and disease, edited by Dimopoullos, G.T. Ann. N.Y. Acad. Sci. 94: 250.

Lederer, M. (1955). An introduction to paper electrophoresis and related methods. Elsevier Publishing Co., Amsterdam.

Lemon, M. and Barker, S. (1967). Changes in milk composition of the red kangaroo, Megaleia rufa (Desmarest), during lactation. Aust. J. exp. Biol. med. Sci. 45: 213.

Lintern, S.M. and Barker, S. (1969). Renal retention of urea in the Kangaroo Island wallaby, Protemnodon eugenii (Desmarest). Aust. J. exp. Biol. med. Sci. 47: 243.

McDonald, I.W. and Hall, R.J. (1957). The conversion of casein into microbial proteins in the rumen. Biochem. J. 67: 400.

McEwan, E.H. and Whitehead, P.E. (1969). Changes in the blood constituents of reindeer and caribou occurring with age. Can. J. Zool. 47: 557.

MacFarlane, R.G. and Robb-Smith, R.H.T. (1961). Functions of the blood. Academic Press, New York.

MacFarlane, W.V., Morris, R.J.H., Howard, B., McDonald, J. and Budtz-Olsen, O.E. (1961). Water and electrolyte changes in tropical merino sheep exposed to dehydration during summer. Aust. J. agric. Res. 12: 889.

Main, A.R. (1968). Physiology in the management of kangaroos and wallabies. Proc. ecol. Soc. Aust. 3: 96.

Masters, C.J. and Horgan, D.J. (1962). Some functional manifestations of the protein depletion of sheep tissues. Aust. J. agric. Res. 13: 1082.

Mawson, P.M. (1955). Some parasites of Australian vertebrates. Trans. R. Soc. S. Aust. 78: 1.

Meacham, T.N., Warnick, A.C., Cunha, T.J., Hentges, J.F. (Jr). and Shirley, R.L. (1964). Hematological and histological changes in young beef bulls fed low protein rations. J. Anim. Sci. 23: 380.

Miller, L.L. and Bale, W.F. (1954). Synthesis of all plasma protein fractions except gamma globulins by the liver. J. exp. Med. 99: 125.

Miller, L.L., Bly, C.G. and Bale, W.F. (1954). Plasma and tissue proteins produced by non-hepatic rat organs as studied with lysine-ε-C^{14}. J. exp. Med. 99: 133.

Moore, D.H., Shen, S.C. and Alexander, C.S. (1945). The plasma of developing chick and pig embryos. Proc. Soc. exp. Biol. Med. 58: 307.

Morgan, E.H. (1968). Plasma protein turnover and transmission to the milk in the rat. Biochim. biophys. Acta 154: 478.

Morgan, E.H. (1969). Transferrin and albumin distribution and turnover in the rabbit. Aust. J. exp. Biol. med. Sci. 47: 361.

Nasser, S.S. and Platt, B.S. (1968). Development of anaemia in protein-calorie deficiency. Proc. Nutr. Soc. 27: 56A.

Ortega, L.G. and Mellors, R.C. (1957), Cellular sites of formation of gamma globulin. J. exp. Med. 106: 627.

Osserman, E.F. and Lawlor, D. (1961). Immunoelectrophoretic characterization of the serum and urinary proteins in plasma cell myeloma and Waldenström's macroglobulinemia, in Plasma proteins in health and disease edited by Dimopoullos, G.T. Ann. N.Y. Acad. Sci. 94: 93.

Phillips, R.A., Van Slyke, D.D., Hamilton, P.B., Dole, V.P., Emerson, K. (Jr.) and Archibald, R.M. (1950). Measurement of specific gravities of whole blood and plasma by standard copper sulphate solutions. J. biol. Chem. 183: 305.

Pond, W.G., Barnes, R.H., Bradfield, R.B., Kwong, E. and Krook, L. (1965). Effect of dietary energy intake on protein deficiency symptoms and body composition of baby pigs fed equalized but suboptimal amounts of protein. J. Nutr. 85: 57.

Pond, W.G., van Vleck, L.D., Walker, E.F. (Jr.), Eisenhard, C.F. and O'Connor, J.R. (1969). Changes in body weight and composition of adult nongravid female rats deprived of dietary protein. J. Nutr. 97: 343.

Putnam, F.W. (ed.) (1960). The plasma proteins. Vols. I and II. Academic Press, New York and London.

Reinhold, J.G. (1953). In Standard methods in clinical chemistry, Vol. I, edited by Reiner, M. Academic Press, New York - cited by Varley (1962).

Ribeiro, L.P., Mitidieri, E. and Affonso, O.R. (1961). Paper electrophoresis. A review of methods and results. Elsevier Publishing Co., Amsterdam, New York.

Robinson, D.S. (1964). The physiological significance of the plasma triglycerides in fat transport, in Metabolism and physiological significance of lipids, edited by Dawson, R.M.C. and Rhodes, D.N. John Wiley and Sons, London.

