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HYPOGLYCAEMIA DUE TO ETHYL ALCOHOL

In vitro studies using human liver slices

by

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Being a Thesis submitted for the Degree
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Ye that are drunken, but not with wine,
That stagger, but not with strong drink.

Isaiah iixi:9.

The modes of mania are infinite in species, but one alone in genus. For it is altogether a chronic derangement of the mind, without fever. For if the fever at any time should come on, it would not owe its peculiarity to the mania, but to some other incident. Thus wine inflames to delirium in drunkenness; and certain edibles, such as mandragora and hyoscyamus, induce madness; but these affections are never called mania; for springing from a temporary cause, they quickly subside, but madness has something confirmed in it.

Aretaeus, the Cappadocian.
Edited and translated by Francis Adams.
London - The Sydenham Society, 1856.

To my wife, Adele, without whose mostly patient forbearance, these studies could not have been undertaken or completed.

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Liver slices obtained from human subjects undergoing elective surgery were incubated in 2ml. of Krebs Ringer Phosphate or Krebs Ringer Bicarbonate in pure oxygen or 95% oxygen - 5% CO_2 respectively. The following radioactive gluconeogenic precursors were employed in concentrations of 0.01 to 10.0 micromoles per ml. (0.01 to 10.0mM). C^{14} - labelled L - alanine; L - glutamic acid; glycerol and D, L - lactic acid. Experimental vessels were further supplemented with 1 to 10 mM of unlabelled ethyl alcohol or sodium acetate. After 2 or 3 hours of incubation, radioactive assays were performed to assess the effects of ethyl alcohol or sodium acetate upon the disposition of each labelled gluconeogenic precursor.

The following aspects were studied:

(a) Formation of glucose by measuring glucose-C^{14} in the suspending media and as tissue glycogen:

(b) Oxidative decarboxylation by collecting $C^{14}O_2$

(c) Reductive retention as a 3 - carbon fragment by measuring the release of lactic acid - C^{14}

(d) Where possible, these parameters were related to the "uptake" of the C^{14} - labelled precursor as judged by its disappearance from the suspending media.

Ethyl alcohol (10mM) reduced the formation of glucose and the evolution of $C^{14}O_2$ from all of the gluconeogenic precursors studied. "Uptake" was also decreased and the

formation of lactic acid-C^{14} variably augmented. The incorporation of glucose-C^{14} into glycogen as well as the release of glucose-C^{14} into the suspending medium were inhibited by ethyl alcohol indicating that gluconeogenesis had been interrupted prior to the formation of glucose-6-phosphate.

In manometric studies ethyl alcohol did not depress oxygen consumption more than 20% at the termination of 3 hour experiments indicating that some mechanism other than histotoxic anoxia was involved.

Parallel experiments using sodium acetate (10mM) failed to simulate the changes due to ethyl alcohol suggesting that the effects were not due to simple dilution with 2-carbon fragments.

An attempt is made to explain these findings of the effects of ethyl alcohol in terms of changes in the ratio of cytoplasmic ($NADH_2/NAD^+$) and the size and turnover of the 3 carbon pool.

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

Alex Cohen.

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

This work was carried out in the Thorndike Memorial Laboratory of Harvard Medical School in the Boston City Hospital during 1962 and 1963. The author was in receipt of a Travelling Fellowship in Medicine from the Royal Perth Hospital, Perth, Western Australia, withoutwhich the whole project would have been impossible. A further Training Grant of the United States Public Health Services was made during my period of residence in America and is most gratefully achnowledged.

As must be apparent from the flavour and essence of this thesis, a large amount of study on the problem of hypoglycaemia due to alcohol had been conducted already by the group at the Thorndike Laboratory. For Dr. Norbert Freinkel, who had instigated most of the previous studies and under whose direction I worked, I feel an overwhelming sense of gratitude. His patience, stimulation, good-humour and immense knowledge have permeated my approach to medicine since my period of study in his laboratory.

My immediate colleagues - Drs. Ronald Arky and Sheldon Bleicher - gave me much help with the problems of in vitro methods, but it is from the many periods of informal discussion, argument and debate that I have gained so much of their knowledge and experience. This wealth was extended by the whole communal atmosphere of the Thorndike Laboratory with Dr. William B. Castle formerly at its head and later Dr. Maxwell Finland from both of whom emanated the spirit of enquiry, mutual assistance and critical uncompromisation

which has made apprenticeship in that Laboratory a source of keen pride to all of its recipients.

I am indebted to Miss Rosalie Krasnoff and Miss Prudence Bunker for technical assistance during the work. The statistical treatment of the results has been prepared by Mr. N.S. Stenhouse, Biostatistician attached to the Raine Medical Research Foundation in the University of Western Australia and his patient attention to my many queries and problems is much appreciated. The Department of Medical Illustration at the Royal Perth Hospital kindly prepared the illustrations.

I am particularly grateful to Dr. Ivan Oliver, Reader in Biochemistry, University of Western Australia, and to Dr. David Curnow, Head of the Department of Biochemistry, Royal Perth Hospital, for their critical reading of the manuscript and for their helpful suggestions thereon.

C H A P T E R 1

I N T R O D U C T I O N

A concept of Hypoglycaemia long awaited an understanding of Hyperglycaemia. Diabetes mellitus is a disease of antiquity but the theories as to its aetiology were bizarre and wide of the mark until Mathew Dobson in 1775 recognised the sweet material in diabetic urine to be sugar and Michel Chevreul in 1815 characterised the sugar as glucose - the same as that in grapes.

In 1849 Claude Bernard established the glycogenic function of the liver by demonstrating sugar in the hepatic vein of dogs fed either sugar or protein. His isolation of glycogen from the liver added substance to the previous evidence that the body could synthesise its own chemicals. He stressed the importance of the liver as a source of peripheral glucose supply and its ability to maintain this in the absence of carbohydrate from the diet. In addition, he drew attention to the threshold phenomenon of renal glycosuria. Bernard Naunyn and his co-workers (1898) correlated the glycosuria due to the elevated blood sugar with the diagnosis of diabetes mellitus.

The localising pathology of the condition was indicated by Opie (1901) who postulated that diabetes, the antithesis of hyperinsulinism, is due to alterations in the Islets of Langerhans. Nicholls (1902) recorded the findings in the first neoplasm to be identified as an islet cell tumor. Allen and associates (1920) directed attention to the

function of the islet cells in their work with experimental diabetes. With the recovery of an hypoglycaemic agent from the anatomically isolated cells of the pancreatic islets by Banting and Best (1922) the deductive chain was forged and within months of the clinical usage of 'Insulin', Campbell and Fletcher (1923) had observed the symptoms of hypoglycaemia following an overdose of the material.

Joslin (1921) had recognised the possibility of hypoglycaemia in diabetics who were being strenuously treated by undernutrition. In 1924, Gibson and Larimer observed fasting hypoglycaemia in a young diabetic woman whose disease had been present for ten years and who was not receiving insulin. Repetitive glucose challenges to this patient resulted in progressively profound hypoglycaemic responses which they attributed to impaired glycogenesis and increased urinary wastage of glucose. Most importantly, they concluded that clinical conditions existed in which the hypoglycaemic states occurred without the administration of exogenous insulin.

The term 'Hyperinsulinism' was introduced shortly afterwards by Harris (1924) to explain the situation wherein symptoms of hypoglycaemia occurred in the absence of injected insulin. His conclusions, incidentally, adumbrated by many years the now established concept of hypoglycaemia due to the sustained action of endogenous insulin in the stage of latent diabetes (Allen, 1953; Seltzer et al., 1956). Finally, Wilder and his associates (1927) established the anatomical concept of organic hyperinsulinism when they reported the first islet cell tumor to be removed from a patient. Large amounts of insulin were recovered from the

parent tumor and from metastases in the liver. From Wilder's observations and subsequent experience, the hypersecretion of such tumors was established. Howland et al. (1929) reported the first cure of a case of hyperinsulinism following the removal of an adenoma of the Islets of Langerhans, and Whipple, up until 1938, was able to collect 74 cases of such tumors from the literature of which 56 were localised at operation and 18 at autopsy.

Rapidly widening experience with the use of insulin in diabetes mellitus and to a lesser extent in psychiatric conditions such as schizophrenia made clinicians aware of the clinical picture of hypoglycaemia in its commonest form and led to increasing documentation of cases in which insulin was not the mediating factor. A clearly delineated sequential analysis of the various phases of cerebral cortical deprivation of glucose was presented by Himwich in 1944.

Animal experimentation had long preceded clinical appreciation. Marked attention had been focused on the liver as a source of body glucose reserves. Experimental hepatectomy or exclusion of the liver from the circulation had been practised since the first experiments of Bock and Hoffman (1874). Minkowski (1886) in his classical research on the effect of the removal of the liver in geese noted a decrease in blood sugar. Further experiments in dogs (Seegen, 1881; Schenk, 1921) amply confirmed this important observation but most workers attributed little significance to the fact. The liver was not removed surgically from any of the animals except the goose. The different methods of exclusion entailed a great alteration in the circulation and,

except in those experiments in which the circulation was restricted to the anterior parts of the body, great loss of blood must have occurred from congestion. The method of experimentation and length of life after the operation, with the exception of the experiments on geese, precluded any correlation between the reduction of blood sugar and the development of symptoms.

The elegant experiments of Mann and Magath (1922) provided the first opportunity for detailed and unhurried study of the animal with surgically induced chronic liver insufficiency or following total hepatectomy. Using a graduated operative procedure by means of prior porto-caval anastomoses they were able to occlude the hepatic circulation at a time when the experimental animals had recovered from the initial manipulations and in this way were able to use the same animal as its own control in circumstances preliminary to the final occlusion.

These workers noted an exact correlation between the decline in blood sugar, the emergence of symptoms and the onset of death. This was to such an extent that the amount of blood sugar could be predicted correctly after a clinical appraisal of the condition of the animal as the symptoms appeared. These observations were followed by the first administration of intravenous glucose for hypoglycaemia with dramatic reversal of the deteriorating clinical condition. Moreover, a quantitative relationship was established whereby varying amounts of glucose per kilogram body weight could restore the animals to corresponding degrees of well being. Although this was subject to individual variation, the response was relatively constant, albeit influenced by the length of time following operation

and the amount of glucose previously administered. Physical activity and the ambient temperature were important factors in glucose requirements.

Soskin and his co-workers (1938) refined the observations of Bernard and those following him and demonstrated that the perfused dog liver removed glucose from the blood stream when portal blood contained high glucose levels. Conversely, when the portal blood was low in glucose this substance was produced by the liver.

The observations have been amply confirmed and extended. Spontaneous hypoglycaemia occurs in a wide variety of hepatic disorders which, for the most part, are due to toxins of chemical or infectious nature; to replacement of functioning with non-functioning tissue in the liver or to disturbances of function resulting from vascular changes (Freinkel and Bleicher, 1963). However, hypoglycaemia is not to be considered a characteristic of these states (Zimmerman, 1953) and even though a reduced blood sugar is suspected as a complication of hepatic disease its occurrence is unusual but not rare. This infrequency might belie its seriousness. Since it reflects a reduced capacity for glycogenesis, gluconeogenesis and glycogenolysis on the part of the liver it is usually an indication of grave and diffuse parenchymal disease.

For centuries, infusions of various plants were used in the treatment of diabetes mellitus. Such preparations may significantly reduce raised blood sugar levels as is now known, but usually by undesirable means such as hepatocullular damage. Several organic chemicals act similarly.

TABLE I

OTHER SUBSTANCES ALLEGED TO PRODUCE HYPOGLYCAEMIA IN MAN

SUBSTANCE	SOURCE	AUTHORS	CLINICAL DETAILS	POSTULATED AETIOLOGY
PHOSPHORUS	CHEMICAL	FRANK & ISAAC, 1910		DIRECT HEPATIC INJURY
HYDRAZINE	EXPERIMENTAL. ACCIDENTAL	IZUME & LEWIS 1926		FAILURE OF GLUCONEOGENESIS SECONDARY TO HEPATIC INJURY
PARATHION	INSECTICIDE	HRUBAN ET AL., 1963	CHILD, ROOM SPRAYED WITH CHLORDANE. NEUROLOGIC AND HYPOGLYCAEMIC SIGNS. B.S.L. 17 MG% DIED	DIRECT STIMULATION OF ISLET CELLS BY PARASYMPATHETIC OVERACTIVITY
DISODIUM-ETHYLENE-DIAMINE TETRA-ACETIC ACID.	CHELATING AGENT.	MELTZER ET AL. 1961	NO EFFECT ON NORMALS AND NO EFFECT ON DIABETICS NOT ON INSULIN-TAKING DIABETICS. ACTION WITHIN AN HOUR.	CHELATION OF ZINC THUS INCREASING THE SOLUBILITY OF EXTRAPANCREATIC (I.E., CIRCULATING INSULIN).
POTASSIUM	INFUSED K	SAGILD, 1963	IN RECOVERY PHASE OF PERIODIC PARALYSIS. 2 CASES.	RAPID TRANSPORT OF GLUCOSE FROM EXTRA TO INTRACELLULAR SPACE.
MANGANESE	ALFALFA (LUCERNE) MEDICAGO SATIVA.	RUBINSTEIN ET AL., 1962.	MUSCULAR MALE, DIABETIC. REPRODUCED BY ORGANIC MN. UNTIL PARTIAL PANCREATECTOMY.	UNKNOWN. ? INCREASED PERIPHERAL UTILISATION. ? INHIBITION OF GLUCAGON. ? INCREASED INSULIN RELEASE.

TABLE I (CONTD)

OTHER SUBSTANCES ALLEGED TO PRODUCE HYPOGLYCAEMIA IN MAN

SUBSTANCE	SOURCE	AUTHORS	CLINICAL DETAILS	POSTULATED AETIOLOGY
FRUCTOSE & ITS DI- & POLY SACCH-ARIDES	DIETARY	FROESCH ET AL., 1969 NIKKILA ET AL., 1962,	HYPOGLYCAEMIA WITH MARKED DECREASE OF SERUM POTASSIUM AND INORGANIC PHOSPHATE.	DEFICIENT FRUCTOSE-1-PHOSPHATE ALDOLASE WITH ACCUMULATION OF FRUCTOSE-1-PHOSPHATE IN THE LIVER.
HYPOGLYCIN A & B	ACKEE NUT (BLIGHIA SAPIDA)	HASSAL & REYLE, 1955.	USUALLY CHILDREN OF POOR NUTRITION. MAY BE EPIDEMIC BUT IN-EXPLICABLY CAPRICIOUS.	UNKNOWN.
VEGETABLE OR PLANT ROOT. (NOT ACKEE)	NOT ES-TABLISHED. TRINIDAD & TOBAGG.	FISTEIN. 1960.	2 CHILDREN, POORLY NOURISHED. BOTH CONVULSED. 1 DIED, LIVER FUNCTION TESTS ABNORMAL IN SURVIVOR	UNKNOWN. SENSITISATION TO HEPATOTOXIN BY MALNUTRITION AND POSSIBLY VITAMIN B DEFICIENCY.
MEBANAZINE 'ACTOMOL' HYDRAZINE DERIVATIVE & METHYL BENZYLHYD-RAZINE.	ANTI-DEPRESSIVE. MONO AMINE OXIDASE INHIBITOR.	COOPER & KEDDIE. 1964.	DRUG PRODUCED SEVERE HYPOTENSIVE EPISODES IN 3 OF 7 CASES IN 1 A DIABETIC - EPISODES OF HYPOGLYCAEMIA. NO B.S.L. GIVEN. INSULIN REQUIREMENT GREATLY REDUCED DURING THERAPY.	INTERFERENCE WITH CATECHOLAMINE DISCHARGE AND FAILURE TO BUFFER INSULIN ACTION.
SALICYLATE	THERAPY	COTTON & FAHLBERG, 1964.	2 CASES IN INFANTS, BOTH HAD BEEN FASTING DUE TO ILLNESS. B.S.L.S 0MG% AND 5MG%. SOME ACIDOSIS. NEUROLOGICAL SIGNS ANDCOMA DIS-PERSED BY GLUCOSE.	REDUCTION OF HEPATIC GLYCOGEN. INTERFERENCE WITH A.T.P. PRODUCTION. INCREASED UTILISATION WITH DECREASED FORMATION OF GLUCOSE.

TABLE 1 (CONTD)

OTHER SUBSTANCES ALLEGED TO PRODUCE HYPOGLYCAEMIA IN MAN

SUBSTANCE	SOURCE	AUTHORS	CLINICAL DETAILS	POSTULATED AETIOLOGY
LEUCINE	DIETARY, CHEMICAL	COCHRANE ET AL., 1956	MINOR RESPONSE IN NORMALS. MARKED RESPONE IN SENSITIVE SUBJECTS, FAMILIAL HYPOGLYCAEMIAS AND THOSE PRESENSITISED WITH SULPHONYLUREAS.	POTENTIATION OF INSULIN SECRETION BY A METHOD DIFFERENT FROM THAT OF THE SULPHONYLUREAS. POSSIBLY SOME DIRECT ACTION ON THE LIVER.
GALACTOSE	DIETARY, BREAST MILK.	BRUCK & RAPEPORT, 1945		MECHANISM UNKNOWN. TOTAL "TRUE SUGAR" ELEVATED BUT GLUCOSE REDUCED. ? PANCREATIC ISLET STIMULATION. ? EFFECT ON HEPATIC GLUCOSE OUTPUT. ? FAILURE TO DISTINGUISH BETWEEN TOTAL SUGAR AND TRUE GLUCOSE. ? INHIBITION OF PHOSPHOGLUCOMUTASE BY GALACTOSE-1-PHOSPHATE (SIDBURY, 1961)
D-RIBOSE	PURE CHEMICAL INTRAVENOUSLY.	SEGAL ET AL., 1957.	MARKED DECREASE IN GLUCOSE LEVEL NOT ASSOCIATED WITH SIGNIFICANT RISE OF PYRUVATE OR FALL IN SERUM INORGANIC PHOSPHATE.	OBSCURE. DATA EXCLUDES INCREASED PERIPHERAL GLUCOSE UTILISATION AND RENAL GLYCOSURIA. POSSIBLE STIMULATION OF INSULIN RELEASE (POZZA ET AL., 1958).
MANNOSE	INVESTIGATIVE INFUSION.	WOOD & CAHILL, 1963.	INFUSION INTO NORMAL MAN WITH MARKED AND PROLONGED DECREASE IN BLOOD GLUCOSE.	PROBABLE ACCUMULATION OF MANNOSE-6-PHOSPHATE DUE TO REDUCED AVAILABILITY OF PHOSPHAMANNOSE-ISOMERASE. POSSIBLE HEPATOTOXICITY.
L-SORBOSE	INVESTIGATIVE INFUSION.	CAHILL, 1964.	INFUSION INTO NORMAL MAN WITH REDUCTION IN BLOOD SUGAR LEVELS.	DIRECT HEPATIC INFLAMMATION AND DECOMPENSATION OF LIVER FUNCTION.

Of these, phosphorus (Frank and Isaac, 1910) arsenic and Hydrazine (Izume and Lewis, 1926) are good examples. More recently, pure vegetable sources of hypoglycaemic material such as the Ackee nut (Blighia sapida) which causes Jamaican vomiting sickness (Hassal and Reyle, 1955; Fistein, 1960) and possibly the manganese content of Lucerne (Medicagosativa) have been characterised (Rubinstein et al., 1962; Lancet, 1963). However, the mechanism of action of most of the botanical hypoglycaemic agents is unknown and this storehouse of former folk medicine may include drugs of clinical value and hypoglycaemic mechanisms of wide interest. With the introduction and acceptance of complex and marginally toxic inorganic chemicals to the therapeutic field it is probable that more examples of such drug action will be brought to light. The literature concerning the actions of the sulphonylurea group of drugs is now extensive and well summarised (Frawley et al., 1959) and neither these nor the biguanide preparations will be further discussed. Table 1 annotates those other materials in which an hypoglycaemic action has been satisfactorily substantiated.

Despite the plethora of vicissitudes attributed to its use, alcohol has never been so incriminated. Certainly alcohol has been desultorily used in the therapy of diabetes mellitus. In 1922, Allen and Wishart reported their experience with it in two diabetic subjects. Their studies were instituted because of the known absence of direct convertibility between alcohol and sugar; because of the lack of immediate relationship between alcohol and the formation of acetone bodies and, as a critical trial of the vaguely salutory effects attributed to this substance. Their review of the literature to that time suggested that

alcohol alone was never responsible for glycosuria. They cited Von Noorden (1891) as showing that alcohol spares protein on a protein-rich diet but not on a protein-poor diet. They suggested that Mosenthal and Harrop (1918) had contributed the only accurate study until then of the food value of alcohol in diabetics. In this review they cited Neubauer (1906) as reporting that wine, representing 65 to 135 grams of alcohol per day when added to diabetic diets had no distinct effect upon mild cases with trivial acidosis but caused a diminution of both glycosuria and acidosis in more severe cases. They further noted Benedict and Torok (1906) as showing that when fat was withdrawn, the acetone and sugar increased, but that when alcohol was added the acetone, sugar and ammonia diminished. In the two patients studied by Allen and Wishart in this report the following observations were made.

Detailed caloric and iso-caloric substitution values are shown and the conclusion reached that ethyl alcohol is not converted into sugar in the body. The addition of calories in the form of alcohol in excess of the patient's caloric tolerance produced and return of glycosuria in other diabetic diets. The addition of alcohol or a mixture of fat and alcohol to a standard diet gave rise to very much less acidosis that similarly excessive diets built up by addition of fat alone with consequently less danger of acidosis when part of the fat of a high caloric diet was substituted by alcohol. Since alcohol was clearly recognised as incapable of conversion into sugar or acetone in the body, they claimed that these experiments showed alcohol to adversely influence the diabetic balance in a manner independent of its caloric content. Any actual hypoglycaemic action of alcohol was not recognised or stressed.

Leclerq (1922) studied two juvenile diabetics in a psychiatric institution. They were both relatively aglycosuric and ketone body free when maintained on a 1,000 calorie diet but suffered a marked augmentation of hyperglycaemia and ketonuria when this diet was increased to 2,000 calories. This change and deterioration was occasioned regardless of whether the calorie increase was attained by alcohol, fat or a mixture of the two and the study thus reinforced the findings of Allen and Wishart (1922).

Shortly after this, Fuller (1922) reported studies on seven diabetics of varying severity to whom alcohol was given. The subjects received 20 to 30 ccs of alcohol by mouth. In five cases a definite reduction of blood sugar was observed, in each during the first or second hour. In a further study of five cases he was able to confirm these findings and differentiate the maximal hypoglycaemic effect of isocaloric substitution of alcohol for fat as against the lesser response when the alcohol was only additional to it. In all instances the milder diabetics were more responsive than those with severe disease.

Normal subjects maintained on an adequate diet of calories but high in fat and low in carbohydrate sufficient to induce ketonaemia and ketonuria were not affected by the exhibition of alcohol (Nebauer, 1906; Benedict and Torok, 1906; Higgins et al., 1916). In a re-examination of this whole problem, Arky and Freinkel (1964), have demonstrated an attenuation of the rising blood sugar and an arrest of the progressive ketonaemia in "juvenile" diabetics after the intravenous infusion of ethyl alcohol when food was withheld.

C H A P T E R 11

The Clinical Syndrome.

In a review of twelve cases of ketone acidosis in nondiabetic adults, Dillon et al., (1940) delineated four cases in whom considerable hypoglycaemia was detected. All of the subjects in the series were chronic alcoholics and much undernourished but only in four of these were levels of blood sugar, ranging from 20 to 42 mg. per cent recorded. All were revived by the administration of glucose after a period of cerebral obfuscation ranging from confusion to coma. Whilst the role of alcohol was briefly mentioned, no firm aetiological relationship between this and the hypoglycaemia was suggested.

A year later Brown and Harvey (1941) drew attention to six cases of hypoglycaemia occurring in patients who had recently ingested 'smoke', an intoxicating beverage containing denatured alcohol solvents. Their experience had extended over a number of years but closer observations of subsequent admissions confirmed the association of the metabolic state with the ingestant. After this report, Tucker and Porter (1942) studied four cases in whom the precipitating material was 'Solox', a shellac solvent containing approximately 90% ethyl alcohol. From these two papers the clinical and biochemical syndrome has been extended, critically investigated and materially clarified. Table 2 incorporates a precis of the reported cases up until mid-1967 on which the ensuing description of 'Alcohol Hypoglycaemia' is based.

Although the condition was originally ascribed to 'smoke' or, more specifically, denatured alcohol solvents, subsequent reports have excluded all but ethyl alcohol as the common ingredient and Freinkel et al., (1963) have reproduced all features of the syndrome with ethyl alcohol alone. Thus Bottura et al., (1949) reporting the first cases in which ethyl alcohol can be clearly implicated, and in which measurements of blood alcohol were obtained, were able to exclude the presence of methyl alcohol from all samples of the intoxicant 'aguardent' which was the source of exposure. Later reports have excluded ethyl acetate, gasoline or methyl isobutyl ketone (Neves et al., 1950; Peluffo et al., 1958; Jeune et al., 1960 Cummins, 1961 and Neame and Joubert, 1961).

Most incidents have been described in chronic alcoholics but the repeated descriptions in children indicates that addiction is not a prerequisite. In the 20 cases reported from Brazil by Neves et al., (1950) two were of acute alcoholic intoxication in nonalcoholics. Whilst all patients have consumed excessive ethyl alcohol in one form or another, great variation in the time interval between its ingestion and the development of symptoms is recorded. Tucker and Porter (1942) report a lapse of 12 hours and Kahil et al., (1964) one of 18 hours. In other instances (Hammack, 1957) the patients were acutely alcoholic.

Nutritional impairment is common but not invariable. The very nature of the exposure dictates that most subjects have been heavy drinkers if not actually chronic alcoholics in the accepted sociological sense and the conditioning sequence of poor dietary intake is paramount in this group.

13a TABLE 2. A REVIEW OF THE LITERATURE OF CASES OF ALCOHOLIC HYPOGLYCAEMIA

AUTHORS	No. OF CASES	PARTICULARS	INGESTANT	CLINICAL FEATURES	TEMP.	B.P.	B.S.L.	URINE ACETONE	CO_2	BLOOD ALCOHOL	TIME INTERVAL	L.F.T.'S	LIVER BIOPSY OR AUTOPSY	G.T.T.	OUTCOME & COMMENT.
DILLON ET AL., 1940	3	FEMALE 59 YR	-	COMATOSE ACIDOTIC	-	-	30MG%	+	31V%	-	-	IMPAIRED	-	-	ALL REVIVED BY GLUCOSE REPORTS OTHER CASES IN WHICH KETOSIS MORE SEVERE BUT BLOOD SUGAR LESSDEPRESSED.
		FEMALE 40 YR	-	UNCONSCIOUS	-	-	22MG%	+	35V%	-	-	IMPAIRED	-	-	
		MALE 54 YR ALCOHOLICS	-	CONFUSED	-	-	42MG%	+	33V%	-	-		-	-	
BROWN & HARVEY 1941	6	CHRONIC ALCOHOLICS 26-68 YRS 5 NEGROES 1 WHITE	"SMOKE" * DEFINITELY IN 3	COMA, CEREBRAL IRRITATION TRISMUS	95.4	1 IN 2 CASES LATER NORMAL	22MG% 20MG% 33MG% 43MG% 38MG% 21MG%	+ IN 2 PATIENTS	NO ACIDOSIS	-	-	NORMAL IN 2 OTHERS NOT DONE	-	NORMAL IN 3	2 RECOVERED SPONTANEOUSLY. RAPID COMPLETE RECOVERY AFTER DEXTROSE IN REST. ONE CASE HAD HYPOGLYCAEMIA ON MORE THAN ONE OCCASION.
TUCKER & PORTER, 1942	4	CHRONIC ALCOHOLICS 32-41 YRS. 1 WHITE 3 NEGROES	"SOLOX"**	COMA CONVULSIONS RIGIDITY.	95.6	NORMAL	22MG% 20MG%	+ IN 3	SLIGHT LOWERING	-	1 CASE 6 HOURS LAPSE	MILDLY IMPAIRED 1 CASE	-	NORMAL OR SLIGHTLY FLAT	RAPID RECOVERY AFTER DEXTROSE. AUTHORS INVOKE LIVER DAMAGE.
BOTTURA ET AL., 1949.	11	CHRONIC ALCOHOLICS	"AGUARDENT" AND WINE.	-	36°C 35.5	-	18MG% 42MG%	-	-	82MG% 172MG%	-	-	FATTY INFILTRATION IN 2 AT AUTOPSY	-	DRAMATIC RESPONSE TO SMALL DOSES OF DEXTROSE. 3 DIED.
NEVES ET AL., 1950	20	2 IN ACUTE ALCOHOLISM. REMAINDER CHRONIC ALCOHOLICS	VARIOUS	COMA	-	-	11MG% TO 65MG%	11 OF 12	-	482MG%	6	-	FATTY INFILTRATION 2. NORMAL 1. (BIOPSY)	ABNORMAL IN 1 CASE.	3 DIED. PANCREAS NORMAL IN 2 AUTOPSIED CASES. FASTING ALONE IS NOT SUFFICIENT TO PRODUCE SYNDROME.
TAYLOR, 1955	1	CHRONIC ALCOHOLIC 54 YEARS	-	COMA SWEATING	-	-	62MG%	STRONG +VE.	-	-	-	SLIGHTLY ABNORMAL PLASMA PROTEINS.	-	NORMAL RENAL GLYCOSURIA.	RAPID RECOVERY AFTER INTRAVENOUS DEXTROSE. RECURRENCE 5 MONTHS LATER.
HAMMACK, 1957	6	MOST CHRONIC ALCOHOLICS 2 NEGROES 4 WHITE	"SOLOX"	COMA CONVULSIONS	-	-	13MG% 17MG%	MILD EXCESS IN SOME	REDUCED	-	ACUTELY ALCOHOLIC	NORMAL	FATTY INFILTRATION (AUTOPSY)	-	REPORTS 36 CASES OF "SOLOX" INTOXICATION OF WHOM 13 DIED. ONLY 6 HAVE DOCUMENTED HYPOGLYCAEMIA.

* "SMOKE" RESULTS FROM THE ADDITION OF DENATURED ALCOHOLS TO WATER. IT HAS THE FOLLOWING AVERAGE COMPOSITION BY VOLUME: 86.5% ETHYL ALCOHOL; 4.4% METHYL ALCOHOL; 0.75% GASOLINE; 3.5 G. PER 100 ML. ETHYL ACETATE.

** "SOLOX" IS THE TRADE NAME FOR A SHELLAC SOLVENT, FORMULA: PURE ETHYL ALCOHOL, 100 GAL; DENATURED GRADE WOOD ALCOHOL, 5 GAL; GASOLINE, 1 GAL. ETHYL ACETATE, UNDENATURED, 1 GAL.

TABLE 2 (CONTD) A REVIEW OF THE LITERATURE OF CASES OF ALCOHOLIC HYPOGLYCAEMIA

AUTHORS	NO. OF CASES	PARTICULARS	INGESTANT	CLINICAL FEATURES	TEMP	B.P.	B.S.L.	URINE ACETONE	CO_2	BLOOD ALCOHOL	TIME INTERVAL	L.F.T.'s	LIVER BIOPSY OR AUTOPSY	G.T.T.	OUTCOME & COMMENT
PELUFFO ET AL., 1958.	2	CHILDREN URUGUAY 6 YRS. 4 YRS.	"CANA" *** 250 ML. WINE	-	35°C	-	35MG%	STRONG +VE.	-	90MG%	-	-	-	-	RESPONDED PROMPTLY TO 10ML. HYPERTONIC GLUCOSE. RAPID RECOVERY AFTER DEXTROSE INFUSION IN SECOND CASE. INSULIN STIMULATION SUGGESTED.
GADSDEN ET AL., 1958/	3	ADULTS CHRONIC ALCOHOLICS	"SCRAP IRON" ****	HYPO-GLYCAEMIC SHOCK.	-	-	10MG% 30MG% 33MG%	+VE IN 1	-	-	SUGGEST DELAY	NORMAL	-	NORMAL	ALL RECOVERED. CASES POSSIBLY DUE TO "SOLOX" IN RETROSPECT. EMPHASISE DELAYED ACTION OF " SOLOX".
HED, 1958.	2	20 YEARS 35 YEARS	ALCOHOL AS WINE	SLIGHT COMA.	-	-	54MG% 57MG%	-	-	AROUND 200MG%	-	NORMAL	SLIGHT FATTY IN-FILTRATION (BIOPSY)	NORMAL NORMAL	INCIDENTAL COMMENT AS PART OF REVIEW OF ALCOHOLISM AND DIABETES.
JEUNE ET AL., 1960.	1	4½ YRS. LIGHT MEAL 16 HOURS BEFORE.	WINE-10% ALCOHOL.	COMA HYPOTONIA TRISMUS.	-	-	20MG%	-	20.8V%	97MG%	-	-	-	NORMAL	FAILURE TO REPRODUCE SYNDROME DESPITE REPLICATION OF CONDITIONS.
WEILL & GOROUBEN, 1960.	1	3½ YRS.	WINE ½ LITRE.	DEEP COMA HYPOTONIA PLANTARS FLEXOR. NYSTAGMUS.	34°C	-	15MG%	+	-	260MG%	10 HRS.	-	-	NORMAL	RAPID RESPONSE TO GLUCOSE NECESSITY FOR PRECEDING FAST IS STRESSED.
CUMMINS, 1961.	2	6 YRS NEGRO	GIN	COMA CONVULSIONS	-	UP	15MG%	NONE	20.5V%	20MG% 20 HRS LATER.	7 HRS.	-	-	-	DIED. HOWEVER, 350 MG AMYTAL ADMINISTERED TO CONTROL CONVULSIONS.
		3 YRS NEGRESS	EAU DE COLOGNE 20ML. (85% DE-NATURED ALCOHOL)	SEMI COMA THEN CON-VULSIONS.	-	-	22.5MG%	STRONG +VE.	9.1V%						RAPID RECOVERY AFTER DEXTROSE.
NEAME & JOUBERT, 1961	23	AFRICAN 21. INDIAN 2. MOSTLY OF POOR NUT-RITION.	MOSTLY AFRICAN BEER.	COMA IN 21, - CONFUSION IN 2. CONVULSIONS REFLEX CHANGE	UP IN 6 LOW IN 5.	9MG% TO 47MG%	+++ TO -VE	-	-	-	OCCASION AL IM-PAIRMENT.	14 NORMAL 3 CIRRHOSIS (BIOPSY); 3 FATTY IN-FILTRATION (AUTOPSY)	MINOR AB-NORM-ALITIES.	MOST PATIENTS COULD NOT BE TERMED CHRONIC ALCOHOLICS. INADEQUATE DIETARY INTAKE IS IMPORTANT. MILD BIO-CHEMICAL HEPATITIS IN SOME CASES.

*** "CANA" CONTAINS 40% ETHYL ALCOHOL.

**** "SCRAP IRON" SO NAMED BECAUSE OF ITS METALLIC TASTE. CHIEF INGREDIENTS: ISOPROPYL ALCOHOL AND NAPHTHALENE. ISOPROPYL ALCOHOL 14.6-25%; ETHYL ALCOHOL 23-46.7%. ZINC CONTENT 1.02-4.36 UG/1.

13c TABLE 2 (CONTD). A REVIEW OF THE LITERATURE OF CASES OF ALCOHOLIC HYPOGLYCAEMIA.

AUTHORS	NO. OF CASES	PARTICULARS	INGESTANT	CLINICAL FEATURES	TEMP.	B.P.	B.S.L.	URINE ACETONE	CO_2	BLOOD ALCOHOL	TIME INTERVAL	L.F.T.'S	LIVER BIOPSY OR AUTOPSY.	G.T.T.	OUTCOME AND COMMENT
TEELUCK-SINGH & SYMONDS, 1962.	7	CHILDREN 3-5 YRS.	RUM	COMA, STUPOR, HEMIPARESIS.	-	-	10MG% TO 40MG%	-	-	-	3 HRS TO 24 HRS.	-	-	-	5 RECOVERED AFTER GLUCOSE. 1 DIED AFTER 10 HOURS OF UNCONSCIOUSNESS. 1 DIED FROM BRONCHOPNEUMONIA POSSIBLY AFTER STOMACH WASHOUT.
FREDERICKS & LAZOR, 1963.	1	FEMALE 53 PREVIOUS PARTIAL GASTRECTOMY ALCOHOLIC.	WHISKEY	COMA, SPASTICITY	-	-	15MG%	-	-	-	-	NORMAL	NORMAL (BIOPSY)	ALIMENT-ARY GLYCOS-URIA.	RAPID RELIEF WITH DEXTROSE. 3 PREVIOUS EPISODES.
FREINKEL ET AL 1963.	9	2 FEMALE 6 MALE CHRONIC ALCOHOLICS. SCHOOLBOY 16 YEARS.	VARIOUS	COMA	RE-DUCED.	REDUC-ED IN EXPER-IMENTAL PERIOD	6MG% TO 34MG%	-	-	-	VARIED	NORMAL OR MARGIN-ALLY AB-NORMAL.	-	NORMAL 2 MILDLY ABNORMAL 3 DIABETIC 4	EXPERIMENTAL REPRODUCTION OF THE SYNDROME.
FIELD ET AL., 1963	1	52 WHITE MALE. PRO-LONGED INTAKE EPISODES OF HYPOGLYCAEMIA FOR 10 YEARS.	WHISKEY	SUBCUT-ANEOUS LIPON-ATOSIS. SENSORY NEURO-PATHY.	-	-	25MG%	-	-	-	-	NORMAL	-	NORMAL	ETHYL ALCOHOL INDICATED. FAST ESSENTIAL.
NEAME & JOUBERT 1963	1	MALE AFRICAN 4 YEARS.	GAVINE	SEMI-COMATOSE. BRADY-CARDIA.	-	-	19MG%	MODERATE	-	-	-	SERUM ALK. PHOS. ELEV-ATED.	-	NORMAL	IMMEDIATE RESPONSE TO 25ML 50% DEXTROSE. ELEVATION OF SERUM ALKALINE PHOSPHATASE AND TRANSAMINASE DURING FIRST FEW DAYS SUGGEST HEPATOTOXICITY.
ROCHE ET AL., 1963.	1	FEMALE 32 YEARS ALCOHOLIC	"ALCOHOL"	COMATOSE	35.5°C	NORMAL	30MG%	HEAVY	-	96MG%	12 HRS.	NORMAL	-	NORMAL	RECOVERED AFTER GLUCOSE
RAMON-GUERRA ET AL. 1963.	2	-	"ALCOHOL"	-	-	-	33MG%	-	-	-	-	-	-	-	EXPERIMENTAL INDUCTION OF THE SYNDROME IN TWO SUBJECTS PREVIOUSLY AFFLICTED.

13 d TABLE 2 (CONTD). A REVIEW OF THE LITERATURE OF CASES OF ALCOHOLIC HYPOGLYCAEMIA.

AUTHORS	NO. OF CASES	PARTICULARS	INGESTANT	CLINICAL FEATURES	TEMP,	B.P.	B.S.L.	URINE ACETONE	CO_2	BLOOD ALCOHOL	TIME INTERVAL	L.F.T.'s	LIVER BIOPSY OR AUTOPSY.	G.T.T.	OUTCOME AND COMMENT
MARKS & MEDD 1964.	1	FEMALE 53 ALCOHOLIC PREVIOUS PARTIAL GASTRECTOMY	-	COMA SPASTICITY	-	-	22MG%	-	-	-	24 HRS.	SERUM ALK. PHOS. SLIGHTLY RAISED.	NORMAL (BIOPSY).	-	COMA SUPERVENED AFTER ADMISSION IN ABSENCE OF ALCOHOL AND REQUIRED CONTINUOUS INTRAVENOUS DEXTROSE. HYPOGLYCAEMIA WAS REPRODUCED BY ORAL ALCOHOL 10% AFTER 40 HOURS FAST. GLUCAGON AND TOLBUTAMIDE TOLERANCE NORMAL.
GUMPEL & KAUFMAN, 1964	1	MALE NEGRO 47 YEARS. ALCOHOLIC. EPILEPTIC. PAST KORSAKOFF PSYCHOSIS.	-	COMA RIGIDITY.	94°F	LOW	12 MG%	-	-	-	-	NORMAL	-	NORMAL AFTER CHO FEEDING	PROMPT RECOVERY AFTER I.V. DEXTROSE. ATTEMPTED REPLICATION OF SYNDROME NOT POSSIBLE.
KAHIL ET AL., 1964.	1	NEGRESS 30 YEARS.	WHISKEY & WINE	EMACIATED CONDITION. LIVER PALABLE.	-	NORMAL	25MG%	-	-	-	18 HRS.	NORMAL	FATTY INFILTRATION (BIOPSY)	NORMAL	POINTS UP SIMILARITY BETWEEN INSULINOMA AND ALCOHOL HYPOGLYCAEMIA IN RESPONSE TO TOLBUTAMIDE TOLERANCE.
TOLIS, 1965.	4	CHILDREN 2½-6 YRS.	TSIPOURO OUZO	COMA, CONVULSIONS	-	-	25MG% TO 40MG%	-	-	-	RAPID	-	-	-	3 RECOVERED AFTER GLUCOSE. 1 DIED AFTER 3 HOURS DESPITE GLUCOSE AND CORTISONE.

However, the previously noted occurrence in children (Peluffo, 1958; Jeune et al., 1960; Neame and Joubert, 1963) contains two references (Weill and Gorouben, 1960 and Cummins, 1961) in whom adequate nutrition is documented. All of the cases of Freinkel et al., (1963) were reasonably nourished.

The overall clinical features of alcoholic hypoglycaemia do not vary intrinsically from those due to hypoglycaemia of other cause although marked findings of sympathetic excitation directed towards enhancement of hepatic glycogenolysis are not prominent. The degree of mental impairment varies widely from mild confusion to complete coma and relatively rapid excursions through these stages have been observed. In the case reported by Marks and Medd, (1964) the subject lapsed into coma after admission to hospital despite further abstinence from alcohol for 30 hours and intravenous therapy was required for 12 hours. Most patients were unconscious. Convulsive seizures, extension spasms, coarse tremors, trismus and conjugate deviations of the eyes were found. Some of these features may be attributable to depressed levels of magnesium (Mc Collister et al., 1960; Frankushen et al., 1964). A few, especially children, had depressed vital signs, muscular flaccidity and absent deep tendon reflexes. The plantar response was usually flexor but rough correlation can be made between the description of the depth of coma and the development of extensor reflexes in accord with the presence of the more ominous 'medullary syndrome' (Himwich, 1944). Sweating is not frequently reported. Pupillary changes were rare. The pulse and blood pressure varied but were usually slow and somewhat depressed respectively.

Hypothermia was commonly observed where temperatures are noted and recovery has often disclosed an unsuspected pyrexia. The hypothermia may reflect the central origin of the hypoglycaemia as recognised in affliction of the posterior hypothalamus. Hypothermia as a sign of hypoglycaemia has been commented upon by Kedes and Field, (1964) and has been confirmed by further studies of this author (Cohen, unpublished observations). Such studies have shown, conversely, that potentiation of the hypoglycaemia induced by ethyl alcohol may be obtained by exposure of the subject to cold. In this respect it is interesting to note that Masson (1941) first described the greater hypoglycaemic action of insulin in rats fasted for 24 hours during exposure to $-2^{\circ}C$ to $+2^{\circ}C$ for 1, 2, 6 and 9 days. The effects became greater the longer the exposure. Other workers have noted a depletion of hepatic glycogen in the liver of the rat after initial exposure to cold and an increase in glucose-6-phosphatase* activity. The turnover and oxidation of glucose is also increased (British Medical Bulletin, 1961).

The urine contained acetone in many cases and slight reduction of serum bicarbonate suggested the presence of acidosis in some cases. The blood sugar was reduced in all instances but varied widely depending on the method of estimation, the time interval between exposure to

*Throughout this thesis the trivial names of enzymes are employed. These are as defined by The Report of the Commission on Enzymes of the International Union of Biochemistry, 1961. I.U.B. Series, Volume 20, Pergamon Press.

alcohol and the drawing of blood and sometimes the intervention of emergency glucose administration. Values as low as 6 mg.% (Freinkel et al., 1963) and 9 mg.% (Neame and Joubert, 1961) are not uncommon.

Blood alcohol levels, where estimated, were surprisingly low and have not exceeded the level of mild introxication regarded as about 200 mg.%. Exceptions were the cases of Neves et al., (1950) and Weill and Gorouben, (1960) where levels of 482 and 260 mg.% respectively were recorded.

Almost all cases have responded rapidly and completely to the administration of glucose and spontaneous recovery was noted in two of the six cases reported by Brown and Harvey, (1941) without specific therapy. These are the cases observed at the Johns Hopkins Hospital in the period 1934-1940. In the first, spontaneous recovery occurred in 5½ hours after admittance with a rise of blood sugar from 15 to 105 mg.% and the temperature from 96°F to 101.6°F. In the other, a slow spontaneous recovery required 2 days and no further details are given. In the case reported by Marks and Medd, (1964) prolonged and vigorous dextrose therapy was required to prevent relapse. Of the 3 fatal cases in the series of 11 reported by Bottura et al., (1949) all received glucose and insulin as therapy.

Hypoglycaemia unrelated to alcohol consumption occurs in a wide variety of hepatic disorders and is an indication of grave and diffuse parenchymal disease (Hed, 1958). Much discussion has therefore naturally centred around the role of the liver in this clinical syndrome. Brown and Harvey, (1941) noted demonstrable evidence of damage to the liver

in just one of six cases and Tucker and Porter, (1942) commenting on the possible presence of cirrhosis noted that "little was found after recovery". Where autopsies have been performed, the most common abnormal finding has been fatty infiltration of the liver which would not be unexpected in any group of malnourished, alcohol-imbibing cases. Those patients reported by Hammack (1957) of whom 13 out of 36 died, were for the most part acutely or dangerously intoxicated. Hypoglycaemia was reported in only 6 cases and whilst fatty infiltration of the liver was present in the autopsy material, pancreatic haemorrhages could not be correlated with the hypoglycaemic picture.

On the other hand, most biopsy studies have revealed absent or minimal hepatic parenchymal changes. The liver was normal in the first biopsy reported in the literature by Neves et al., (1950) and subsequently Neame and Joubert (1961) performed 20 liver biopsies in their series of 23 cases of which 17 were available for study. Of these 17 biopsies, only 3 were abnormal, showing evidence of cirrhosis. The remainder showed a normal architectural pattern. Sufficient tissue was obtained for fat staining in 4 cases and in 3 of these significant fatty infiltration was observed. In the 3 cases where liver biopsy was performed on admission, absence of glycogen was noted, while in the case where the liver biopsy was performed an hour and a half after the administration of dextrose, minimal glycogen deposition was observed.