Roman, W. (1966). Serum proteins. Aust. J. Sci. 28: 265.

Rosenblum, W.I. and Asofsky, R.M. (1967). Effects of dehydration on blood viscosity and on distribution of plasma proteins in experimental macroglobulinaemia. Nature 216: 1327.

Rosenmann, M. and Morrison, P. (1967). Some effects of water deprivation in reindeer. Physiol. Zoöl. 40: 134.

Rothschild, M.A., Oratz, M. and Schreiber, S.S. (1967). Factors regulating serum protein metabolism, in Protides of the Biological Fluids, Proceedings of the 14th Colloquium, Bruges, 1966, edited by Peeters, H. Elsevier Publishing Co., Amsterdam.

Ryoo, H. and Tarver, H. (1968). Studies on plasma protein synthesis with a new liver perfusion apparatus. Proc. Soc. exp. Biol. Med. 128: 760.

Schmidt-Nielsen, K. (1964). Desert animals. Physiological problems of heat and water. Oxford Univ. Press.

Schultze, H.E. and Heremans, J.F. (1966). Molecular biology of human proteins with special reference to plasma proteins. Vol. I. Nature and metabolism of extracellular proteins. Elsevier Publishing Co., Amsterdam.

Scrimshaw, N.S. and Behar, M. (1961). Protein malnutrition in young children. Science 133: 2039.

Scrimshaw, N.S., Taylor, C.E. and Gordon, J.E. (1959). Interactions of nutrition and infection. Am. J. med. Sci. 237: 367.

Seal, U.S. and Erickson, A.W. (1969). Hematology, blood chemistry and protein polymorphisms in the white-tailed deer (Odocoileus virginianus). Comp. Biochem. Physiol. 30: 695.

Searcy, R.L. and Bergquist, L.M. (1962). Lipoprotein chemistry in health and disease. Thomas, Springfield, Illinois.

Sharman, G.B., Frith, H.J. and Calaby, J.H. (1964). Growth of the pouch young, tooth eruption and age determination in the red kangaroo, Megaleia rufa. CSIRO Wildl. Res. 9: 20.

Sheriff, D., Clapp, K.H. and Reid, M.A. (1966). Eperythrozoon ovis infection in South Australia. Aust. vet. J. 42: 169.

Shield, J.W. (1958). Aspects of field ecology of the quokka (Setonix brachyurus Quoy and Gaimard). Ph.D. thesis, University of Western Australia.

Shield, J.W. and Woolley, P. (1961). Age estimation by measurement of pouch young of the quokka (Setonix brachyurus). Aust. J. Zool. 9: 14.

Shoemaker, W.C. and Elwyn, D.H. (1969). Liver: functional interactions within the intact animal. A. Rev. Physiol. 31: 227.

Smith, I. (1960) (ed.) Chromatographic and electrophoretic techniques. Vol. II. Zone electrophoresis. Heinemann, London.

Van Slyke, D., Hiller, A., Phillips, R.A., Hamilton, P.B., Dole, V.P., Archibald, R.M. and Eder, H.A. (1950). The estimation of plasma protein concentration from plasma specific gravity. J. biol. Chem. 183: 331.

van Tets, P. and Cowan, I.McT. (1966). Some sources of variation in the blood sera of deer (Odocoileus) as revealed by starch gel electrophoresis. Can. J. Zool. 44: 631.

Varley, H. (1962). Practical clinical biochemistry, 3rd ed. Heinemann, London.

Wannemacher, R.W. (Jr.), Russell, T.J. and Allison, J.B. (1963). Serum and liver protein metabolism in protein-depleted dogs. J. Nutr. 80: 315.

Wehmeyer, P. (1954). Variation in the composition of the blood in cows during thirst, after intake of water, and on hungring. Acta path. microbiol. scand. 34: 518.

Weinman, D. (1944). Infectious anaemias due to Bartonella and related red cell parasites. Trans. Am. phil. Soc. 33(3).

Wintrobe, M.M. (1967). Clinical hematology, 6th ed. Lea and Febiger, Philadelphia.

Wolf, A.V. (1958). Thirst. Physiology of the urge to drink and problems of water lack. Thomas, Springfield, Illinois.

Wolstenholme, G.E.W. and Millar, E.C.P. (1956)(eds.). Paper electrophoresis. Ciba foundation symposium. J. and A. Churchill Ltd., London.

Wu, D.Y. and Wu, H. (1951). Determination of urea in saliva. Proc. Soc. exp. Biol. Med. 76: 130.

Wunderly, Ch. (1953). Chimia 7: 145 - cited by Lederer (1955).

Zeldis, L.J., Alling, E.L., McCoord, A.B. and Kulka, J.P. (1945). Plasma protein metabolism - electrophoretic studies. J. exp. Med. 82: 157.