Biochemical tests of liver function have been non-specific, but most cases have yielded normal results and in the remainder, where some malfunction has been observed,

the tendency has been for its correction soon after the acute episode has subsided. Elevation of the serum alkaline phosphatase has been the most commonly reported abnormality and levels of serum glutamic oxaloacetic transaminase have also been elevated in some cases. The latter estimation would seem the most sensitive in the assessment of the effects of alcohol on the liver. Bang et al., (1958) observed elevation of serum glutamic oxaloacetic transaminase activity following the ingesting of alcohol by chronic alcoholic patients and concluded that such changes were sensitive indices of liver damage; they failed to show any such rise in healthy volunteers. Madsen and his colleagues (1959) confirmed these observations finding the transaminases elevated in 27 of 35 chronic alcoholics after alcohol. The icteric index, thymol extinction and plasma proteins remained normal. Transaminase activity remained elevated from 2 hours to 11 days, usually falling to normal by the 4th day. There was rough correlation between the blood alcohol and the maximum serum glutamic oxaloacetic transaminase. Beckett et al., (1962) found similar elevations of the transaminases in 3 of 4 cases in whom all other liver function tests were normal and concluded that "no test of liver function other than the serum enzyme tests constantly indicates liver cell damage." They further concluded that accurate diagnosis of liver dysfunction was only possible after liver biopsy. However, Neame and Joubert (1961), as already mentioned, had 14 normal liver biopsies out of 17 cases and several of their patients showed minor elevations of serum transaminase activity. These elevations were again induced in one of their cases to whom alcohol was given after recovery.

This point has been more extensively treated since it is obviously of considerable importance. Opinions vary widely as to whether alcohol is "toxic" to the liver. Some authors believe there to be no direct action and the work of Summerskill et al., (1957) would seem to confirm this. On careful observation of liver function tests and repeated liver biopsies they were unable to demonstrate any deterioration after the administration of alcohol. They did not however study changes in serum transaminases. Madsen et al., (1959) and Beckett et al., (1962) believe that varying degrees of hepatotoxicity may ensue. Most of the reported cases of hypoglycaemia due to alcohol have had critical tests of liver function performed including, in many cases, liver biopsy (see Table 2). The overall impression gained from this assessment does not suggest active liver damage as a factor in its development.

Attention to carbohydrate metabolism was inevitable but surprisingly few abnormalities have been documented. Tucker and Porter (1942) performed glucose tolerance tests on 3 of their 4 cases. In the first, a 35 year old negress, the response to glucose challenge on the day after admission was normal except for a low fasting level of 60 mg.% but retesting on the 8th and 13th hospital days revealed a flat-curve response. The other two cases responded normally. Glucose tolerance tests have been performed in many of the reported cases (Table 2) and have been recorded as normal in most instances. Some tendency to difficulty in handling a glucose load might be expected since this is a fairly common finding in chronic alcoholics (Hed, 1958). In the series reported by Freinkel et al., (1963) oral glucose tolerance was normal in 2 of 9 patients, marginally abnormal in a further 3 and frankly abnormal in 4.

Repeat observations were possible in 6 patients; all had gained weight in the interval. In 2 of the subjects whose glucose tolerance was mildly abnormal improvement to normalcy had occurred but in the other 4 subjects glucose disposition remained retarded despite some improvement. These authors further challenged glucose homeostasis with intravenous insulin, tolbutamide and the effects of 72 hours fasting. Abnormalities in one or more of these aspects were exhibited by all cases. All showed a tendency to excessive responsiveness after intravenous insulin and all but one displayed some degree of hypoglycaemic unresponsiveness so that restoration to the initial concentration of plasma glucose was not achieved within 120 minutes. In the 4 patients in whom the test was repeated the patterns persisted despite the interval administration of an adequate diet and some consequent gain in weight. Jeune and his group (1960) noted a normal response to 0.1 units of insulin per kg. body weight in the subject tested. As previously noted however, three of the fatal cases of Bottura et al., (1949) had received glucose and insulin.

Freinkel and his colleagues (1963) further assessed the response to intravenous Tolbutamide after overnight fasting. A diabetic tendency was confirmed in the 3 marginal cases who all showed a delay in initial blood glucose fall. In all 9 cases the plasma glucose continued to decline so that maximal reductions did not occur until 60 or more minutes after the injection of Tolbutamide. Such delayed nadirs might have been anticipated in the 3 patients with the diabetic type of 30 minute response (Unger and Madison, 1958) but in the others the prolonged reductions were contrary to the usual experience in non-diabetic subjects.

In 3 of their patients the hypoglycaemia was prolonged to an extent that is classically associated with massive hepatic parenchymal disease or islet-cell tumors. Gumpel and Kaufman (1964) found a similar steep decline in blood sugar after Tolbutamide and Kahil et al., (1964) were constrained to make a diagnosis of islet-cell tumor in their case of hypoglycaemia due to alcohol because of this exaggerated response.

The important role of poor nutrition or immediate dietary deprivation in the genesis of hypoglycaemia due to alcohol was recognised immediately by the earlier authors. Brown and Harvey (1941) caused their 6 patients to fast for 48 hours without obtaining a fall in blood sugar. Both Bottura et al., (1949) and Neves et al., (1950) concluded that fasting alone was insufficient to initiate the phenomenon although the duration of the fasting in the cases reported by the latter authors was no longer than 24 hours. Jeune et al., (1960) tried to reproduce the hypoglycaemia in their 4 year old boy but were unsuccessful. Weill and Gorouben (1960) stressed "the indispensable combination of alcohol ingestion and the prolonged preceding fast". However, Neves et al., (1950) cite the case of one chronic alcoholic who developed hypoglycaemia after a prolonged fast without antecedent alcohol and with no demonstrable alcohol in the blood. Freinkel et al., (1963) were able to induce significantly lowered levels of venous plasma glucose at the end of 72 hours fasting in their subjects. These levels were significantly lower than in agematched controls. This reduction of blood sugar was not further enhanced by exercise as might have been the case in the presence of islet-cell adenoma or hyperplasia.

The potentiating action of fasting on the development of the hypoglycaemia has been demonstrated experimentally in dogs by Clark et al., (1961). Using pure ethyl alcohol and the shellac solvent 'Solox' they were able to obtain significant depression of blood glucose levels and found that the necessary dosage was greatly reduced by prior fasting. Brown and Harvey (1941) using dogs and Bennett (1953) using rabbits had failed to demonstrate such an effect even when fasting was employed. Fasting is also recognised as an important pre-requisite for the blood sugar-lowering action of Blighida sapiens, the material responsible for Jamaican vomiting sickness (Hassal, 1954).

On the other hand, Haggard and Greenberg (1937) have demonstrated that the toxicity of alcohol is inversely influenced by the concentration of sugar in the blood. Elevation of the blood sugar by only 30 mg.% in their rats required a corresponding alcohol dosage increase of 20% to cause lethal effects.

The group at the Thorndike Laboratory first attained the experimental reproduction of the syndrome in man. Using intravenous alcohol or alcohol regularly administered by stomach tube significant reductions of blood sugar were obtained. (Freinkel et al., 1963). Their subjects were prepared by an overnight fast. In 5 of their 7 cases the plasma glucose was reduced below 50 mg.% and the other features of the clinical syndrome were reproduced. Hypoglycaemia occurred late in the phase of alcohol administration and frequently when the blood alcohol concentration was declining. When induced after a 3 day fast, the hypoglycaemia was more pronounced and acute.

Field et al., (1963) substantiated these findings in their case and noted the difficulty in producing significant reductions after an overnight fast compared with the simplicity after a 44 hour fast. Such difficulty had been foreshadowed by the studies of Tennent (1941) and Lieber et al., (1962) in normal subjects.

C H A P T E R 111

Aetiological Mechanisms

Despite the fact that over one hundred cases of hypoglycaemia due to alcohol have been reported, the physiological causation remains unknown. Most investigators have put forward suggestions based on theory, attempted replication in the patient or animal experimentation. None has fully elucidated all of the surrounding aspects.

Brown and Harvey (1941) postulated that reduction of hepatic glycogen by starvation associated with continued drinking may have been contributory but remarked that starvation alone has not been known to produce hypoglycaemia in so short a period. They could not discern "evidence of either wide-spread damage to the liver or of hyperinsulinism". They thought it possible that a poisonous substance might have caused the increased utilisation of carbohydrate with resulting hypoglycaemia under the particular circumstances of depleted glycogen supply or that such a substance might temporarily inhibit gluconeogenesis interrupting the normal transformation of protein to carbohydrate. Attempts to reproduce the syndrome in two dogs starved from 48 hours and then kept in a state of acute alcoholic intoxication for a further period of 24 hours by the administration of "smoke" through a stomach tube were unsuccessful.

Tucker and Porter (1942) agreed broadly with the foregoing but considered that the denaturing substances present in 'Solox' were most likely responsible in view of the transient nature of the disturbance. Because of the rarity of the condition and the absence of obvious manifestations of liver disease or endocrine imbalance, they suggested that there might be an inherent abnormality of carbohydrate metabolism and quoted in support of this the tendency for the condition to recur in the same individual. Such susceptibility has been confirmed by Ramon Guerra and his colleagues (1963). Either temporary interference with liver function or more specific inhibition of gluconeogenesis were regarded as the most likely cause.

In the 11 cases reported by Bottura et al., (1949), chronic alcoholism, vitamin deficiency, hepatomegaly and prolonged prior fasting prompted the authors to invoke fatty infiltration of the liver as a mediating cause. Taylor, (1955) concurred. On the other hand, Neves et al., (1950) found an absence of fatty change in their one case biopsied and drew attention to the fact that fasting alone was insufficient to induce hypoglycaemia. Some combination of alcohol and food deprivation was obviously required. The 4 year old child described by Jeune et al., (1960) was subjected at first to a further amount of alcohol without lowering of blood sugar and an increased amount after a period of fasting simulating the initial experience was again without effect. Both Bottura et al., (1949) and Neves et al., (1950) regarded defective synthesis of glycogen due to interference with Krebs cycle activity as a predisposing cause.

Peluffo et al., (1958) proposed a mechanism similar to the 'dumping syndrome' following partial gastrectomy. They suggested that the rapid transition of alcohol into the small intestine provokes a massive release of insulin. This, in the absence of available glucose mobilisation from glycogen, would permit the development of hypoglycaemia. However, no stimulation of the pancreas has been documented following alcohol administration and Freinkel et al., (1963) were unable to discover increased amounts immunologically reactive insulin in humans nor were the correlative biochemical findings of insulin action demonstrable in their cases. Bleicher et al., (1964) also failed to demonstrate a significant rise in plasma insulin in their alcohol treated dogs and Lochner et al., (1967) report a mean decline of mean plasma insulin levels from 20 to 15 uU/ml. during ethyl alcohol infusion in dogs. Kahil et al., (1964) noted an excessive response to the administration of Tolbutamide in their undernourished dogs maintained on alcohol additions to the diet, but because of a rise in the urinary excretion of 17-ketogenic steroids and a fall in the excretion of catcholamines suggested that impairment of the adrenal counter-regulatory mechanisms to hypoglycaemia rather than excessive insulin secretion were responsible.

Suppression of a central diencephalic mechanism was invoked by Weill and Gorouben (1960). After citing an excellent review of the evidence for a central glycaemic centre in the hypothalamus they suggested that an autonomic mechanism arises during fasting which acts to reduce hypoglycaemia. They suggested that the administration of alcohol or one of its derivatives exerts an inhibitory action on the diencephalic centre and that certain individuals are unduly sensitive to this action.

Heelpien et al., (1954) considered that thiamine deficiency had a potentiating effect on the production of hypoglycaemia with alcohol. However, the fact that alcohol can produce hypoglycaemia in acute studies in normal persons (Field et al., 1963) makes this hypothesis unlikely. Moreover, when Clark and his colleagues (1961) produced hypoglycaemia in fasted dogs given 'Solox' it was found that the hypoglycaemic response was not influenced by pre-treatment with thiamine.

A reduction of hepatic glycogen or some inhibition of its coversion to glucose has been postulated by many investigators. The importance of glycogen depletion is suggested by earlier studies in animals in which hypoglycaemia was produced only in those whose livers were devoid of glycogen (Matanuga, 1942; Tennent, 1941). Large doses of ethyl alcohol in animals have been found to produce a decrease in liver glycogen (Forbes and Duncan, 1960). Neame and Joubert, (1961) reported liver glycogen absent from biopsy specimens obtained from their patients during periods of hypoglycaemia. No significant rise in blood sugar was obtained with glucagon in the experimentally-induced alcohol-hypoglycaemia by Field et al., (1963) or Freinkel et al., (1963) whereas the latter group were able to demonstrate a significant response in the same subjects receiving a control infusion of isotonic saline. However, glycogen depletion is a well documented consequence of even moderate fasting and certainly after such stressful occurrences in man as major surgery (Sunzel and Ekdahl, 1963). It is important to note that hepatic glycogen provides only a meagre reserve for the continuing carbohydrate requirements of the organism and Myers, (1950) has calculated that the total hepatic glycogen in man

(about 75 gram) is only sufficient to supply approximately 12 hours of splanchnic glucose output. After a 2 to 3 day period of starvation, the output of glucose by the liver is derived almost entirely by gluconeogenesis (Renold et al., 1953; Wood et al., 1960).

Previous studies by the group at the Thorndike Laboratory in Boston (Freinkel et al., 1963; Cohen et al., 1963; and Bleicher et al., 1964) had prompted the suggestion that ethyl alcohol might directly impair gluconeogenesis, and the subsequent formation of glycogen, from non-carbohydrate sources within the liver. This suggestion was supported by the 4 in vitro studies of Field et al., (1963) which showed a 50 per cent reduction in hepatic glucose production by the isolated rat liver perfused with ethyl alcohol at a concentration of 410mg /100ml. In the same experiments a decreased incorporation of glucose-C^{14} into glycogen was observed. However, this concentration of ethyl alcohol clinically is associated with profound coma and is not far removed from blood levels that may be lethal in humans. Coppage et al., (1964) showed interference by ethyl alcohol with the normally expected blood sugar elevating and glycogenic response after the infusion of amino acids in man.

In 1963, Lochner and Madison demonstrated a decrease in hepatic glucose output in fasted dogs rendered hypoglycaemic after alcohol infusion. These animals had chronic end-to-side portocaval shunts in order to permit the measurement of hepatic rather than splanchnic glucose balance but subsequent experiments (Lochner et al., 1967) not only confirmed these findings but demonstrated a similar albeit lesser effect in intact, healthy animals in the fasting state. In these later studies, some inhibition of peripheral glucose

utilisation was also noted but hypoglycaemia only developed when this effect was less than that on hepatic glucose output. The latter was usually the case.

Freinkel ascribed a mediating role in this interruption of gluconeogenesis to the effects of intrahepatic oxidation of alcohol on the ratio of reduced nicotinamide-adenine nucleotide ($NADH_2$) to the oxidised form (NAD^+) in the soluble cytoplasm (Freinkel, 1962). Further critical studies utilising rat liver slices under conditions of varying availability of endogenous substrate have supported this hypothesis (Freinkel et al., 1965). The paper reporting these studies is to be found in Appendix 2. The complementary investigations using human liver slices were carried out at the same time and form the basis of the work hereafter to be described.

Recently, Madison et al., (1967) have adduced further support for the concept of an alteration in $NADH_2/NAD^+$ ratios being responsible for the suppression hepatic gluconeogenesis. Using dogs with chronic end-to-side portocaval shunts they have demonstrated that fructose, a non-NAD dependant precursor of glucose, produced a rapid restoration of hepatic glucose output during ethyl alcohol infusion. The administration of glutamate and a keto-glutarate, both NAD dependant precursors of glucose, failed to augment the depressed hepatic glucose output induced by ethyl alcohol. Finally, the addition of methylene blue, a redox dye which oxidises $NADH_2$ to NAD^+, not only prevented the expected fall in hepatic glucose output when infused simultaneously with ethyl alcohol, but also produced a rapid restoration of hepatic glucose output previously depressed by ethyl alcohol

administration in the fasting dogs. Whilst changes in hepatic glucose output are not necessarily indicative of changes in hepatic gluconeogenesis, these findings are highly suggestive that the new formation of glucose has been influenced.

C H A P T E R IV

Materials and Methods

The series studied comprises 22 patients of whom 13 were women and 9 men. The number of experiments is 27 since in some instances the same liver biopsy was used under different conditions of added substrate or mode of incubation.

Selection of Material:

Biopsies of human liver were obtained at laparotomy from patients on the Surgical Services of the Boston City Hospital. The subjects were seen personally prior to operation and a full history and physical examination performed. Cases of overt liver disease, malnutrition or diabetes mellitus were rejected from the study. Following clinical assessment, blood was drawn from the estimation of serum alkaline phosphatase, pro-thrombin time, serum bilirubin and cephalin-cholesterol flocculation. Where some doubt was entertained as to the suitability of the subject for *in vitro* liver studfes, ancillary tests such as bromsulphthalein retention and plasma protein levels were carried out.

Patients undergoing surgery at the Boston City Hospital are fasted for 12 to 15 hours preoperatively. All cases were operated upon during the morning. The preoperative medication consisted of atropine and pethidine in dosage proportionate to the body weight. Anaesthesia was induced

with intravenous pentothal and nitrous oxide and maintained with cyclopropane. Wherever possible the liver biopsy was obtained before the administration of the latter gas and, in all instances, as the first surgical procedure after opening the peritoneum. The average time from the induction of anaesthesia to the removal of the liver wedge was 15 minutes.

Preparation of the tissue:

The excised liver was placed directly into a polyethylene bag and enclosed in ice. Immediately following its removal a specimen of venous blood was withdrawn without stasis from an antecubital vein into a heparinised tube for subsequent analysis of blood sugar and non-esterified fatty acids. The material was then transported to the laboratory for slicing - usually within ten minutes.

Wedges of excised liver were freed of capsule. A separate aliquot was introduced directly, after weighing, into boiling 30% KOH for the estimation of initial glycogen by the method of Good as modified by Stadie (1951). Immediately thereafter, the remaining liver was sectioned free-hand into slices of 0.4 to 0.5 mm. thickness, rinsed briefly in saline to remove adherent detritus, plasma proteins and extracellular substrates and floated at room temperature in the same medium that was to be employed for the subsequent incubation - i.e., Krebs Ringer Bicarbonate, pH 7.4 or Krebs Ringer Phosphate, pH 7.4. This manoeuvre resulted in greater uniformity of response during the experimental period.

Slices were briefly blotted, weighed on a torsion balance and 150 to 250 mg. portions were introduced into vessels

filled with 2ml. of the suspending medium. The slices were incubated for 3 or sometimes 2 hours in stoppered vessels containing 2 ml of KRB (pH 7.4) or KRP (pH 7.4). The KRB and KRP were modified to contain 0.8 mM Ca^{++}.

Warburg vessels were used for the manometric studies. All other experiments were performed in glass vials sealed with rubber serum caps from which plastic cups were suspended for the collection of $C^{14}O_2$ (vide infra) Figure 1. All preparative manipulations were performed at room temperature; more than half of the initial glycogen was lost in the process.

Preparation of Media:

Suspending media were variously supplemented with:

(a) 0 to 10.0 millimolar ethyl alcohol (i.e., 0 to 10 micromoles per ml.

(b) 0 to 10.0 millimolar sodium acetate.

(c) 1.0 microcuries per ml of one of the following labelled substrates in concentration 0.01 to 10.0 millimolar:-

(i) Uniformly labelled L-alanine-C^{14}, (Alanine-U-C^{14}).

(ii) Uniformly labelled L-glutamic acid-C^{14}, (Glutamate-U-C^{14}).

(iii) Glycerol-C^{14} labelled in the 1 and 3 positions, (Glycerol 1, 3-C^{14}).

(iv) D-L lactate-1-C^{14}.

All chemicals were of reagent grade. The ethyl alcohol was obtained from freshly opened bottles at each experiment.

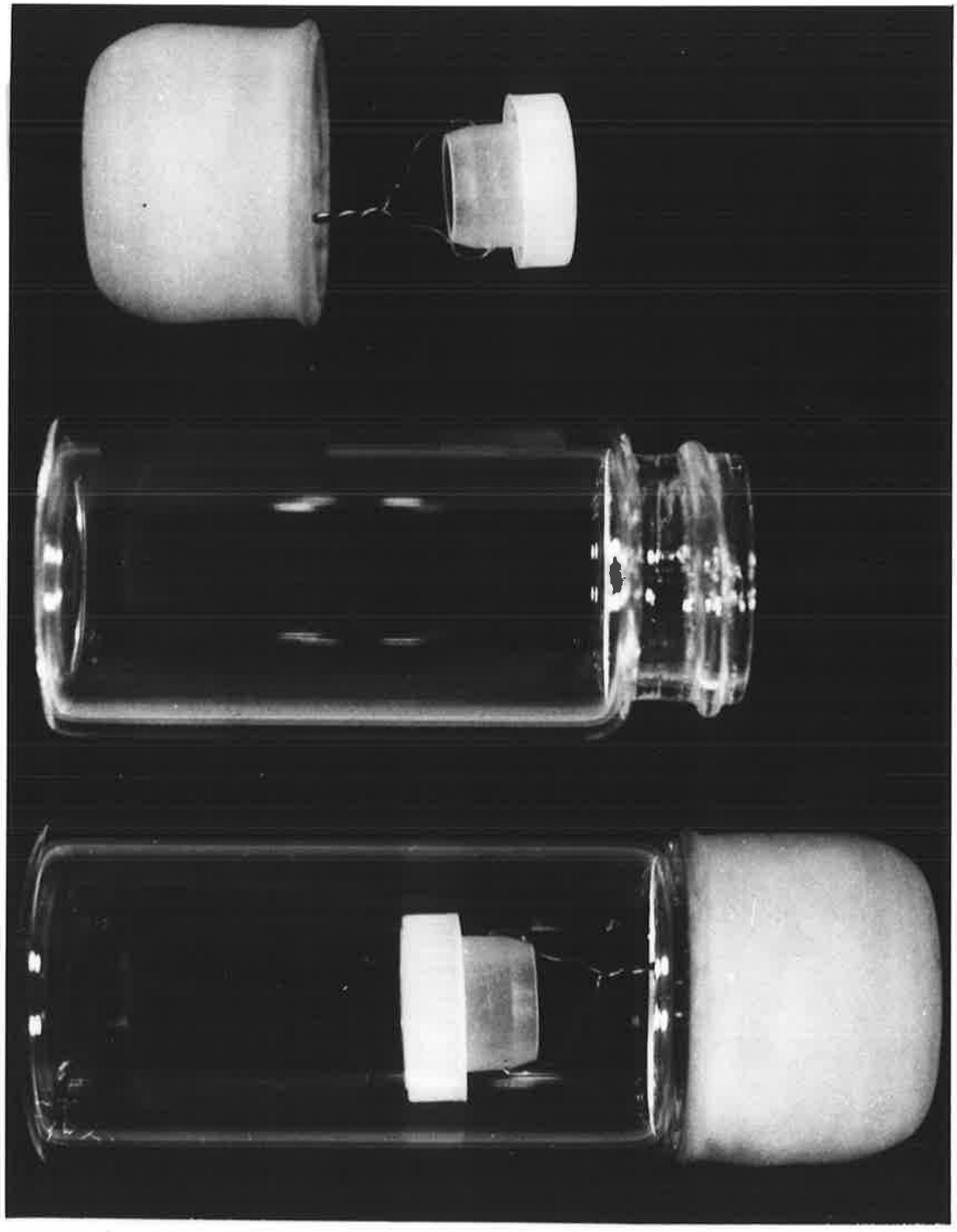

Fig. 1. Hydroxide of Hyamine was introduced by hypodermic syringe through the rubber cap into the plastic cup for the "trapping" of $C^{14}O_2$ at the end of the experimental period.

Labelled alanine, lactic and glutamic acids were obtained from the New England Nuclear Corporation, Boston, Massachusetts; glycerol 1,3-C^{14} was secured from Volk Chemicals, Chicago, Illinois. In one experiment the suspending media were supplemented by fructose 10 millimoles and sorbitol 10 millimoles and pyruvate 10 millimoles. In all supplemented media osmolality was maintained constant by appropriate dilutions of NaCl and the pH was preserved at 7.4 by adjustment with 0.3N NaOH or HCl prior to adding bicarbonate or phosphate buffer.

Incubation procedures:

Incubation for 90 to 180 minutes at 38°C was performed in the Warburg apparatus (shaking rate, 104 cycles per minute) with conventional 15ml. Warburg vessels, or in the Dubnoff metabolic incubator (shaking rate, 90 to 100 cycles per minute) using glass vials as previously described and depicted in figure 1. Except for 3 experiments in which a 2 hour period was employed, all incubations lasted 3 hours.

In KRP studies, oxygen consumption was measured at 10 minute intervals by standard manometry and $C^{14}O_2$ was collected continuously in NaOH contained within the sidearms of the Warburg vessels. For the KRB experiments, $C^{14}O_2$ was evolved at the end of incubation by the introduction of 0.2ml. of $N.H_2SO_4$ into the sealed vessels. The $C^{14}O_2$ from KRB as well as KRP studies was finally "trapped" in Hydroxide of Hyamine by gently shaking for 90 minutes at room temperature. "Blank" vessels from which slices had been omitted throughout the incubation were processed in a similar fashion to check the alanine-U-C^{14} for volatile contaminants which were soluble in Hyamine. Although such

contamination averaged only 0.054 ± 0.008% (Mean ± Standard error of the mean), appropriate corrections were instituted in the estimation of $C^{14}O_2$ (Freinkel et al., 1965).

Processing of Media and Tissues:

After $C^{14}O_2$ had been evolved, the slices were removed from the vessels, blotted, weighed and frozen in liquid nitrogen. Media were centrifuged to remove insoluble debris and stored at -18°C.

To estimate glycogen, frozen slices were digested in boiling KOH and twice precipitated with 60-65% alcohol (Good et al., 1933). The precipitated glycogen was then hydrolysed in 5N H_2SO_4 for 30 minutes, neutralised with 5N NaOH or with powdered $BaCO_3$. Where necessary, carrier quantities of unlabelled glycogen were added prior to precipitation. In some experiments, fat free aqeous extracts of the tissues were prepared by homogenising liver slices in 70% ethyl alcohol and treating the extracts with carbon tetrachloride (Freinkel, 1961). In these studies, the insoluble residues were digested with alkali and glycogen was isolated as above.

Duplicate aliquots of the tissue extracts, neutralised glycogen hydrolysates and final suspending media were analysed for glucose enzymatically (Huggett and Nixon, 1957) and the measurements were related to the "initial wet weight" of the slices (i.e., micrograms of glucose per 100 mg. of tissue). Previous studies (Freinkel et al., 1964) had shown that the relationship of the "initial wet weight" with the "final wet" and "final dry" weights were quite constant and unaffected by the inclusion of ethyl alcohol in the suspending media. Accordingly, "initial wet weight" of the slices

was employed as the reference standard for all measurements.

Radioactive estimations:

(a) Paper Chromatography:

One-inch wide strips from 18"x22½" sheets of Whatman No. 1 filter paper were employed for the quantitative one or two dimensional chromatography. The neutralised glycogen hydrolysates were applied onto paper directly; tissue extracts and final suspending media were first deproteinised by precipitation with equal volumes of acetone at 2°. Aliquots of 100 microlitre were applied in duplicate and spots were overlaid with 20 microgram of unlabelled glucose.

Ascending chromatography in isopropanol: pyridine: acetic acid: water (8:8:1:4, v/v) was utilised as the primary system because inorganic salts do not appreciably affect resolution in this solvent (Gordon et al., 1958). For further characterisation, ascending chromatography in secondary butanol: formic acid: water (75:15:15 v/v) (Beloff-Chain et al., 1959), or phenol: NH_3 (phenol saturated with 0.1 NH_3 v/v), and high voltage ionophoresis (5,000 volts) at pH 3.7 in pyridine: acetic acid: water (1:10:289 v/v) (Katz et al., 1959), were employed. Chromatograms and ionophoretograms were dried at room temperature. Radio-active components were located by radioautography for 8 hours to 2 weeks in contact with X-ray film, in metal casettes, at 4°. The glucose in glycogen hydrolysates was insufficiently labelled for radioautographic detection, therefore, the glycogen hydrolysates were chromatographed with paired reference strips carrying glucose. The glucose markers were identi-

fied by spraying with benzidine (Horrocks and Manning, (1949) and corresponding areas on the "glycogen" strips were analysed for radio-activity.

(b) Radioactive assay:

$C^{14}O_2$ was counted by introducing the Hyamine into liquid scintillation media containing 12% methanol, 0.4% 2, 5-diphenyloxazole (PPO), 0.005% 1,4-bis-2-(5-phenyloxazoyl) -benzene (POPOP) and toluene. Counting standards were prepared by directly assaying aliquots of suspending media which had been incubated without tissues (i.e.,"blank media"). Chromatograms were counted by cutting out appropriate spots and introducing these segments into vials containing PPO, POPOP and toluene without methanol. Counting standards were prepared by assaying aliquots of "bland" media which had been dried on Whatman No. 1 filter paper. To minimise the geometric variation in the assay of the paper spots, the vials were counted at least twice with 90° variation in their position relative to the photomultiplier tubes of the automatic Packard instrument.

Samples were counted sufficiently long to reduce the error to $\pm$ 3% i.e., 3,000 counts above background were observed.

Radioactive measurements were expressed as a function of the appropriate counting standard (i.e., as "percentage of the total counts per gram of tissue"). Since the experimental vessels usually contained 100-200 mg of tissue this mode of expression has sometimes resulted in values for "uptake" being greater than 100%. This relationship to a gram of tissue has been retained throughout all the tables in order to maintain uniformily expressed results.

Characterisation of the <u>in vitro</u> system:

Radioautographs of one-dimensional chromatograms of the final suspending media developed in isopropanol: pyridine: acetic acid: water, disclosed 6 radioactive spots with the following Rf characteristics: *1 = 0.04-0.07: *2 = 0.10-0.13: *3 = 0.24-0.30: *4 = 0.42-0.48: *5 = 0.51-0.59: *6 = 0.65-0.75. A seventh spot (Rf 0.81-0.92) was inconstantly discernible. Chromatography (Figure 2,) two dimensional chromatography (Figure 3), ionophoresis (Figure 4) or ionophoresis in a second dimension (Figure 5) and co-chromatography with known marker, indicated that spots *1, 3, 5 and 6 were homogenous and consisted of aspartic acid-C^{14}: alanine-C^{14}: glucose-C^{14} and lactic acid-C^{14} respectively. Spot *2 contained two radioactive components; the most heavily labelled (i.e., spot *2A) was identified as glutamic acid-C^{14}.

Spots *4 and *7 may have been artefacts of the preparative technique; spot *4 was variable in intensity and correlated most closely with the labelling of glutamic acid (*2A). It was found inconstantly during chromatography of acidified KRB solutions containing only glutamic acid-C^{14} and has been identified provisionally in consequence as a salt derivative of glutamic acid. Spot *7 appeared only in acidified media and was also reproduced by acidifying KRB solutions containing only lactic acid-C^{14}.

Recoveries of glucose-C^{14} and alanine-C^{14} (when taken through all of the preparative manipulations) ranged from 94% to 104%. Recoveries of lactic acid-C^{14} were more erratic. Experiments with 3 lots of lactic acid-1-C^{14} in varying concentrations showed that:

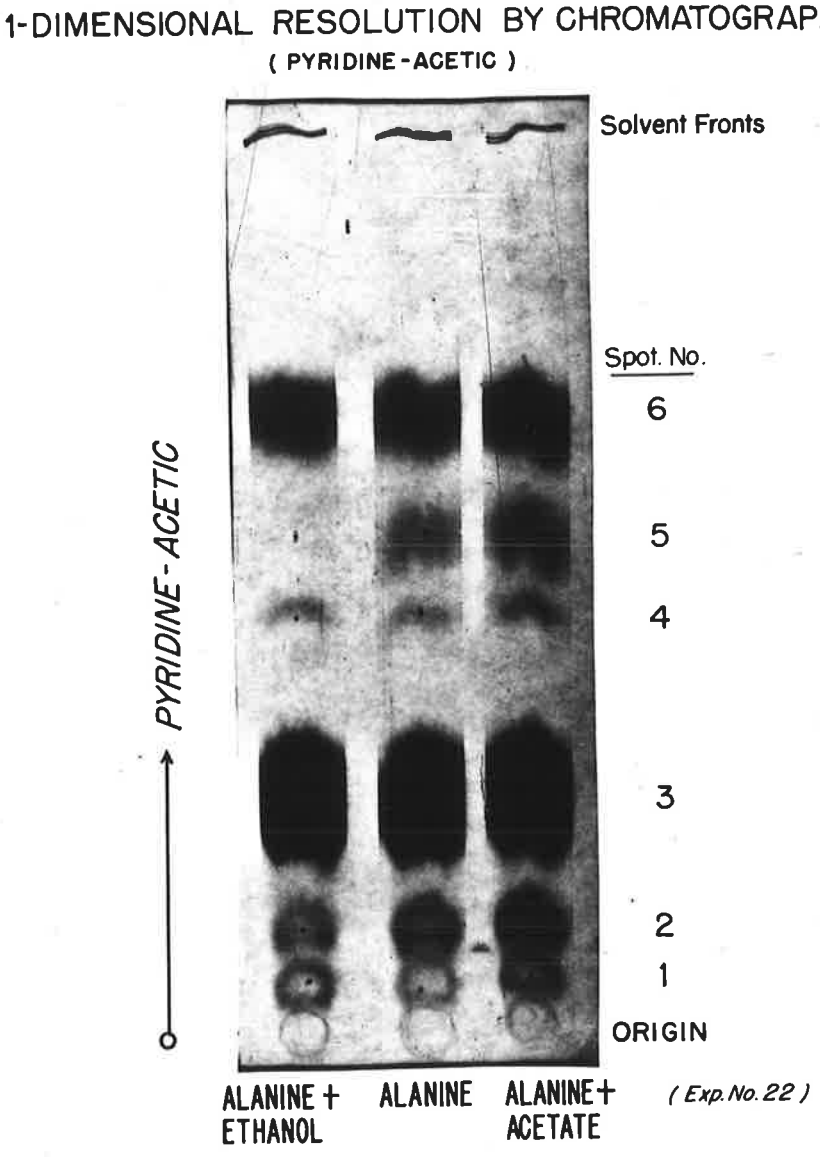

Fig. 2. Radioautograph of 1-dimensional chromatograms of final suspending media. Human liver slices were incubated in KRB with 10.0 mM amounts of alanine-U-C^{14} (1.0 μc/ml). Experimental vessels were supplemented with 10.0mM ethyl alcohol or 10.0mM sodium acetate. Individual radioactive components are designated by the numbers employed in the text. Spot# 7 is not here visible.

2-DIMENSIONAL RESOLUTION BY CHROMATOGRAPHY
IN PYRIDINE-ACETIC & PHENOL-AMMONIA

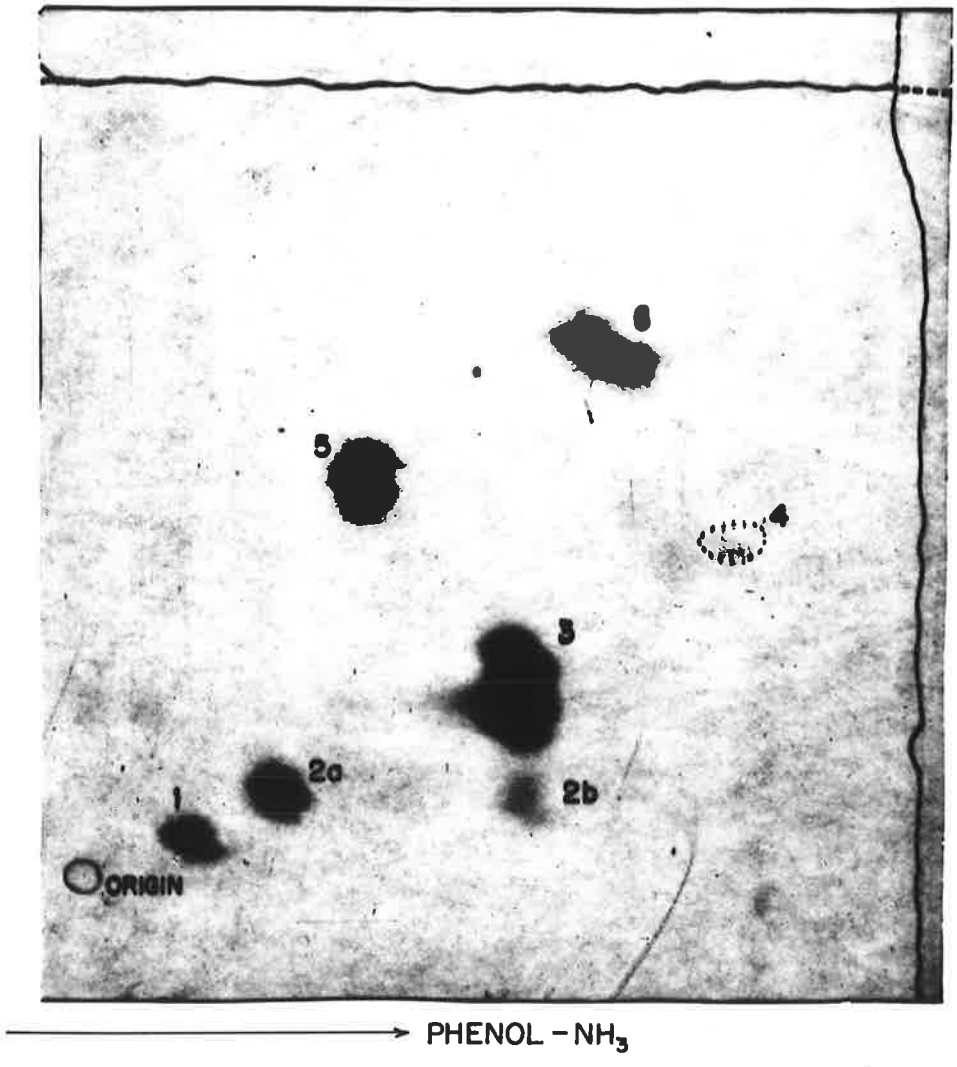

Fig. 3. Radioautographs of 2-dimensional resolution of final suspending media. Following initial chromatography in isopropanol:pyridine:acetic acid:water, secondary resolution was effected by chromatography in phenol:ammonia. The dissociation of Spot 2 can be appreciated. (Vessel supplemented with acetate 10mM; alanine-U-C^{14}. Expt #22).

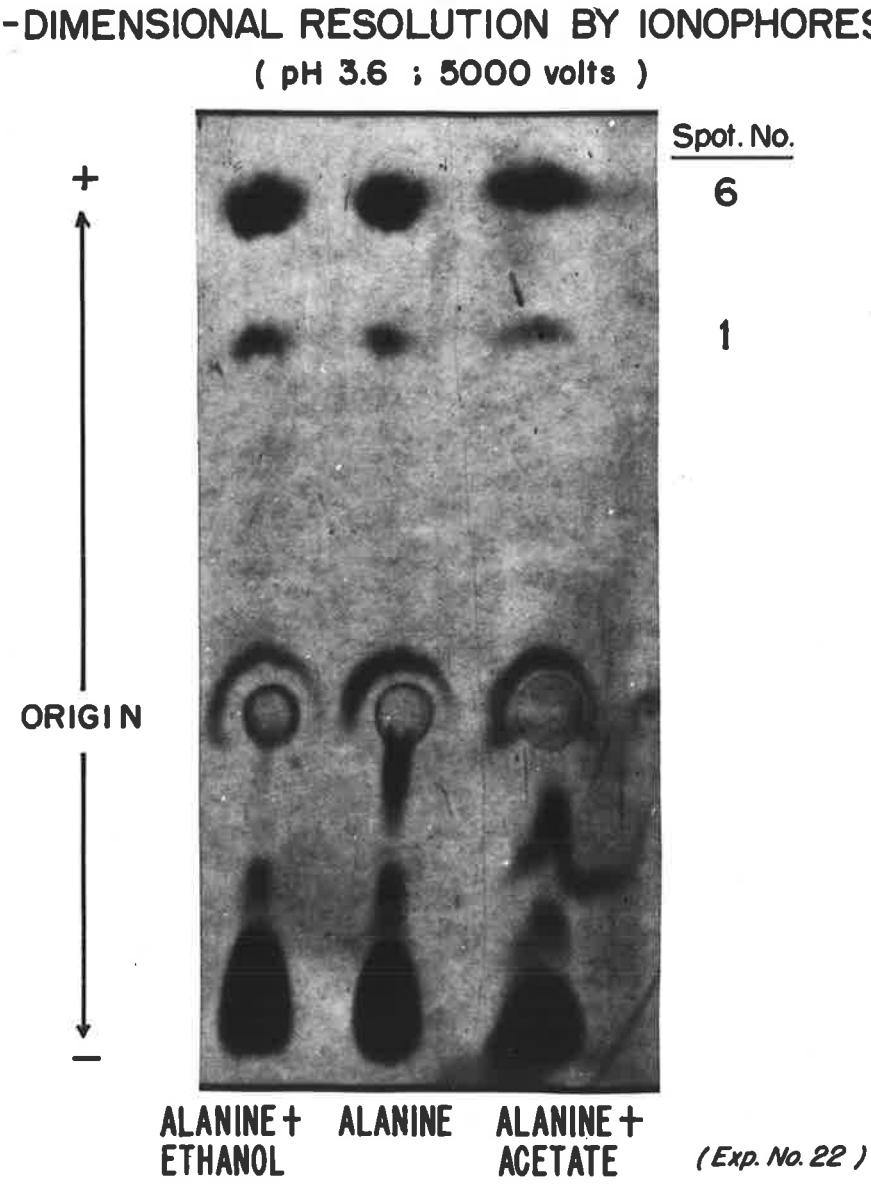

Fig. 4. Radioautograph of 1-dimensional ionophoresis using buffer pH 3.6 at 5,000 volts for 45 minutes. Lactic acid (spot#6) and aspartic acid (spot#1 - pK 2.98) become negatively charged and migrate towards the anode. Glutamic acid (pK 3.08) may be represented by the eliptical shadow about the origin. Alanine (pK 6.1) is accumulated at the cathode. KRB supplemented with alanine-U-C^{14} 10mM 1μc/ml). Expt# 22

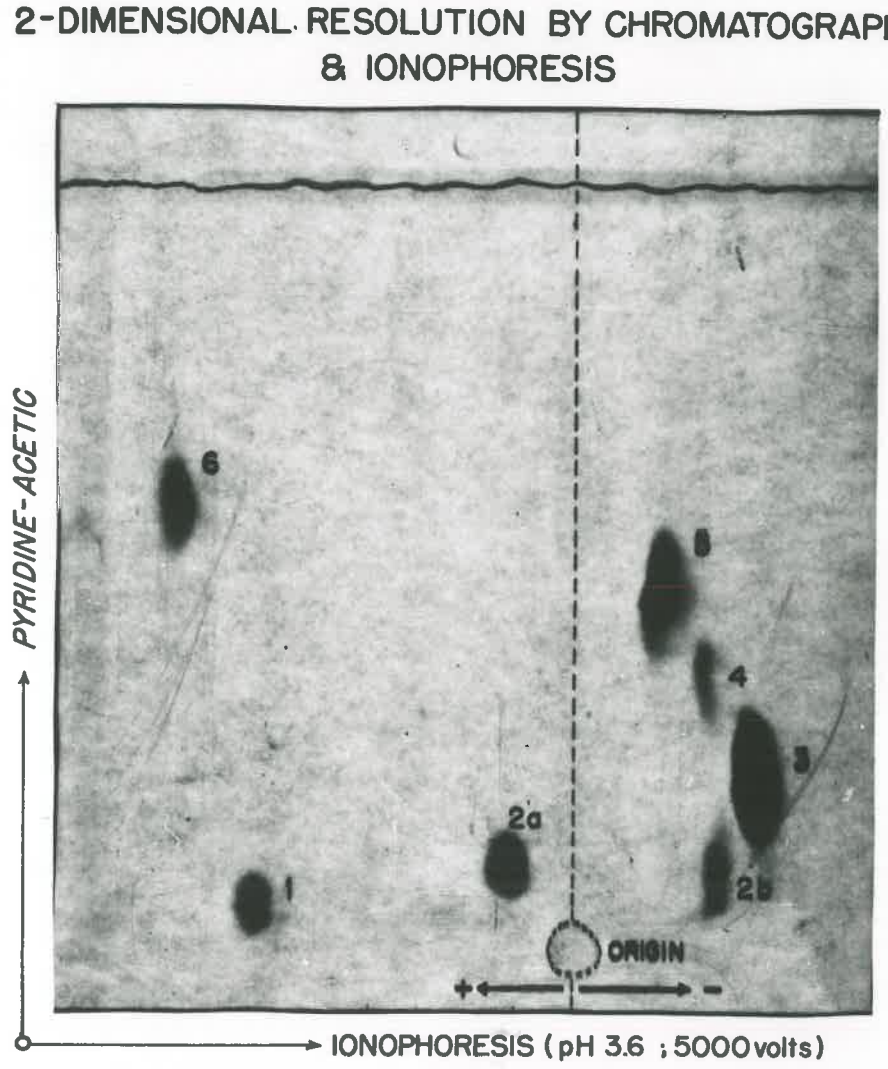

Fig. 5. Radioautograph of final suspending medium-KRB supplemented with alanine 10.0mM (Iμc/ml) but no other substrate. Conventional chromatogram in pyridine:acetic followed by ionophoresis (buffer pH 3.6) at 5,000 volts for 45 minutes. Relative positions of the individual radioactive components are denoted by the numbers employed in the text. The homogeneity of Spots 1,3,4,5 and 6 was preserved during secondary resolution on the basis of solubility characteristics or electrical charge. Expt # 22

(a) 3-5% may be lost during application of dilute solution (< 1mM) onto paper;

(b) 5-15% may be lost during chromatography in neutral or acid solvents;

(c) as much as 50% may be lost during prolonged storage of developed chromatograms.

The latter loss is conditioned by ambient temperatures and can be obviated by maintaining chromatograms at 4°C during radioautography.

Previous studies (Freinkel et al., 1965) had shown that for rat liver slices in KRB and KRP, 90% of the labelled glucose derived from alanine-C^{14} can be recovered in the suspending media. They also showed that ethyl alcohol does not appreciably influence the partition of newly formed glucose between slice and medium. In view of the meagre glucose production into the medium in experiments with human liver (see Results) these substantive experiments on glucose production were not repeated but the partition of stable glucose between medium and tissue without substrate was 39.37% / 60.63% ($\pm$ 3.34%) whilst under the influence of ethyl alcohol it was 43.12% / 56.88% ($\pm$ 6.69%) suggesting that no change in partition had occurred. On the basis of the above considerations, simple resolution of the final suspending media by duplicate one-dimensional chromatography in isopropanol: pyridine: acetic acid: water was employed for most experiments.

"Uptake"

To assess the net assimilation of alanine-C^{14} (i.e., "uptake"), the difference between "blank" media and media which had been incubated with tissues was measured.

Since this difference was often small, and since it was derived from chromatographic analyses of two separate media for spot *3 (i.e., alanine) the estimates of "uptake" constituted the least reliable radioactive measurement.

Net formation of glucose-C^{14} (spot *5), lactic acid-C^{14} (the sum of spot *6 and spot *7 when present) and aspartic acid (spot *1) was estimated on the basis of the radioactivity in the appropriate segments of the chromatograms on the final media. Glutamic acid-C^{14} was not measured because of its variable chromatographic distribution.

C H A P T E R V.

Results

The content of initial glycogen in each human liver biopsy together with the amount present at the end of the incubation and the quantity of glucose recovered from the medium are shown in Table 3. From these results is derived the net delta or total disappearance which is represented by the sum of the final glycogen and final glucose in the medium, subtracted from the initial glycogen since no glucose was present in the initial medium. It will be noted that in all instances there is a deficit with less total glucose being recovered than the amount as initial glycogen.

The experimental data in this table are confined to those results in which a direct comparison can be made between the initial glycogen and the final glycogen and in which the incubation time was 3 hours, however the figure for initial glycogen of 24,201 micrograms per gram initial weight as a mean of 9 experiments compares well with that of 23,390 for the whole 19 subjects in whom this analysis was performed. The average percentage loss over the incubation period was 40%. There is a positive and strong correlation between the amount of initial glycogen and final glycogen ($r = 0.898$; $p < .001$) and the initial glycogen and the total disappearance ($r = 0.9709$; $p < .001$) suggesting that the release of glycogen as glucose into the medium was uniform under control conditions.

TABLE 9

HUMAN LIVER GLYCOGEN.*

DISTRIBUTION IN UNSUPPLEMENTED KRB

EXPT. NO.	INITIAL GLYCOGEN	FINAL GLYCOGEN	FINAL MEDIUM GLUCOSE	NET △ (DISAPPEARANCE)
3	15612	5284	4853	5474
6	41086	12039	9890	19157
7	11691	6181	1848	3662
9	56967	20260	6591	30116
11	7483	1906	3342	2235
12	15986	8048	5441	2500
13	40014	9513	5421	25080
15	9930	3873	2011	4046
18	19551	10510	4466	4575
22	23691	5621	7510	10560
MEAN	24201	8324	5137	10741
± S.E.M.	5176	1649	781	3254

* GLYCOGEN EXPRESSED AS MICROGRAM OF GLUCOSE PER GRAM INITIAL WEIGHT OF LIVER.
ALL INCUBATIONS PERFORMED FOR 3 HOURS.

A significantly greater proportion of glycogen was retained in the liver slice than was released into the medium as glucose ($r = 0.7962$; $p < .01$). However, all of these correlations may have been influenced by the considerable loss of initial glycogen during the preparative manipulations.

Table 4 sets out the distribution of stable glucose under each of the experimental conditions in which alanine was used as substrate for 3 hours. Again, as in Table 3, the initial glycogen is related to the final glycogen and final medium glucose from whose sum the total disappearance is derived. The residual glycogen was higher in 7 out of 8 instances when the medium was supplemented with alanine but the difference is only marginally significant ($p < .05$). The presence of alanine significantly modified the total disappearance of glucose from the slices ($p < .01$) but induced no such change in the amounts of medium glucose. The total glucose derived from the final glycogen combined with the amount in the medium did not significantly differ whether the medium was plain or supplemented with alanine. The inference, whilst only partially substantiated, is that alanine, by providing a direct and graduated release of pyruvate tends to reduce glycogen loss from the liver slices. The addition of sodium acetate had a marginally protective influence on this glycogen loss ($p < .05$).

The recovery of glucose from the medium was less than the initial glycogen in all experiments and only in one instance was the sum of the total residual glycogen medium glucose greater than the

TABLE 4

DISPOSITION OF STABLE GLUCOSE* USING SUBSTRATE ALANINE-U-C^{14}**

EXPT.	INITIAL GLYCOGEN	FINAL GLYCOGEN				FINAL MEDIUM GLUCOSE				NET Δ (TOTAL DISAPPEARANCE)			
		-ALANINE	+ALANINE	+ALCOHOL +ALANINE	+ACETATE +ALANINE	-ALANINE	+ALANINE	+ALCOHOL +ALANINE	+ACETATE +ALANINE	-ALANINE	+ALANINE	+ALCOHOL +ALANINE	+ACETATE +ALANINE
2	16671	-	9926	13119	-	6430	5918	6442	-	-	827	2890	-
3	15612	5284	7100	4757	-	4853	4213	3832	-	5474	4298	7203	-
7	11691	6181	4533	-	-	1848	2690	-	-	3662	4467	-	-
9	56967	20260	22905	-	-	6591	4638	-	-	30116	29423	-	-
11	7483	1906	2765	2360	3033	3342	4075	3568	-	2235	643	1715	783
13	40014	9513	12710	17383	14160	5421	5218	6711	4851	28081	22086	15920	21003
15	9930	3873	8069	-	-	2011	1187	-	-	4046	674	-	-
22	23691	5621	11209	12015	9504	7510	6589	7956	6671	10560	5893	3720	7516
MEAN	22757	7520	9902	9927	8899	4751	4316	5702.	5761	12025#	8539	6290#	9767#
±S.E.M.	6091	2296	2194	2776	3226	758	615	857	910	4524	3884	2576	5945

* GLUCOSE EXPRESSED AS MICROGRAM PER GRAM INITIAL WEIGHT OF HUMAN LIVER.

** ALANINE, ETHYL ALCOHOL AND SODIUM ACETATE IN CONCENTRATION 10 MICROMOLES PER MILLILITRE. INCUBATION WAS FOR 3 HOURS IN EACH INSTANCE.

THE RESULTS OF NET Δ ARE DERIVED FROM THE INDIVIDUAL SUBSTRATIONS OF FINAL TOTAL GLUCOSE (I.E., FINAL GLYCOGEN + GLUCOSE) FROM THE INITIAL GLYCOGEN IN EACH EXPERIMENT. BECAUSE OF THE VARYING NUMBER OF OBSERVATIONS, THE FINAL TOTAL MEANS DO NOT THEREFORE NECESSARILY CORRELATE.

initial glycogen. These findings show that no production of glucose had occured from the human liver slices. This is in contradistinction to the findings in the rat where, during incubation without alanine, glucose progressively appears into the suspending medium. In KRB this glucose frequently exceeds the amount initially present within the tissues as glycogen or "free" glucose, and when the suspending medium is enhanced with alanine the net synthesis from endogenous precursors is increased even further (Freinkel et al., 1965). This production of glucose from endogenous as well as exogenous precursors by rat liver slices has been demonstrated before (Haynes, 1962) and the inability of the human liver slices to act similarly may be an interesting species difference as well as a reflection of different rates of metabolic turnover.

It must be emphasised at this point that evidence of stable glucose formation cannot be obtained from <u>in vitro</u> systems when the final total glucose does not exceed the initial glycogen. In techniques employing non-isotopic glucose, even when changes in glycogen are encompassed, net balance across the liver rather than synthesis of new glucose is measured. Figure 6 demonstrates the progressive decline in tissue glycogen with a slow rise in medium glucose in an experiment where periodic sampling was performed. The combined sum of these does not exceed the initial glycogen level which shows that no net accumulation of glucose has occurred. Without knowledge of the derivation of the medium glucose however it is not possible to state that no gluconeogenesis has occurred.

Table 5 relates the disposition of the alanine-U-C^{14}

to the initial glycogen in the control vessels. There is a negative correlation between the initial glycogen and the "uptake" ($r = -0.8288$; $p < .05$). The assimilation of exogenous gluconeogenic precursor may be reduced therefore by the presence of adequate liver glycogen. No correlation could be found between the initial glycogen and any of the other parameters nor they between themselves except in the case of $C^{14}O_2$ and the medium glucose where $r = 0.7718$; $p < .05$ which is as might be expected inasmuch as the enhanced evolution of carbon dioxide is a reflection of increased tricarboxylic acid cycle activity.

The effect of ethyl alcohol on tissue respiration was examined in four experiments. Such documentation was obviously essential to exclude toxic action on tissues and anoxia as a cause of the altered metabolism. Table 6 compares the consumption of oxygen in microlitres per milligram wet weight of tissue over the time interval of the experiments. The values are expressed beneath as a percentage of the control vessels. By this convention 100% denotes no effect. Although the results are variable, at no time in the first hour did ethyl alcohol depress oxygen consumption more than 12% of the control and in three instances no depression was evident. By the end of the third hour the depression of oxygen consumption did not materially differ from that in the second in three experiments, and only in one instance was it depressed to approximately 20% of the control. The figures are too few to allow for formal statistical analysis but they do show that alcohol has no grossly deleterious effect on oxygen uptake.

Expt. No. 6

TIME CURVE FOR TOTAL GLUCOSE IN UNSUPPLEMENTED VESSELS

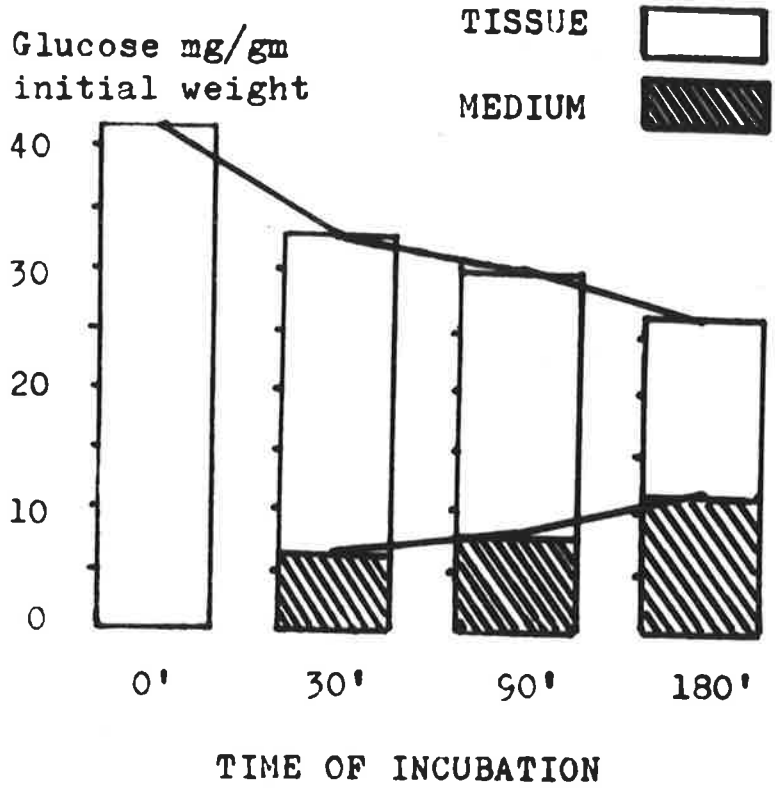

Fig. 6. Medium KRB human liver slice. No net production of glucose has been demonstrated.

TABLE 5

CORRELATION BETWEEN INITIAL GLYCOGEN AND DISPOSITION OF ALANINE-U-C^{14}

EXPT.	INITIAL GLYCOGEN (UG/GM INITIAL WEIGHT)	"UPTAKE"*	$C^{14}O_2$	GLUCOSE-C^{14}	LACTIC ACID-C^{14}	GLYCOGEN-C^{14}
		(PER CENT TOTAL COUNTS PER GRAM INITIAL WEIGHT)				
2	16671	255.3	21.0	4.1	34.6	0.137
3	15612	223.7	69.8	26.6	42.3	3.910
7	11691	-	29.5	7.8	29.2	1.850
9	56967	134.2	42.3	7.3	40.0	1.000
11	7483	349.1	115.4	55.4	45.2	3.570
13	40014	-	30.09	15.4	47.1	1.57
15	9930	295.1	80.0	10.3	35.8	17.50
22	23691	199.3	52.2	34.7	58.9	1.340
23	27562	145.5	54.4	11.8	43.7	0.902
MEAN:	23291	228.9	54.96	19.27	41.87	3.531
±S.E.M.	5398	29.43	9.92	5.59	2.85	1.793

* "UPTAKE" = RADIOACTIVITY IN BLANK MEDIA - RADIOACTIVITY IN MEDIA CONTAINING HUMAN LIVER. VALUES EXCEED 100% IN MOST INSTANCES BECAUSE SLICES AVERAGED 100-200MG. AND VALUES ARE RELATED TO GRAM INITIAL WEIGHT.

N FOR GLYCOGEN VS "UPTAKE" = 7, FOR ALL OTHER CORRELATIONS, N=9.
INITIAL GLYCOGEN VS "UPTAKE" - R = -0.8288 : P .05

$C^{14}O_2$ VS GLUCOSE-C^{14} R = 0.7718 : P .05

NO OTHER SIGNIFICANT CORRELATIONS.

Figure 7 shows graphically the utilisation of oxygen in one experiment and the close relationship to the control is well demonstrated. The increase in respiration of the slice in the presence of sodium acetate is noteworthy, averaging 16% more than the control in the four experiments. This average figure is perhaps influenced by the greatly increased result in experiment 16 which is depicted in Figure 7, but the increase in QO_2 is shown in all experiments, although in lesser degree. The reasons for this increase are obscure and no attempt was made at further elucidation. It may purely reflect the ready availability of sodium acetate as a substrate. However, in the following studies on incorporation, no significant difference was found in the behaviour of the liver slice when the substrate sodium acetate was compared with the radioactive substrates alanine, glutamic acid, lactic acid or glycerol.

THE EFFECTS OF ETHYL ALCOHOL ON THE DISPOSITION OF ALANINE-U-C^{14}.

Experiments were performed with liver slices from 8 subjects. Table 4 reveals the influence of alcohol on the disposition of stable glucose when the substrate was alanine-U-C^{14} 10mM. Alcohol did not influence significantly the distribution of glucose in the tissue or medium in comparison with the control. Using experiments 3, 11, 13 and 22 the rate of change between the alcohol vessels and the supplemented and plain control vessels was not statistically significant. The partition of medium and tissue glucose did not differ significantly in the alcohol vessels from those of the controls.

TABLE 6

SUMMARY OF QO_2 * VALUES

EXPT.	SUBSTRATE	CONTROL 1°	CONTROL 2°	CONTROL 3°	ALCOHOL 1°	ALCOHOL 2°	ALCOHOL 3°	ACETATE 1°	ACETATE 2°	ACETATE 3°
16	0	.300	.257	.238	.276	.217	.189	.450	.384	.331
17	0	.430	.322	.340	.445	.297	.309	.472	.360	.372
	ALANINE	.346	.333	.304	.570	.441	.330	.356	.382	.343
21	ALANINE	.386	.347	-	.337	.279	-	.481	.429	-
28	ALANINE	.512	.482	.430	.513	.422	.377	.496	.496	.455

QO_2 VALUES AS % OF CONTROL

16	0	92	84.4	79.4	150	149.4	139.1
17	0	103.5	92.2	90.9	109.8	111.8	109.4
	ALANINE	164.7	132.4	108.5	102.9	114.7	112.8
21	ALANINE	87.3	80.4	-	124.6	123.6	-
28	ALANINE	100.2	87.6	87.7	96.9	102.9	105.8
MEAN		95.75	86.15	86.0	116.8	120.5	116.8
±S.E.M.		3.712	2.497	3.427	9.49	7.95	7.57

*QO_2 = MICROLITRE OF OXYGEN CONSUMED PER MILLIGRAM WET WEIGHT OF LIVER SLICES.

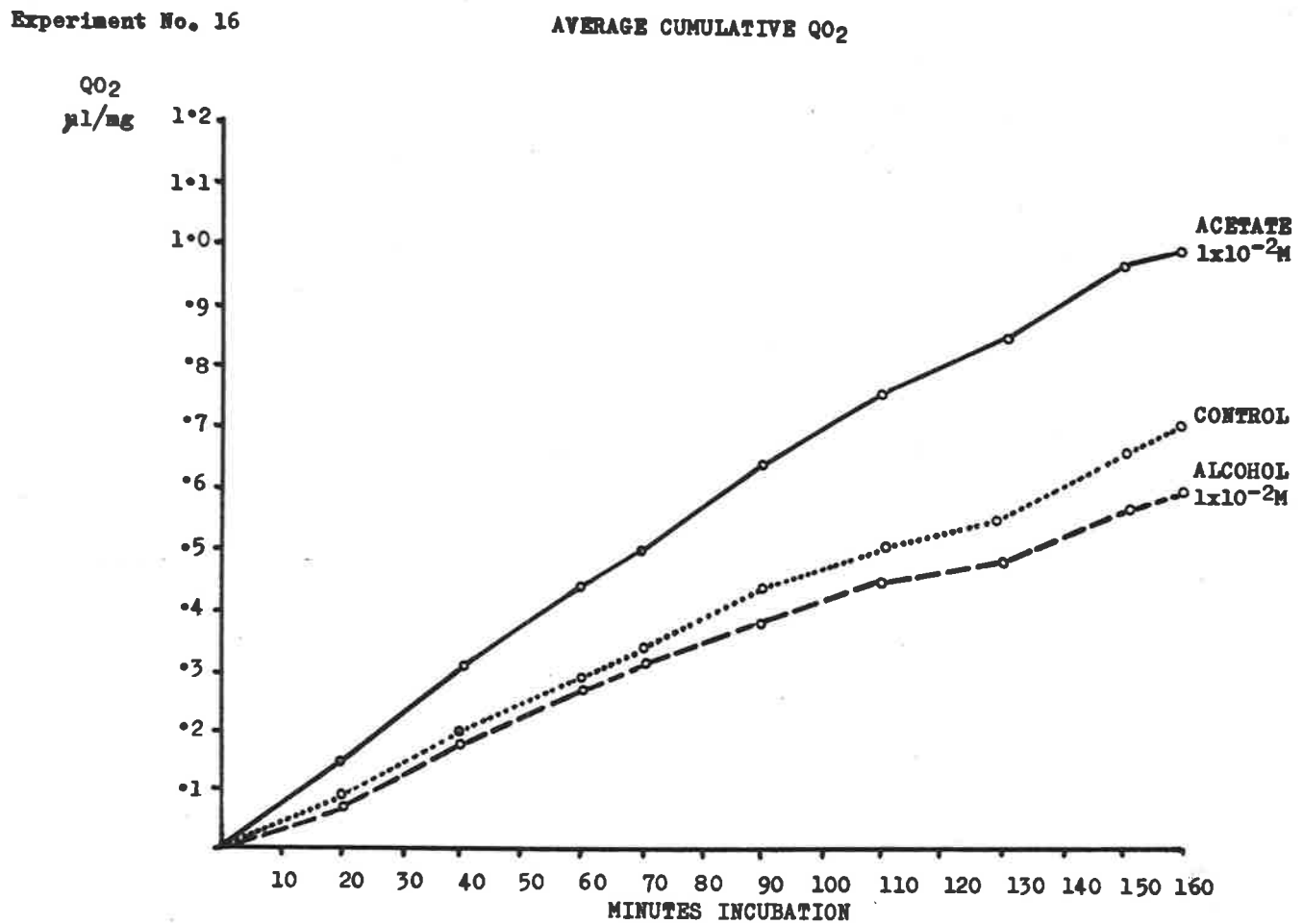

Fig. 7. Oxygen consumption (µl/mg) of human liver slices under acetate, alcohol and control conditions.

Table 7 summariese the findings in KRB when supplemented with 10mM alanine-U-C^{14} expressed as a percentage of the total counts per gram initial weight. A comparison with the effects of 10mM sodium acetate is included.

Ethyl alcohol 10mM depressed the "uptake" of alanine ($p < .01$). Significant depressions of $C^{14}O_2$ evolution and glycogen formation in the liver slices were observed ($p < .01$ in both instances) and moderate depression of glucose release in the medium occurred ($p < .05$). Lactic acid formation was not significantly influenced. The effects of ethyl alcohol upon glucose-C^{14} were observable at all times throughout the incubation and the specific activities (i.e., counts per microgram) of medium glucose and tissue glycogen were consistently depressed in its presence. The variance of both of these latter parameters was related to the mean. Analyses performed on log transforms on the original variates revealed a significant reduction in the specific activity of the glucose ($T = 3.347$ of df, $p < .05$) and in the specific activity of glycogen ($T = 7.255$ on 7 df, $p < .001$). These findings suggest that ethyl alcohol truly inhibits gluconeogenesis rather than merely accelerating the intrahepatic utilisation of glucose-C^{14} and that this inhibition is mediated prior to the renewal of the glucose-6-phosphate from smaller precursors.

As discussed in the section on methods (Chapter IV) the calculation of the "uptake", being dependent on two individual measurements was most prone to error. Nevertheless, the "uptake" was significantly depressed in all experimental situations where ethyl alcohol was employed. Two representative examples from experiments with human liver are depicted in Figure 8. Absolute values for "uptake"

TABLE 7

THE EFFECT OF ETHYL ALCOHOL ON THE METABOLISM OF ALANINE-U-C^{14} IN HUMAN LIVER SLICES*

DISPOSITION OF ALANINE-U-C^{14} EXPRESSED AS % TOTAL COUNTS PER GRAM INITIAL WEIGHT.
(MEAN, ± S.E.M. AND STATISTICAL EVALUATION ARE DOCUMENTED, OVERALL FIGURES IN APPENDIX 4)

CONTROL:

8 EXPTS.	"UPTAKE"	$\dot{C}^{14}O_2$	GLUCOSE C^{14}	LACTIC ACID-C^{14}	GLYCOGEN C^{14}	S.A.** GLUCOSE	S.A.** GLYCOGEN	ASPARTIC-C^{14}
MEAN:	215.84	49.7	21.489	41.85	2.085	16203	1220.1	14.92
±S.E.M:	28.23	12.08	5.92	3.318	0.487	5329	541.6	2.58

ALCOHOL:

8 EXPTS.	"UPTAKE"	$C^{14}O_2$	GLUCOSE C^{14}	LACTIC ACID-C^{14}	GLYCOGEN C^{14}	S.A.** GLUCOSE	S.A.** GLYCOGEN	ASPARTIC-C^{14}
MEAN:	148.44	11.034	3.746	50.725	0.4128	3658	228.5	20.40
±S.E.M:	23.52	2.21	0.677	8.499	0.159	811	91.2	3.76
T	5.12	4.087	3.103	1.236	4.061	2.446	2.115	2.203
P	.01	.01	.05	N.S.	.01	.05	N.S.***	N.S.

ACETATE:

5 EXPTS.	"UPTAKE"	$\dot{C}^{14}O_2$	GLUCOSE C^{14}	LACTIC ACID-C^{14}	GLYCOGEN C^{14}	S.A.** GLUCOSE	S.A.** GLYCOGEN	ASPARTIC-C^{14}
MEAN:	257.35	62.1	22.46	42.5	2.195	17909	1428	21.24
±S.E.M:	39.24	17.29	7.46	2.13	0.77	8263	1020	4.24
T	1.368	0.567	1.463	1.358	0.89	0.507	0.843	3.349
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	.05

* INCUBATION WAS PERFORMED FOR 3 HOURS IN KRB EXCEPT IN EXPERIMENTS 21 AND 23 WHERE 2 HOURS WERE EMPLOYED. ALL VESSELS CONTAINED 10MM ALANINE, ALCOHOL VESSELS WERE SUPPLEMENTED WITH 10MM ETHYL ALCOHOL (I.E. 46MG%) ACETATE VESSELS WITH 10MM SODIUM ACETATE.

** S.A. = SPECIFIC ACTIVITY = COUNTS GLUCOSE-C^{14} PER ML. MEDIUM/UG GLUCOSE PER ML. MEDIUM.

*** LOG TRANSFORMATIONS WERE ANALYSED BECAUSE OF MARKED VARIANCE OF THE ORIGINAL MEANS AND SIGNIFICANT REDUCTION WAS DEMONSTRABLE. SEE TEXT OPPOSITE.

in the control and ethyl-alcohol-containing vessels are shown at the head of the figure expressed as micromoles per gram initial weight. To compensate for the variable reductions in uptake, the values for $C^{14}O_2$ and lactic acid-C^{14} have been expressed in two ways. Firstly, as a percentage of the total radioactivity within each flask in the upper diagram and secondly, in relative terms as a percentage of the net "uptake" by the slices in each flask as shown in the lower diagram. It can be seen in experiment - 4 on the right that the absolute formation of lactic acid-C^{14} from labelled alanine was still markedly increased despite the reduced uptake. However, more often, the absolute increase was not so pronounced as shown in experiment 11 and the overall changes in lactic acid-C^{14} in the whole experimental series were not significantly greater than those from the controls. The general tendency nevertheless, as shown in both of these experiments was for a greater proportion of the assimilated counts to be diverted to lactate. Moreover, in all situations, ethyl alcohol caused a far smaller proportion of the "uptake" to be evolved as $C^{14}O_2$ or transformed to glucose-C^{14}. Thus the reductions in oxidative decarboxylation and gluconeogenesis were disproportionate to the reductions in "uptake" and it would appear that ethyl alcohol induces intracellular changes in metabolism which are relatively independent of fluctuations in "uptake".

When expressed as a percentage of the control valve, the inhibition of $C^{14}O_2$ by alcohol was always greater than the effects on "uptake" so that relative lack of substrate for decarboxylation cannot be the reason for the consistent effect.

THE EFFECT OF ETHANOL ON THE DISPOSITION OF ALANINE-U-C^{14} (10 mM) BY HUMAN LIVER SLICES

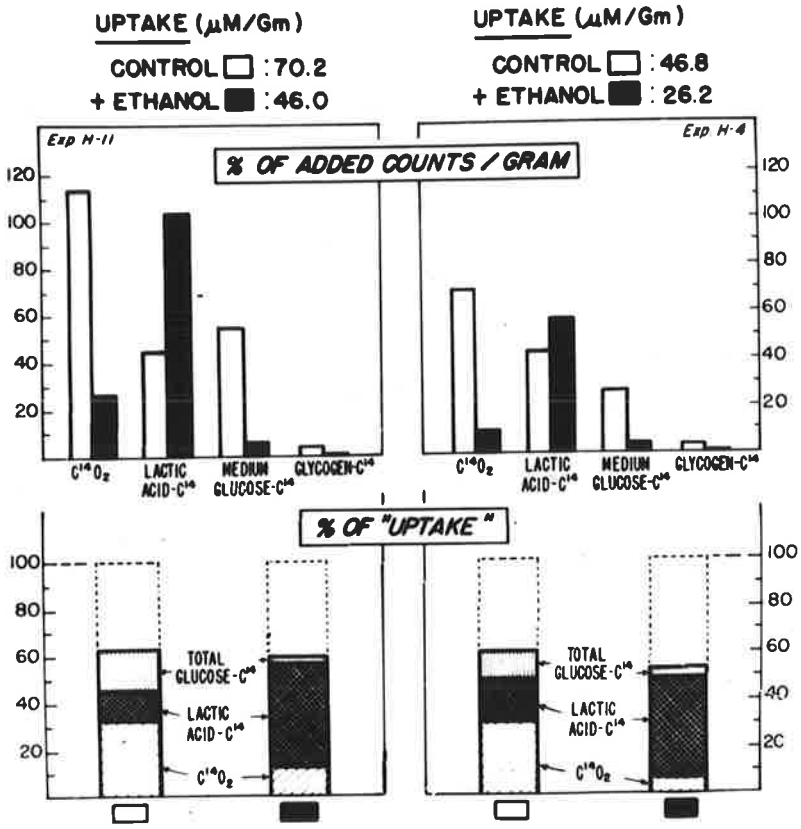

Fig. 8. Effects of ethyl alcohol (10.0mM) on the disposition of L-alanine-U-C^{14} (10.0mM) by human liver. In both experiments as shown above, slices were incubated for 3 hours in KRB. The upper portion of the illustration depicts results expressed as a percentage of the total number of counts that were added to each vessel. The lower portion depicts the same values as a function of the net assimilation (i.e. "uptake") of alanine-C^{14} from the suspending medium. Ethyl alcohol depressed "uptake" 34.5% in experiment # 11 and 44.0% in experiment #4. As can be seen from the lower graphs, the recoveries of $C^{14}O_2$, glucose-C^{14} and lactic acid-C^{14} accounted for more than 50% of the total counts that were assimilated under all experimental conditions. For further discussion see text.

The upper portion of figure 8 reinforces a point already made. It can be seen that alcohol inhibited the incorporation of glucose-C^{14} into the suspending medium and that the percentange inhibitions of medium glucose and tissue glycogen were approximately equal. This dual reduction would indicate that the inhibitory effects of ethyl alcohol on gluconeogenesis may be limited to steps preceding the renewal of the glucose-6-phosphate pool.

The relationships between ethyl alcohol depression of "uptake", $C^{14}O_2$ and glucose-C^{14} were studied by fitting an exponential regression. Depression in this regard was obtained by subtracting the actual value obtained under alcohol from the control valve. A significant regression was obtained for the depression of $C^{14}O_2$ and "uptake" of alanine the equation being $y = 19.86 + 0.19e^{0.0544x}$ where y is $C^{14}O_2$ depression and x is "uptake" depression. The regression is significant, ($F = 10.512$ on 2, 4df; $p < .05$) and the curvilinear relationship is depicted in figure 9 suggesting that beyond 50% depression of uptake, marked reduction of $C^{14}O_2$ evolution occurs. No relationship could be demonstrated between glucose-C^{14} depression and that of "uptake" and whilst an exponential regression existed for the depressions of $C^{14}O_2$ and glucose-C^{14}, a linear relationship was better.

Significant variation in the ratios of metabolic products were observed under the influence of alcohol and are listed in Table 8. The reductions of $C^{14}O_2$ / lactic acid-C^{14} ($T = 3.925$ on 7 df; p .01) and glucose-C^{14} / lactic acid-C^{14} ($T = 3.163$ on 7 df; $p < .05$) are due to the dual depressions of the numerators.

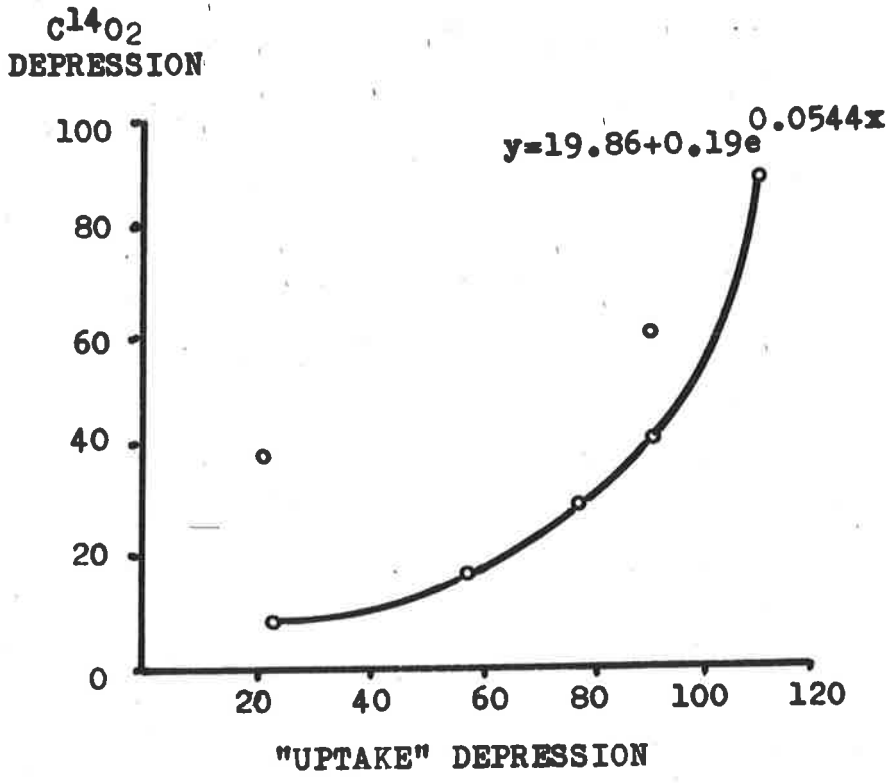

Fig. 9. The relationship between $C^{14}O_2$ depression (y) and depression of "uptake" of alanine-U-C^{14} in control and alcohol vessels.

Aspartic acid-C^{14} was not significantly altered by the presence of alcohol in these human liver experiments although the amount of its increase compares with that of acetate where marginal significance is evident.

THE EFFECTS OF ETHYL ALCOHOL ON THE DISPOSITION OF OTHER SUBSTRATES.

A. Sodium Acetate

Because the initial metabolism of alcohol involves the formation of acetaldehyde and acetate, equimolar concentrations of sodium acetate were employed as well as the control vessels in most experiments to obviate the dilutional effects of the 2 carbon fragments released by acetate metabolism. Acetate did not reduplicate the effects of ethyl alcohol on $C^{14}O_2$, glucose-C^{14}, lactic acid-C^{14} or "uptake" and the action of ethyl alcohol cannot be attributed to this cause. The slight but significant increase in aspartic acid has already been noted.

B. Other gluconeogenic precursors.

1. L-glutamic acid.

Experiments were performed on 5 occasions with slices of human liver exposed to the action of ethyl alcohol in which the substrate was l-glutamic acid-C^{14}. The concentration used was 10mM. The results are expressed as a percentage of the total values obtained in the control vessels. By this convention 100% denotes no effect. The "uptake" of glutamic acid was significantly depressed by ethyl alcohol ($p < .01$) as was the formation of glucose-C^{14} ($p < .05$) and

TABLE 8

RATIOS OF METABOLIC PRODUCTS*
FOR ALANINE-U-C^{14}

	CONTROL			ALCOHOL		
EXPT.	$\frac{C^{14}O_2}{\text{LACTIC-}C^{14}}$	$\frac{C^{14}O_2}{\text{GLUCOSE-}C^{14}}$	$\frac{\text{GLUCOSE-}C^{14}}{\text{LACTIC-}C^{14}}$	$\frac{C^{14}O_2}{\text{LACTIC-}C^{14}}$	$\frac{C^{14}O_2}{\text{GLUCOSE-}C^{14}}$	$\frac{\text{GLUCOSE-}C^{14}}{\text{LACTIC-}C^{14}}$
1	0.66	1.44	0.46	0.43	1.41	0.303
2	0.61	5.12	0.12	0.11	3.44	0.033
3	1.65	2.62	0.63	0.16	2.50	0.063
11	2.55	2.08	1.22	0.24	4.26	0.055
13	0.66	2.01	0.33	0.14	2.48	0.057
21	0.99	3.06	0.32	0.18	3.12	0.089
22	0.89	1.50	0.59	0.22	4.34	0.051
23	1.24	4.61	0.27	0.33	3.25	0.102
MEAN	1.03	2.81	0.4925	0.2269	3.10	0.0904
± S.E.M.	0.235	0.4899	0.1204	0.037	0.341	0.0310
T				3.925	0.534	3.163
P.				< .01	N.S.	< .05

* VALUES ORIGINALLY EXPRESSED AS PERCENTAGE TOTAL COUNTS PER GRAM INITIAL WEIGHT OF LIVER.

TABLE 9

THE EFFECT OF ETHYL ALCOHOL ON THE METABOLISM OF L-GLUTAMIC ACID-C^{14} IN HUMAN LIVER SLICES*

EXPT. NO.	"UPTAKE"	$C^{14}O_2$	GLUCOSE $-C^{14}$	LACTIC ACID-C^{14}	GLYCOGEN-C^{14}	S.A.* GLUCOSE	S.A.** GLUCOSE
CONTROL VESSELS (% OF TOTAL COUNTS/GRAM INITIAL WEIGHT)							
4	88.7	36.5	30.7	6.23	.255	109.13	1.44
8	78.8	23.25	3.89	10.20	.117	41.97	.545
10	126.9	30.14	11.11	18.31	.109	39.82	1.31
12	158.6	45.9	24.32	6.03	.232	189.4	.618
18	117.26	42.2	34.07	5.67	4.25	125.14	8.83
MEAN	112.06	35.58	20.69	9.28	.993	101.09	2.549
± S.E.M.	14.22	4.09	5.72	4.80	.814	28.01	1.579
ALCOHOL VESSELS (% OF CONTROL VALUES)							
4	41.93	64.9	17.26	80.2	21.53	13.86	23.26
12	35.81	62.53	36.1	85.57	48.27	12.20	43.04
18	41.24	46.0	24.74	85.50	-	22.23	-
MEAN	39.66	57.80	26.03	83.76	34.90	16.10	33.15
± S.E.M.	1.93	5.81	5.47	1.78	13.37	3.10	9.88
T	31.179	7.097	13.505	9.133	4.869	27.032	6.759
DF	2	2	2	2	1	2	1
P	<.01	<.05	<.01	<.05	N.S.	<.01	<N.S.
ACETATE VESSELS (% OF CONTROL VALUES)							
12	135.9	104.1	55.9	88.2	128.4	57.4	114.2
18	90.6	126.8	90.02	77.6	93.6	80.9	99.09
MEAN	113.2	115.4	72.96	82.9	111.0	69.1	106.64
± S.E.M.	22.7	11.4	17.06	5.2	17.4	11.8	7.56
T	0.584	1.361	1.584	3.226	.632	.265	.879
DF	1	1	1	1	1	1	1
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* INCUBATION WAS PERFORMED FOR 3 HOURS IN K.R.B.
RESULTS OBTAINED IN THE PRESENCE OF ALCOHOL (10MM) OR SODIUM ACETATE (10MM) ARE LISTED AS A PERCENTAGE OF THE VALUES OBTAINED IN THEIR ABSENCE. AVERAGE PERCENTAGE CHANGES (± S.E.M.) ARE TABULATED BENEATH AND EXPRESSED IN TERMS OF THEIR STATISTICAL SIGNIFICANCE.
N.S. = NOT SIGNIFICANT

** S.A. = SPECIFIC ACTIVITY - COUNTS GLUCOSE-C^{14} PER ML MEDIUM/UG GLUCOSE PER ML. MEDIUM.

lactic acid-C^{14} ($p < .05$). The specific activity of glucose was reduced ($p < .01$) and when analysis of the original variates was performed on log transforms for specific activity of glycogen depression, significance ($p < .01$) was also demonstrated here. It was not possible to accurately assess the amounts of aspartic acid by radioautography since the Rf distance of aspartic (spot *1) was usually too close to that of the substrate glutamic acid (spot *2) making accurate discrimination impossible. The general trend of reduced uptake and gluconeogenesis is again revealed by the data obtained with glutamic acid as substrate.

2. Glycerol.

Three experiments were performed with glycerol-1, 3-C^{14}. The results (Table 10) whilst suggestive, are in most instances not numerically sufficient for statistical significance. Thus, although glucose formation, lactic acid production and "uptake" were depressed in all experiments, statistical significance cannot be established. Only in the case of $C^{14}O_2$, tissue glycogen and its specific activity is such significance attained. The marked excess of glycerophosphate-C^{14} is notable and will be commented upon further in discussion.

3. Lactic Acid.

To appraise the effects of ethyl alcohol on an additional gluconeogenic precursor, two experiments were performed using D-l Lactate-U-C^{14}. Depression of "uptake", $C^{14}O_2$ evolution and glucose-C^{14} production were again observed. Since D-l lactate-U-C^{14} was the precursor, the release of lactic acid-C^{14} could not be studied.

TABLE 10

THE EFFECT OF ETHYL ALCOHOL ON THE METABOLISM OF GLYCEROL-1,3-C^{14} IN HUMAN LIVER SLICES*

EXPT. NO.	"UPTAKE"	$C^{14}O_2$	GLUCOSE-C^{14}	LACTIC ACID-C^{14}	GLYCOGEN-C^{14}	S.A. ** GLUCOSE	S.A. ** GLUCOSE	G.P.A.#
CONTROL VESSELS (% OF TOTAL COUNTS/GRAM INITIAL WEIGHT)								
19	235.5	27.32	136.2	8.98	6.85	334.0	11.97	7.52
20	343.0	23.08	91.1	35.52	.848	302.0	4.29	3.72
27	285.0	14.35	41.96	36.16	.173	145.7	-	-
MEAN	287.8	21.58	89.75	26.89	2.624	260.6	8.13	5.62
+-S.E.M.	31.1	3.82	27.21	8.96	2.122	58.2	3.84	1.90
ALCOHOL VESSELS (% OF CONTROL VALUES)								
19	137.3	4.44	19.07	60.6	9.09	38.9	12.58	192.8
20	108.4	7.06	53.20	78.2	38.8	53.5	37.30	278.8
27	29.5	7.53	88.5	93.2	45.9	108.6	56.30	-
MEAN	91.7	6.34	53.6	77.33	31.3	67.0	35.39	235.8
+-S.E.M.	32.2	0.96	20.0	9.42	11.3	21.2	12.66	43.0
T	0.256	97.435	2.315	2.406	6.096	1.554	5.104	3.158
DF	2	2	2	2	2	2	2	1
P	N.S.	.001	N.S.	N.S.	.05	N.S.	.05	N.S.
ACETATE VESSELS (% OF CONTROL VALUES)								
19	87.2	88.7	74.4	81.9	37.7	102.6	50.2	66.9
20	90.4	133.2	114.8	83.9	136.7	87.6	118.2	100.0
27	86.4	146.8	150.6	98.7	65.9	65.7	65.7	-
MEAN	88.0	122.9	113.3	88.2	80.1	85.3	78.0	83.5
+-S.E.M.	1.2	17.5	22.0	5.3	29.4	10.7	20.6	16.6
T	9.819	1.305	0.602	2.233	0.675	0.765	1.067	1.000
DF	2	2	2	2	2	2	2	1
P	.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* INCUBATION WAS PERFORMED FOR 3 HOURS IN KRB
RESULTS OBTAINED IN THE PRESENCE OF ALCOHOL (10MM) OR SODIUM ACETATE (10MM) ARE LISTED AS A PERCENTAGE OF THE VALUES OBTAINED IN THEIR ABSENCE. AVERAGE PERCENTAGE CHANGES (+ S.E.M. ARE TABULATED BENEATH AND EXPRESSED IN TERMS OF THEIR STATISTICAL SIGNIFICANCE.

N.S. NOT SIGNIFICANT

**S.A. SPECIFIC ACTIVITY - COUNTS GLUCOSE-C^{14} PER ML. MEDIUM/UG GLUCOSE PER ML. MEDIUM

\# G.P.A. - GLYCEROPHOSPHATE-C^{14}

Figure 10 is a composite representation of the effects of ethyl alcohol onthe disposition of the three gluconeogenic precursors mentioned in this section. The slices of human liver were incubated with labelled L-glutamate, D-l lactate or glycerol in concentration 10mM. The effects of supplementation with 10mM ethyl alcohol are shown by the dark and light bars respectively. Results are expressed as a percentage of the values obtained in control vessels by which convention 100% denotes no effect. It can be seen that ethyl alcohol profoundly depressed gluconeogenesis and the evolution of $C^{14}O_2$ from all of the substrates. In addition, the uptake of L-glutamate and lactate was depressed. The uptake of glycerol could not be estimated. None of the alterations could be reproduced by acetate and cannot therefore be attributed to simple glutting with 2 carbon fragments.

REDUCTION OF AVAILABLE SUBSTRATE

Experiments were performed on 3 occasions using "tracer" quantities (0.01 mM) of alanine-U-C^{14} in contradistinction to the customary "carrier" quantities (10.0 mM). The same radioactive component (1.0 microcurie) was retained. These were carried out to assess the effect of reduced availability of exogenous substrate and limitation of 3 carbon atom gluconeogenic precursors. The experiments were not strictly comparable with those of the main series since all but one, (experiment 6) were incubated for 2 hours only. They were performed to demonstrate specific effects in a particular environment but the familiar trend of profound

THE EFFECT OF ETHANOL vs. ACETATE ON THE DISPOSITION OF GLUCONEOGENIC PRECURSORS BY HUMAN LIVER SLICES

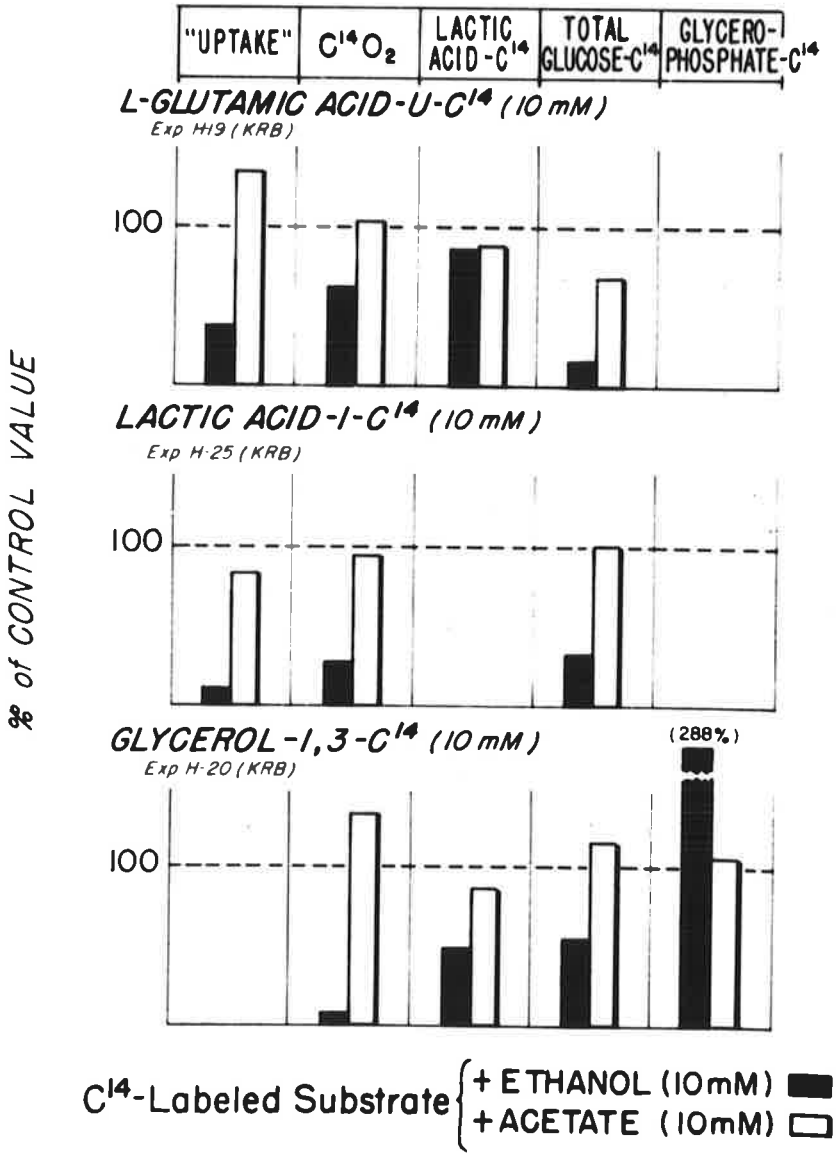

Fig. 10. The effect of ethyl alcohol (10mM) on gluconeogenic precursors other than alanine. Incubation was for 3 hours in KRB. Control studies with sodium acetate (10mM). The uptake of glycerol could not be measured.

$C^{14}O_2$ and glucose-C^{14} depression in the presence of alcohol is evident, Table 11. The greatly heightened uptakes are a consequence of the higher proportion of radioactivity to carrier material.

Experiment No. 23 was performed to contrast the effects of available substrate and the results are shown in Figure 11. Human liver slices were incubated in KRB for 2 hours with 10 micromoles per ml. of ethyl alcohol in the presence of "tracer" and substrate ("Carrier") quantities of alanine-U-C^{14}. The open bars denote the percentages of the added counts that were recovered as $C^{14}O_2$, lactic acid-C^{14} and glucose-C^{14} in control vessels and recoveries in the presence of alcohol are represented by the hatched bars. The proportional inhibitions of gluconeogenesis by alcohol were far greater when the amount of substrate was limited - that for the "tracer" system being approximately 5:1 and that for the "carrier" system 3:1.

TABLE II

THE EFFECT OF ETHYL ALCOHOL ON THE METABOLISM OF ALANINE-U-C^{14} ("TRACER") IN HUMAN LIVER SLICES

(% TOTAL COUNTS PER GRAM INITIAL WEIGHT)

EXPT NO.	TIME	"UPTAKE"	$C^{14}O_2$	LACTIC ACID-C^{14}	GLUCOSE-C^{14}	GLYCOGEN	S.A.** GLYCOGEN
CONTROL VESSELS:							
6	3^0	768.2	175.7	216.5	93.9	5.68	7.60
23	2^0	693.8	142.0	302.7	98.6	0.289	0.60
24	2^0	512.2	133.6	85.2	55.9	1.249	1.561
MEAN 2^0		603.0	137.8	193.9	77.2	0.769	1.08
ALCOHOL VESSELS:							
6	3^0	826.4	95.5	343.5	27.9	4.96	7.19
23	2^0	496.6	86.7	110.8	39.4	1.135	1.409
MEAN 2^0		536.9	68.4	200.6	30.3	0.623	0.796
ACETATE VESSELS:							
23	2^0	648.4	162.2	211.4	89.5	0.319	0.620
23	2^0	515.5	165.8	71.3	62.0	1.037	1.526
MEAN 2^0		581.9	164.0	143.6	75.7	0.678	1.073

* SUBSTRATE ALANINE 0.01MM. ETHYL ALCOHOL AND SODIUM ACETATE 10MM.

** S.A. - SPECIFIC ACTIVITY - COUNTS GLUCOSE-C^{14} PER ML MEDIUM/UG GLUCOSE PER ML. MEDIUM.

Experiment No. 23 **KRB**

SUBSTRATE COMPETITION IN THE DISPOSITION OF L-ALANINE-U-C^{14} BY HUMAN LIVER SLICES.

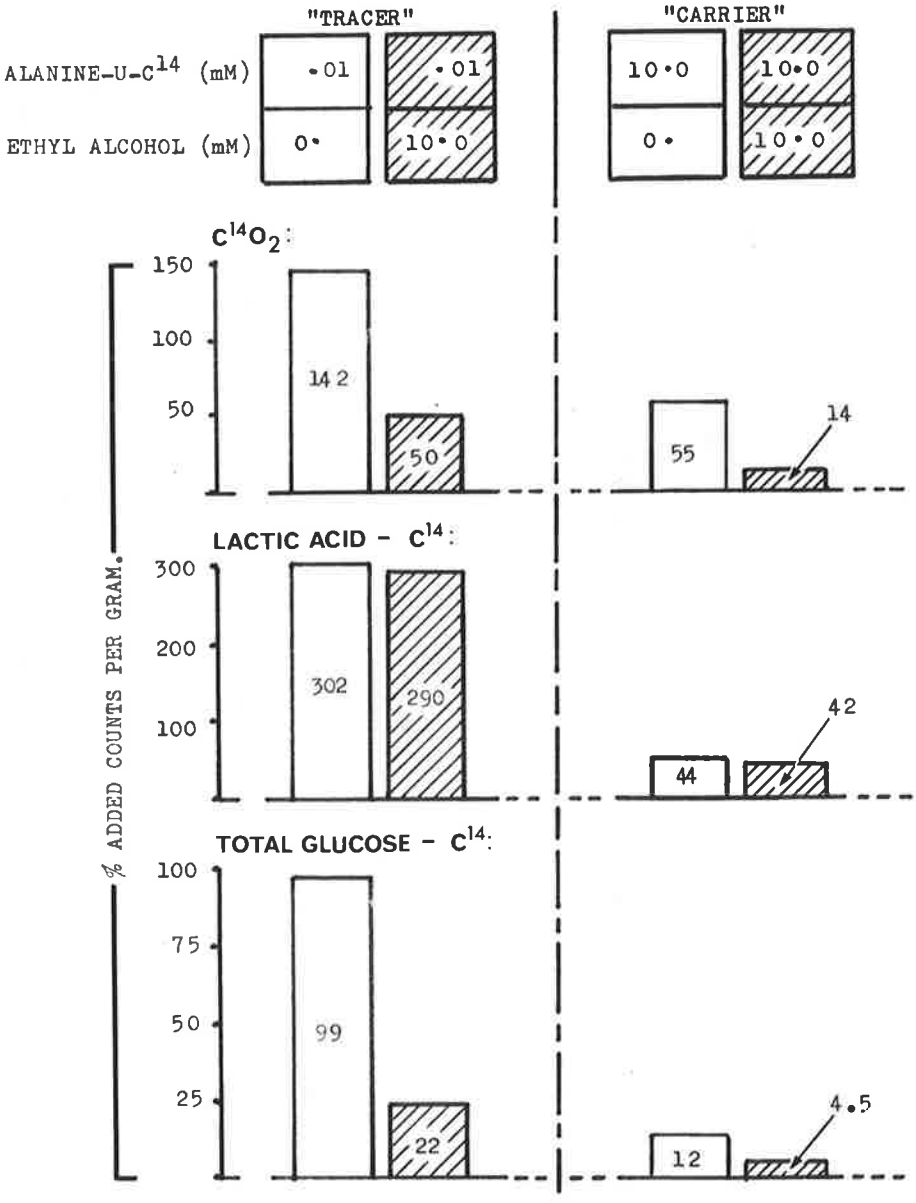

Fig. 11. A comparison between the effects of ethyl alcohol in the presence of "TRACER" (0.0mM) and "CARRIER" (10.0mM) amounts of substrate alanine.
Hatched areas represent results under alcohol.

C H A P T E R VI

Discussion.

The majority of studies concerned with the carbohydrate metabolism of the isolated liver have been carried out on liver slices and the integrated information widely reported. It is recognised that slicing leads to marked trauma of some of the liver cells and that their metabolism is sufficiently disturbed to produce measurable derangements such as the inability of the liver slices to maintain adequate concentration gradients for potassium ions across the cell membrane (Flink et al., 1950) and the non-homogeneous distribution of freshly synthesised glycogen (Deane et al., 1947). Such problems have been thoroughly investigated and partially resolved by suitable adjustments in the incubation medium (Hastings and Buchanan, 1942; Hastings et al., 1952) where the environment of the slice has been altered to simulate the intracellular cationic composition.

Few if any such studies have been carried out with human liver. Lipman et al., (1962) have used human liver slices to study 6B-hydroxy-cortisol production. Cahill, (1964) reports an isolated observation made by Ashmore and himself in 1955 on human liver slices obtained from a patient undergoing excision of a hepatoma under hypothermia. This preparation yielded and expected result in comparison with their animal studies in that insulin decreased glucose production in the slices and halved the conversion of fructose to glucose whilst doubling the incorporation of fructose carbon into fatty acids.

Many preparations in which the architecture of the hepatic cell has not been preserved have been reported but the present study has not been previously attempted and a search of the literature has failed to yield data which could be used for comparison of the metabolic effects.

The reasons for this diffidence in using human liver slices are not hard to find. The isolated organ or tissue - even if relatively intact and maintained in unison with its source of blood supply and drainage - is manifestly artificial and devoid of the subtle complexities of mineral, hormonal and enzymatic influences which regulate and transform the various syntheses it performs. Despite these discrepancies, the use of solitary animal tissues has provided invaluable information for the very reason of their relative isolation which allows of concentration on one or two special points. Extrapolation of data to the events taking place in the whole organism is unjustified, but the combined demonstration of *in vivo* and *in vitro* events in response to similar experimental circumstances immeasurably strengthens the interpretation of clinical and physiological observations. No greater significance is claimed for these studies.

The rat liver slice preparation can be narrowly stabilised. Conditions of age, sex, weight, temperature and prior food intake can be regulated to reduce tremendously the possibility of experimental variation. The preamble to and act of sacrifice can be reduced to a judicial precision, and the predictability of the tissue in terms of metabolic performance has been substantiated by a thousand documented studies.

As is apparent from the details in the clinical methodology of this thesis, the use of human liver for slice techniques is far less capable of standardisation. The age, sex, weight and pedigree of the experimental subjects were randomised and contained only by those broad criteria of relative good health and absence of gross metabolic, nutritional, infectious or malignant disease. The mode of obtaining the liver wedge was no less ill-regulated in the variable period of the preceding fast, the nature of and response to the anaesthetic agent and the elastic period of time between induction of anaesthesia and the removal of the specimen.

These points being conceded however there is no doubt that the behaviour of the human liver slices was similar in most respects to those of rat liver as judged by parallel experiments performed during the course of this work. Moreover, Freinkel et al., (1965) have obtained identical effects with ethyl alcohol in vitro during the incubation of 5 to 8 mg. blocks of human liver obtained by percutaneous biopsy, indicating that the vulnerability of surgically-derived liver to interruption of gluconeogenesis cannot be attributed to anaesthesia. From the point of biopsy onwards, all experimental conditions were as rigidly circumscribed as those with animal tissues.

Ingram, (1962) has shown that the survival of monkey liver tissue was affected very little by setting up the cultures in the cold, changing the medium, rolling the culture tubes or leaving them stationary with the possible exception of changing the medium which appeared to be harmful.

Gassing the cultures with a 95% O_2 - 5% CO_2 mixture had the greatest advantage of any factors tested for their beneficial effect on survival of the tissue. These precautions were observed in these studies on human liver.

There was a marked difference in the metabolic activity between the tissues exposed to prior gassing with 95% O_2 - 5% CO_2 and those utilised for the Warburg studies. In the latter instance CO_2 content is varied by omitting HCO_3 and pCO_2 from the system. The Krebs Ringer Phosphate in this experimental situation poorly supports the oxidation of pyruvate, and such reactions as those involved in gluconeogenesis which require the fixation of atmospheric CO_2 are also retarded. The Warburg studies revealed a mild degree of reduction in tissue oxygen utilisation in the alcohol-containing vessels as compared with the control. With human liver slices, CO_2 was usually depressed 5 - 10% during the first hour and 10 to 20% at the end of the third hour. In view of these comparatively minor changes in respiration mechanisms other than histotoxic anoxia were probably involved. Apparently ethyl alcohol does not by itself cause the oxygen consumption of the liver to rise, either *in vitro* (Leloir and Munoz, 1938) or *in vivo* (Lundquist et al., 1962).

HUMAN LIVER GLYCOGEN.

The problem in determining glycogen concentration in human liver is made difficult because of the rapidity of glycogenolysis. Wedge resections require prior haemostasis which produces anoxia and glycogenolysis. Needle biopsies are also difficult to assess because of their small volume and the possibility of sampling errors. Cahill and Horsely (1964) have commented on the advantages of obtaining

specimens from patients undergoing surgery with hypothermia. Paired wedge biopsies of 0.5 gm. each in patients fasted 1-3 days and analysed for glycogen gave mean values of 6.6; 6.7; 3.5; 3.2; 3.8; 2.6 and 2.9 gm./100 gm liver. Concomitant analyses of glucose and lactate in these tissues gave values within 20% of the concentration of blood glucose and lactate thus ensuring that significant glycogenolysis had not occurred from the time of excision to the time of analysis.

The levels of stable glycogen found in the liver slices of these experimental subjects accord well with those previously described in the literature. Ekdahl and Zederfeldt, (1963) used a biopsy technique very similar to that described here and obtained a mean level of 2.91 gm. per 100 gm. wet weight of liver. From a comparison with other subjects they considered that glycogen levels were lower in patients with chronic gall bladder disease. Sunzel, (1963) studied glycogen levels in a group of patients undergoing partial gastrectomy and found mean amounts of 3.47gm. glycogen per 100 gm. wet liver at the commencement of the operation. He noted the profound glycogen-lowering effect of starvation which was capable of reducing the initial glycogen amounts by 30%. Similar findings were obtained by Abels et al., (1943) and Annamunthodo et al., (1958) but MacIntyre and his colleagues, (1941) could not verify this effect. Edlund and Sunzel, (1964) studied 36 subjects with gall bladder disease of whom half had been jaundiced. Their techniques were almost identical with those of this study. In their control - not jaundiced - group they found a mean glycogen level of 4.0% with a range of 2.40 to 5.83 and S.D. 1.05.

THE STUDY OF GLUCONEOGENESIS UNDER IN VITRO CONDITIONS.

The synthesis of glucose from non-glucose precursors may be most difficult to substantiate under in vitro conditions. Concomitant changes in glycogen, as the storage form of glucose, must always be estimated. An increase of glucose in a system under observation with a corresponding decrease in glycogen may or may not indicate that new glucose formation has occurred. In studies using stable glucose it is not possible to be certain that gluconeogenesis has occurred, although gluconeogenesis from a substrate would seem more likely the greater the net synthesis of glucose that occurs in the presence of the substrate compared with its absence.

When isotopes are employed, care in the interpretation of results is equally necessary. Isotopes permit measurements of incorporation but these cannot be equated with synthesis (Landau, 1960). The effects of changes in pool sizes and the rate of turnover, exchange reactions, transient and steady states must all be considered in the evaluation of isotopic data.

The finding of newly formed glucose derived from the carbons of isotopically labelled alanine, glutamic acid, lactic acid and glycerol in these experiments is suggestive that gluconeogenesis has occurred. However, some of the assumptions inherent in the acceptance of incorporation of C^{14} as a measure of gluconeogenesis should be noted.

Incorporation of C^{14} from a substrate such as alanine into a product such as glucose can presumably occur as a consequence of exchange reactions whereby C^{14} is exchanged for C^{12} in the absence of net synthesis. These reactions may simply represent the equilibration of metabolic intermediates as is found in the equilibration of labelled glyceraldehyde-3-phosphate and dihydroxyacetone phosphate with fructose diphosphate via aldolase. In this case a net synthesis of carbohydrate may or may not occur. Alternatively, they may represent reactions such as the equilibration of labelled glyceraldehyde phosphate with fructose diphosphate via transaldolase (Wood et al., 1959), in which case no net synthesis of carbohydrate is possible. The increased incorporation of label from radioactive alanine into carbohydrate could therefore represent an increase in the rate of such reactions rather than an increased synthesis of carbohydrate from the precursor.

Certain inferences may be utilised to establish that gluconeogenesis has indeed occurred. Thus, the quantity of carbohydrate present initially may serve as a measure of the quantity of carbohydrate available for exchange. If incorporation occurs to the same extent on incubation of two tissues, one with a low initial carbohydrate content and the other with a high initial content, in the former incorporation might be more justifiably equated with gluconeogenesis. The incorporation of isotope into carbohydrate in greater quantity than the amount of carbohydrate initially present may also imply that gluconeogenesis has occurred, since under these circumstances more than exchange reactions would be necessary to account for the incorporation. However, as Landau (1960) remarks, even this additional criterion is not conclusive since

carbohydrate formed from substrates other than labelled substrates could enter exchange reactions and theoretically increase the ultimate yield of carbohydrate. Landau in this paper considers that there are no infallible standards for the establishment of incorporation as a measure of gluconeogenesis.

In the experiments described here, slices from the same human liver showed significant alterations in radioactive glucose and lactate formation between the control and alcohol containing vessels. It is not possible to assert whether the production of lactate and glucose was altered in net amount or merely their rate of labelling, but the reciprocal changes which were noted between lactate and glucose suggests that a true impairment of gluconeogenesis has occurred. Moreover, as has been discussed, ethyl alcohol reduced the glucose - C^{14} recovered in glycogen hydrolysates to the same extent as the glucose - C^{14} recovered in the final suspending media. This dual inhibition of labelling in "stored" as well as "released" glucose suggests that gluconeogenesis has been directly impaired.

ETHYL ALCOHOL AND GLUCONEOGENESIS

In the present series of experiments ethyl alcohol (10mM) diminished the assimilation (i.e., "uptake") of labelled alanine by liver slices from the suspending medium in all experimental situations. Formations of $C^{14}O_2$ and glucose-C^{14} were reduced to an even greater extent coincident with an absolute or relative increase in the evolution of lactic acid-C^{14}. Thus, as is shown in two representative experiments in figure 8, ethyl alcohol caused a smaller proportion of assimilated alanine to be oxidised to $C^{14}O_2$ or transformed to glucose-C^{14}, and a greater proportion to be converted to lactic acid-C^{14}. Ethyl alcohol reduced the amount of glucose-C^{14} recovered in glycogen hydrolysates to the same extent as the glucose-C^{14} recovered in final suspending media. This, as already discussed, implies that gluconeogenesis has been impaired directly and at some stepprior to the renewal of the glucose-6-phosphate pool. Similar results were obtained with other gluconeogenic precursors (Figure 10).

In the intact animal, gluconeogenesis by the liver may be influenced by effects upon:

a) Extrahepatic generation of gluconeogenic precursors via protein catabolism (for amino acids), lipolysis (for glycerol) and glycolysis (for lactate);

b) Delivery of the gluconeogenic precursors via splanchnic blood flow;

c) Assimilation and transformation of the precursors within the liver by specific enzymes and co-factors;

d) Integration of all these aspects by neurohumours and hormones (Freinkel and Bleicher, 1963).

Actions of ethyl alcohol have been described which could influence each of these factors.

Very few published data on the effects of alcohol on amino acid and protein metabolism within the liver are available. Shimizu and Isselbacher, (1964) have shown that alcohol can inhibit the oxidation of an amino acid such as leucine by as much as 60 to 70% in the isolated, perfused rat liver and may interfere with the incorporation of amino acid into perfusate lipoproteins as well as other plasma proteins. The effects of alcohol on lipolysis are well documented as causing increased hepatic fatty acid synthesis and decreases in fatty acid oxidation (Lieber and Schmid, 1961). Levels of free fatty acids in response to alcohol in man have been found to vary depending on the duration of the experiments and the dosage of alcohol (Lieber and Davidson, 1962; Isselbacher and Greenberger, 1964). Increased blood lactate levels after the administration of alcohol to humans is established (Seligson et al., 1953; Mendeloff, 1954; Lieber et al., 1960).

The effect of alcohol on hepatic and other regional blood flows has been the subject of various controversial reports indicating that in man there may be either an increase in hepatic flow (Stein et al., 1960) or no change (Castenfors et al., 1960; Willard and Horvath, 1960).

The assimilation and transformation of gluconeogenic precursors within the liver may be best studied by <u>in vitro</u>

methods. It must be recognised however that most *in vitro* preparations have disadvantages for the study of gluconeogenesis since of the disintegrated preparations only pigeon liver homogenates have been found capable of synthesis of glucose (Krebs et al., 1964) and liver slice techniques, despite the wealth of qualitative information which they have yielded, have proved unsuitable for quantitative work on the *rates* of gluconeogenesis. In this latter regard, sliced kidney cortex has proved much more active than liver, and the increasingly recognised capability of the kidney for gluconeogenesis is a consequence of this finding (Krebs et al., 1963). The role of the kidney in blood glucose homeostasis has not been defined but it should be noted that the kidney is one of the few extra hepatic structures in which there is sufficient alcohol dehydrogenase for any meaningful oxidation of alcohol (Lieber 1967).

Glucose production depends on the functioning of the final common path of gluconeogenesis which encompasses the enzyme steps between lactate and glucose. From the point of view of function, direction of metabolic flow and regulatory significance, the enzyme activities between glucose and lactate can be divided into three groups. (Weber et al., 1965).

1. The key gluconeogenic enzymes.
2. The key glycolytic enzymes.
3. The bifunctional enzymes.

It is the key gluconeogenic enzymes which are most relevant to the discussion in this thesis since only gluconeogenesis has been particularly studied. Nevertheless, the

activities of the glycolytic and bifunctional enzymes may have relevance to the action of alcohol in that their suppression may heighten an otherwise unaltered gluconeogenic activity.

The key gluconeogenic enzymes exert a rate limiting role in the production of glucose from lactate and other gluconeogenic precursors. They have low activities, govern one-way reactions and are involved in circumventing thermodynamic barriers. They are localised chiefly or exclusively in organs capable of gluconeogenesis. (Weber et al., 1965). The enzymes are:

i) Glucose-6-phosphatase
ii) Fructose-1, 6-diphosphatase
iii) Phosphoenol pyruvate kinase
iv) Pyruvate carboxylase.

In the liver there are three steps of glyconeogenesis which are crucial for the reversal of glycolysis (Krebs, 1964). The four key gluconeogenic enzymes operate at these steps as follows:

a) Glucose-6-phosphatase reverses the thermodynamically unfavourable glucokinase reaction by hydrolysis of glucose-6-phosphate.
b) Fructose-1, 6-diphosphatase reverses the similarly unfavourable phosphofructokinase reaction by hydrolysis of fructose-1, 6 diphosphate.
c) Phosphoenol pyruvate kinase and pyruvate carboxylase promote the formation of phosphoenolpyruvate via oxaloacetate.

The last process is the most complex and may have alternative pathways for its accomplishment which vary with different species. It has the most relevance for the formation of glucose from noncarbohydrate sources and particular significance in the studies of the effects of alcohol, for pyruvate stands in a key position at the gateway to gluconeogenesis. If glucose synthesis from pyruvate were to result from simple reversals of the kinase reactions with consumption of ATP, excessively high concentration ratios of ATP/ADP far exceeding physiological amounts would be required (Krebs, 1954).

A search of the literature has failed to reveal experimental studies which have investigated the action of ethyl alcohol on the key gluconeogenic enzymes. Much work has been done on the action of the glucocorticoid hormones in influencing these enzymes and it has been shown by several experimenters that glucose-6-phosphatase and fructose-1, 6 diphosphatase are both increased (Kvam and Parks, 1960; Weber et al., 1963). Using actinomycin and puromycin to block the cortisone-induced enzyme increases, the latter authors were able to show that such increases were due to new enzyme synthesis. This aspect has been further studied by Kenney et al., (1965) who found increases of RNA synthesis in livers of rats treated with hydrocortisone. They claimed that the general nature of the hormone effect on RNA synthesis suggests that enzyme induction may simply reflect a general increase in hepatic protein synthesis. The rapid turnover enzymes, tyrosine transaminase and tryptophan pyrrolase could be induced to a limited extent by ethyl alcohol given as 2ml of a 10% solution.

It has been observed in laboratory animals as well as in certain human subjects that a-ketoglutaric acid concentrations in the liver are increased in acute and chronic alcohol intoxication (Klatskin, 1961; Altschule et al., 1957). Dajani and Kouyoumjian (1966) estimated the levels of isocitric, succinic and glutamic dehydrogenases in the livers of rats given a 20% solution of ethyl alcohol as their sole drinking fluid. Compared with controls, the alcohol-fed rats showed a significant increase in isocitricand glutamic dehydrogenase with a slight increase in succinic dehydrogenase. The authors were unable to state whether these elevations were a consequence of altered protein synthesis.

Recently, Williams (1965) has presented preliminary evidence that ethyl alcohol may also affect enzymes involved in glycogen synthesis (i.e., UDP glucose glucanglucosyl transferase) and breakdown (glucan phosphorylase). However, the physiological relevance remains to be demonstrated since these actions necessitated 500mg% - 2,000 mg% concentrations of alcohol.

In summary it may be said that no specific investigations have been published concerning the action of ethyl alcohol on the key gluconeogenic enzymes. However, in the <u>in vitro</u> studies reported here, the effects are independent of the synthesis of new enzyme although they may be related to the direct inhibition of existing enzymes.

The abundant lactic acid-C^{14} formation in all of these experiments suggested that the depression of $C^{14}O_2$ by ethyl alcohol was not due to major limitations in formation of

pyruvate-C^{14} from labelled alanine. Instead, the reciprocal changes in $C^{14}O_2$ and lactic acid-C^{14} were viewed as evidence that alcohol impaired the oxidation of pyruvate as has been demonstrated by Field et al., (1963) and Forsander, (1961).

In the bulk of the present studies, labelled alanine was employed to provide a modulated generation of pyruvate that might simulate the delivery of gluconeogenic precursors *in vivo*. (Arky and Freinkel, 1964). The subsequent pathways for the intrahepatic disposition of pyruvate that are relevant to gluconeogenesis are summarised in Figure 12.

Under the control circumstances, the labelled pyruvate, newly generated from alanine (reaction *1) has several alternative pathways for metabolic disposition. It can be reduced to lactate by lactic dehydrogenase (reaction *2) in accord with the prevailing concentration ratio of $NADH_2/NAD^+$; oxidised by pyruvate dehydrogenase to acetyl-CoA, again with the influence of the pyridine nucleotides (reaction *3); or converted to phospheonol pyruvate directly by pyruvate kinase (reaction *4). Indirect formation of phosphoenolpyruvate may be accomplished by carboxylation via malic enzyme (reaction *5) or pyruvate carboxylase (reaction *6) with subsequent decarboxylation by phosphoenol-pyruvate carboxykinase (reaction *7). Direct formation of phosphoenolpyruvate from pyruvate is probably insignificant under ordinary circumstances, (Utter, 1963; Topper and Hastings, 1949).

Carboxylation via malic enzyme is probably also of minor import. Avian liver mitochondria, which lack both malic

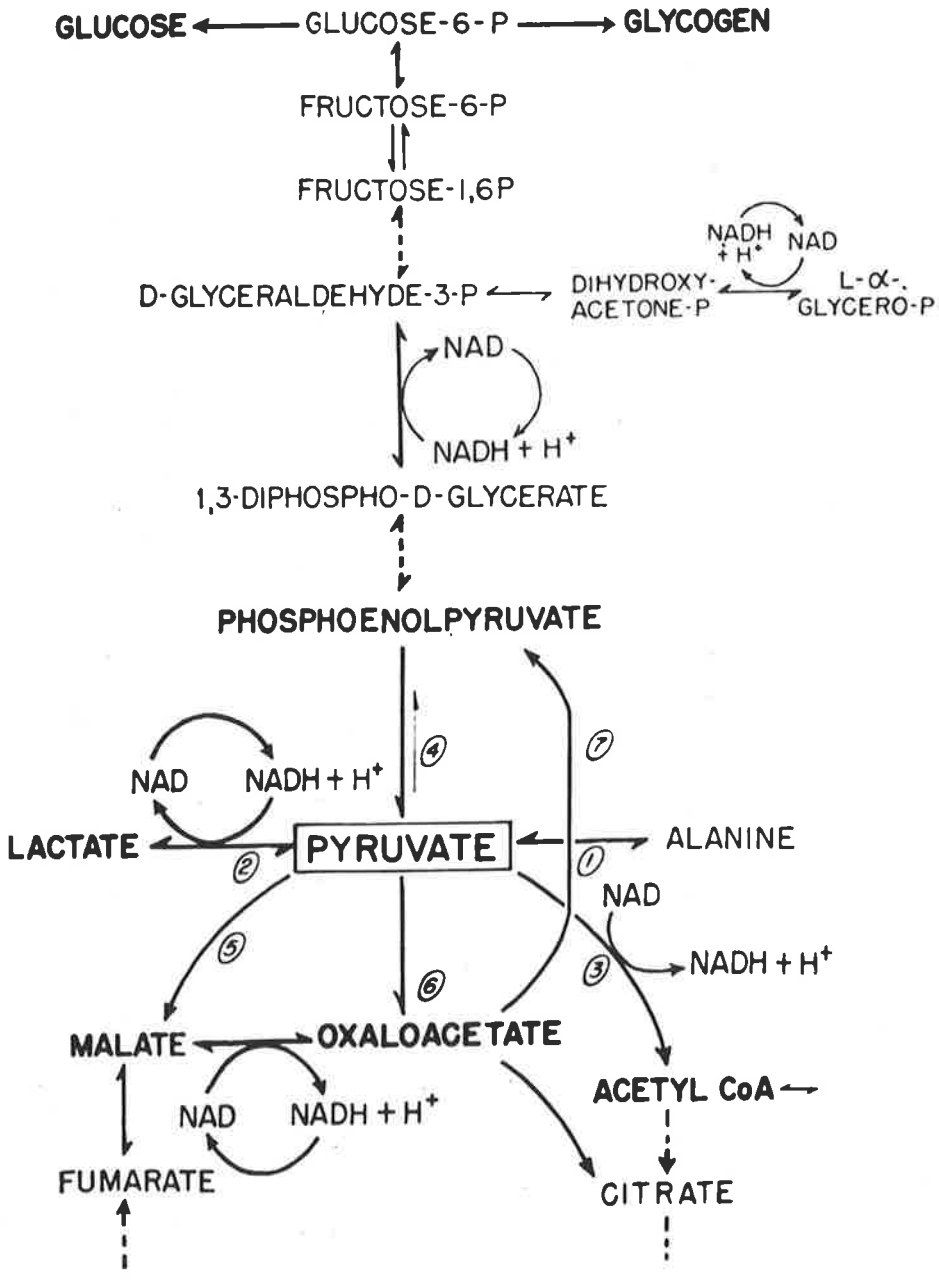

Fig. 12. Alternative pathways for the intrahepatic disposition of pyruvate-C^{14} derived from alanine-C^{14}. For description see text

enzyme and pyruvate kinase, are capable of synthesising considerable amounts of phosphoenolpyruvate from pyruvate. The abbreviated dicarboxylic shuttle utilising the intramitochondrial enzyme pyruvate carboxylase (Keech and Utter, 1963; Utter and Keech, 1963) and phosphoenol pyruvate carboxykinase is probably the most significant pathway of synthesis.

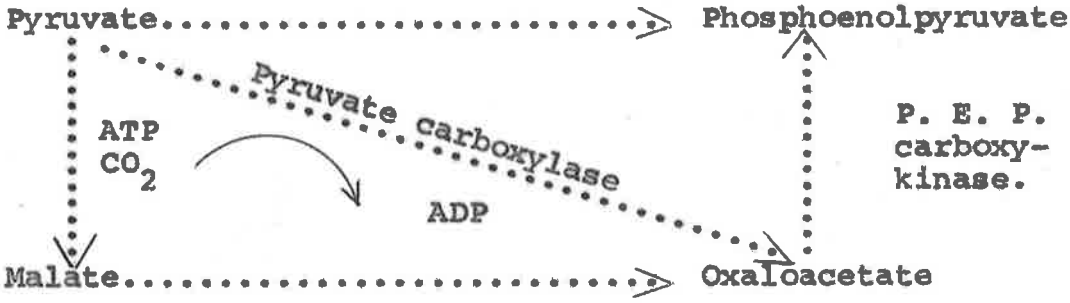

FIGURE 13. Synthesis of phosphoenolpyruvate by an abbreviated dicarboxylic acid shuttle.

The fact that pyruvate carboxylase requires activating amounts of acetyl CoA has provided a direct link between the oxidative decarboxylation of pyruvate and gluconeogenesis (Keech and Utter, 1963; Krebs, 1964).

Reduction to lactate is a metabolic cul-de-sac since lactate cannot be oxidised within the mitochondrion. On the other hand, the acetyl CoA can be employed for the reductive biosynthesis of fat, or for total oxidation within the Krebs cycle by condensing with oxaloacetate to form citrate. Fixation of CO_2 to form malate or oxaloacetate is perhaps the most important step since the generation of these substances can replenish the 4-carbon intermediates of Krebs cycle. More importantly, it

affords the potentiality for net synthesis of 6-carbon compounds since oxaloacetate can be decarboxylated to yield phosphoenolpyruvate and can thus circumvent the thermodynamic barrier which pyruvate kinase poses to the direct formation of phosphoenolpyruvic acid as described above.

The foregoing inter-relationships may be altered in the presence of ethyl alcohol. During the two-step oxidation of 1 mole of alcohol to acetaldehyde and then to acetate (or acetyl CoA) by means of alcohol and aldehyde dehyrogenase respectively, 2 moles of NAD^+ are reduced, (Lieber and Davidson, 1962; Isselbacher and Greenberger, 1963). This reaction takes place within the soluble cytoplasm of the liver and the extra-mitochondrial ratio of $NADH_2/NAD^+$ is increased. This is quite different from the generation of $NADH_2$ which occurs during normal glycolysis since the additional $NADH_2$ from alcohol oxidation is not counterbalanced by a paired generation of 3-carbon fragments. Thus the reoxidation of such $NADH_2$ must be wholly effected by pre-existing hydrogen acceptors, (Freinkel et al., 1965).

At this juncture, in reference to figure 12, the ubiquitousness of the diphosphopyridine nucleotide systems must be noted. They occur in relationship to most of the important redox steps and may at times be rate limiting if their ready conversion from the reduced to the oxidised form is impeded. It is also noteworthy that they are found predominantly in an extramitochondrial location in the cell sap and cannot readily cross the mitochondrial membrane (Lehninger, 1951; Chance, 1963). Wherever redox reactions are occurring, NAD^+ will accept hydrogen to

become $NADH_2$ but some readily available mechanism for reoxidation to NAD^+ must be present to avoid slowing and ultimate cessation of the reaction.

Electrons can be carried from extramitochondrial $NADH_2$ to the intramitochondrial electron transport chain by indirect routes. In principle, any metabolite that can be reduced in the cell sap by $NADH_2$ to a product that is a substrate for mitochondrial oxidation can serve as a link in the transport of reducing equivalents (Boxer and Devlin, 1961) Such carrier systems or "shuttles" are utilisedto regenerate fresh NAD^+.

The glycerophosphate "shuttle" is of considerable importance in this regard. Dihydroxyacetonephosphate and glyceraldehyde-3-phosphate are formed in equimolar amounts as the products of the action of aldolase on fructose-1, 6-diphosphate. The very active triosephosphate isomerase establishes an equilibrium between these products which, by a ratio of 20 to 1, favours dihydroxyacetonephosphate which can, in the presence of the soluble a-glycerolphosphate dehydrogenase of the cell sap, accept electrons from $NADH_2$ to form L-a-glycerophosphate (Boxer and Devlin, 1961). Since $NADH_2$ is formed during the oxidation of glyceraldehydephosphate, the two enzymes glyceraldehydephosphate dehydrogenase, triosephosphate dehydrogenase and glycerolphosphate dehydrogenase, form an effective dismutation system that leads to the continous regeneration of NAD^+ (Green et al., 1937).

Another pathway for the transfer of reducing equivalents from extramitochondrial $NADH_2$ to molecular oxygen,

involving acetoacetate and B-hydroxybutyrate, has been described by Devlin and Bedell (1960). The reversible relationship between oxaloacetate and malate provides a further example (Siegal and Englard, (1962).

In spite of these many routes for the re-oxidation of $NADH_2$, the $NADH_2/NAD^+$ ratio within the hepatic cell increases two to four-fold during the oxidation of ethyl alcohol (Smith and Newman, 1959; Reboucas and Isselbacher, 1961). During starvation, total hepatic NAD^+ plus $NADH_2$ decreases and the $NADH_2/NAD^+$ ratio rises (Smith and Newman, 1959). These workers have also shown a greater increase in $NADH_2/NAD^+$ ratio following ethyl alcohol in the tissues of starved as compared with fed animals. Similar studies have not been performed in man, but an idea of the ratio may be obtained by determinations in the hepatic venous blood of certain metabolites that form parts of redox systems governed by NAD - dependent enzymes. Tygstrup et al., (1965) have demonstrated an increase in the B-hydroxybutyrate/acetoacetate and lactate/pyruvate ratios in hepatic venous effluent blood following infusions of ethyl alcohol in human volunteers. These *in vivo* and *in vitro* data leave little doubt that ethyl alcohol oxidation within the liver results in a diminished availability of NAD^+ and an excess of $NADH_2$.

The entry of glutamic acid into the Krebs cycle is effected by oxidative deamination and transamination to alpha-ketoglutarate. In the presence of relative $NADH_2$ excess and a reduced generation of acetyl CoA and cycling of tricarboxylic acids, the glutamic hydrogenase reaction will be displaced towards glutamate. A reduced

uptake of extracellular glutamate with a diminished amount of gluconeogenesis from this substrate might be expected. Similarly, the prevailing $NADH_2/NAD^+$ ratio conditions the equilibrium of lactic acid dehydrogenase. In the presence of relative $NADH_2$ excess, net hepatic assimilation of lactate would be diminished and gluconeogenesis by way of the Coricycle might be compromised. Finally, the importance of the prevailing $NADH_2/NAD^+$ ratio is also seen in the case of glycerol. Following its phosphorylation by intrahepatic glycerokinase, glycerol may directly equilibrate with glyceraldehyde-3-phosphate via glycerophosphate dehydrongenase and so bypass the reductive step in gluconeogenesis. However, this equilibration is also conditioned by the $NADH_2/NAD^+$ ratio (figure 12) and, in the face of relative $NADH_2$ excess, the already limited availability of glycerol for glucose synthesis and for lactate production might be retarded. Such an interference would be reflected by a relative accumulation of glycerophosphate.

Figure 10 illustrates the depression of gluconeogenesis produced by ethyl alcohol (10mM) on each of these substances - radioactive glutamate, lactate and glycerol. Evolution of $C^{14}O_2$ was similarly reduced as was the uptake of L-glutamate-C^{14} and lactate-^{14}. Although the uptake of glycerol was not measured, the expected accumulation of glycerophosphate-C^{14} occurred. None of these phenomena was observed when sodium acetate was used and therefore cannot be ascribed to simple 2-carbon fragment dilution.

The investigations in this thesis have been designed to show that ethyl alcohol can inhibit gluconeogenesis

from certain glucose precursors in human liver slices. They have not sought specifically to elucidate the mechanism whereby such inhibition is attained. The results quoted above would certainly support the contention that the altered $NADH_2/NAD^+$ ratios occasioned by alcohol administration may be responsible. Madison et al., (1967) have tendered further evidence that disturbances of this ratio cause a partial block at several points in the gluconeogenic pathways in the dog. A toxic role for incomplete oxidation products (Freund and O'Hollaren, (1965), coincidental peroxidations (Kalish and DiLuzio, 1966) novel metabolites (Bloom and Westerfield) 1966, or the effects on enzymes of other non-substrate metabolites (Atkinson, 1966) cannot be excluded entirely.

The addition of NAD^+ itself to the experimental system did not increase gluconeogenesis in the experiments in which it was tried. A small increase of lactate was the only obvious difference. This is not surprising in view of the inability of NAD^+ to cross the mitochondrial membrane (Lehninger, 1951: Chance, 1963). However, Smith and Newman, (1959) obtained increased alcohol oxidation in the liver slices from their starved rats by the addition of NAD^+.

Certain aspects of ethyl alcohol-induced hypoglycaemia remain unexplained. The inconstant occurrence of the condition despite the widespread use of alcohol, the difficulty in reproducing the syndrome in all experimental animals and the variability of response under similar experimental conditions are some of the features not yet fully elucidated. In humans the condition is probably much more common than is recognised or described.

Whilst prior fasting or at least glycogen depletion of the liver are important pre-requisites, there are no doubt more subtle priming situations in the intermediate metabolism of the liver. Variations between the degree of depressions of hepatic glucose output and peripheral glucose utilisation may also contribute to the difficulty in reproducing the syndrome under all conditions.

C O N C L U S I O N S

These studies have shown that in the human liver slice, ethyl alcohol 10mM can reduce the formation of radioactive glucose from the radioactive gluconeogenic precursors alanine, glutamic acid, lactic acid and glycerol. The findings are similar in type but not in degree to those which occur in the rat and rabbit liver slice but the response of human liver in this regard has not been previously investigated.

It is probable that the reduced formation of radioactive glucose reflects a decrease in gluconeogenesis which may be explained by the effect of ethyl alcohol on the ratio of $NADH_2/NAD^+$ within the liver cell. Comparable actions on glucose formation were not achieved with sodium acetate which, like ethyl alcohol, furnishes 2 carbon fragments in its metabolism but does not increase cytoplasmic $NADH_2/NAD^+$.

The concentration of ethyl alcohol in the experiments represents a blood level of 46 mg.% which is not "unphysiological" in terms of toxicity to adult human beings. The mild depression of tissue oxygen consumption in these studies does not provide an explanation for the effect of ethyl alcohol on the formation of glucose from gluconeogenic precursors.

The <u>in vitro</u> findings with human liver slices have

relevance for the clinical aspects of hypoglycaemia due to alcohol:

Firstly, they establish a direct effect on the human liver cell as a cause of the reduced glucose formation:

Secondly, by localising the biochemical lesions to steps preceding the renewal of glucose-6-phosphate from smaller carbon fragments, the data can explain the concomitant inhibition of hepatic glycogenesis and glucose release that are seen in clinical hypoglycaemia due to alcohol:

Thirdly, the findings emphasise the importance of preformed glycogen in protecting the individual from hypoglycaemia induced by the interruption of gluconeogenesis. Presumabley, it is the short-lived maintenance of blood sugar levels by glycolysis which protects the transiently fasted as against the more prolonged fasting patient from the effects of ethyl-alcohol-mediated hypoglycaemia. Moreover, these effects may be masked for varying periods of time depending on the duration of availability of glucose from preformed glycogen thus explaining the described occurrence of hypoglycaemia many hours after the imbibition of alcohol. Such a balance between the blood sugar sustaining action of glycogenolysis and the alcohol-induced-supression of gluconeogenesis may explain the occasional spontaneous return to normoglycaemia which may have been responsible for the earlier failures to distinguish between hypoglycaemic coma and alcoholic stupor. The fact that alcohol

is oxidised at a rate which is relatively independent of blood alcohol levels above a certain concentration (Brown and Harvey, 1941) also serves to emphasise the potentially lethal aspects of the syndrome whenever imbibition has been heavy, or when insulin (which can also restrain the hepatic release of glucose) has been used before the debauch, as therapy for diabetes, or afterwards for "sobering up" as has been described.

Finally, although alcohol can cause hypoglycaemia in otherwise normal subjects, the vulnerability to alcohol hypoglycaemia will be greater in any setting in which endogenous capacities for gluconeogenesis are marginal already. The diagnosis of this state therefore should prompt a search for other factors, particularly endocrine, which might predispose the subject to an interruption of gluconeogenesis.

APPENDIX I

CLINICAL, EXPERIMENTAL AND BIOCHEMICAL DETAILS

EXPT. NO * & SUBSTRATE	CLINICAL DETAILS	PROCEDURE	B.S.L. MG%	N.E.F.A. M EQ/I	L.F.T.'S.	HISTOLOGY
*1 ALANINE	S.F. FEMALE 53 OBESE NO SIGNIFICANT PREVIOUS ILLNESS.	REPAIR OF VENTRAL HERNIA	-	-	-	-
*2 ALANINE	A.V. MALE 58 LONGSTANDING HISTORY OF DUEDENAL ULCER PAIN WITHOUT OTHER COMPLICATIONS.	PARTIAL GASTRECTOMY	115	570.6	ALK. PHOS. 2.1 AMYLASE 62	-
*3 ALANINE	M.D'G. FEMALE 61 RECURRENT BILIOUS ATTACKS FOR 21 YEARS. HYSTERECTOMY 1934	ELECTIVE CHOLECYSTECTOMY	355	480.3	-	MARKEDLY FATTY LIVER WITH FATTY CYSTS. SLIGHT BILE DUCT PROLIFERATION IN PORTAL TRACTS.
*4 GLUTAMIC						
*5 GLUCOSE	J.D. MALE 65 PERFORATED D.U. OVERSEWN 1957 RECENT PAIN INCREASE AND HAEMATEMISIS OF SLIGHT AMOUNT.	PARTIAL GASTRECTOMY	100	599.5	B.S.P. 0% ALK. PHOS. 2.0 AMYLASE 106 BILIRUBIN 0.3	SLIGHT FATTY INFILTRATION.
*6 ALANINE "TRACER"	M.J. FEMALE 58 OBESE ACUTE CHOLECYSTITIS	ELECTIVE CHOLECYSTECTOMY. REMOVAL OF PANCREATIC CYST.	-	393.6	-	NORMAL.

THE SUBSTRATE REFERS TOTHE RADIOACTIVE MATERIAL AS DETAILED IN THE TEXT.

ALKALINE PHOSPHATASE LEVELS EXPRESSED AS BODANSKY UNITS (N 1.5).

APPENDIX I (CONTD)

CLINICAL, EXPERIMENTAL AND BIOCHEMICAL DETAILS

EXPT. NO. * & SUBSTRATE	CLINICAL DETAILS	PROCEDURE	B,S.L. MG%	N.E.F.A. M EQ/I	L.F.T.'S	HISTOLOGY
*7 ALANINE & *8 GLUTAMIC	P.P. MALE 67 INTERMITTENT CLAUDICATION SEVERAL YEARS. PROGRESSIVE OCCLUSION OF L FEMORAL ARTERY. PARTIAL GASTRECTOMY 1956.	ILIAC ARTERY BY-PASS GRAFT	109	563.4	BILIRUBIN 0.5 CEPH. FLOCC 0 THYMOL TURB. 0.8 PROTEINS 6.3/3.8	NORMAL
*9 & *10 GLUTAMIC	T.L. FEMALE 59 POST PRANDIAL DYSPEPSIA 2 YEARS. MULTIPLE GALL STONES.	ELECTIVE CHOLECYSTECTOMY.	107	924.5	PROTEIN 6.0/2.3	MILD FATTY CHANGE
*11	M.O'T. MALE 59 RECENT HAEMATEMESIS. NO PREVIOUS ULCER HISTORY. ACUTE DUODENAL ULCER.	PARTIAL GASTRECTOMY	90	635.6	B.S.P. 4.6%	-
*12 GLUTAMIC	PARTIAL GASTRECTOMY. FOR D.U. 10 YEARS BEFORE. RECENT EPIGASTRIC PAIN AND VOMITING. POSSIBLE PACREATITIS.	LAPAROTOMY. NIL FOUND	127	148.1	ALK. PHOS. 4.6 S.G.O.T. 30 UNITS BILIRUBIN 0.3	NORMAL
*13 ALANINE	V.S. FEMALE 40 RECURRENT DYSPEPSIA AND FAT INTOLERANCE 15 YEARS.	ELECTIVE CHOLECYSTECTOMY.	123	942.6	PROTEINS 7.1/4.6 ALK. PHOS. 1.1 AMYLASE 143 BILIRUBIN 1.3 S.G.O.T.	FOCAL FATTY CHANGE. MILD CELLULAR INFILTRATION.

APPENDIX 1 (CONTD.)

CLINICAL, EXPERIMENTAL AND BIOCHEMICAL DETAILS

EXPT. NO. * & SUBSTRATE.	CLINICAL DETAILS	PROCEDURE	B.S.L. MG%	N.E.F.A. M EQ/l	L.F.T.'S	HISTOLOGY
*14 NO RADIO-ACTIVITY	SIX MONTHS INCREASING DIARRHOEA WITHOUT WEIGHT LOSS. INFECTED MASS ANTERIOR RECTAL WALL. CARCINOMA OF RECTUM.	COMBINED RESECTION. TRANSVERSE COLOSTOMY.	95	455	PROTEINS 6.5/4.6 S.G.O.T. 32 ALK.PHOS. 1.9	MILD FATTY INFILTRATION. SLIGHT INCREASE IN PORTAL CONNECTIVE TISSUE. NO EVIDENCE OF METASTASIS.
*15 ALANINE	I.D. FEMALE 58 MILD RECURRENT DYSPEPSIA WITHOUT JAUNDICE. MULTIPLE GALLSTONES.	ELECTIVE CHOLECYSTECTOMY.	78	642.8	PROTEINS 8.0/3.9 ALK.PHOS. 3.0 BILIRUBIN 0.7 S.G.O.T. 40	MILD FATTY INFILTRATION
*16 NO RADIO-ACTIVITY	D.D. FEMALE 42 SYMPTOMS OF D.U. 8 YEARS. PARTIAL GASTRECTOMY 4 YEARS AGO. RECURRENCE OF SEVERE EPIGASTRIC PAIN. STOMAL ULCER.	VAGOTOMY. EXCISION OF STOMAL ULCER.	103	-	ALK.PHOS. 2 AMYLASE 100 B.S.P. 2.9	MILD FATTY CYST FORMATION & PERI CHOL ANGITIS.
*17	M.B. FEMALE 44 RECURRENT DYSPEPSIA 10 YEARS. PAST HISTORY HEALED GASTRIC ULCER. GALL STONES HIATUS HERNIA.	REPAIR HIATUS HERNIA. CHOLECYSTECTOMY.	107	772.8	PROTEIN 5.9/3.5 ALK.PHOS. 2 BILIRUBIN 0.5 S.G.O.T. 40	-

APPENDIX I (CONTD)

CLINICAL, EXPERIMENTAL AND BIOCHEMICAL DETAILS

EXPT. NO. * & SUBSTRATE	CLINICAL DETAILS	PROCEDURE	B.S.L. MG%	N.E.F.A. M EQ/I	L.F.T.S.	HISTOLOGY
*18 GLUTAMIC & *19 GLYCEROL	H.E. MALE 68 SLOW WEIGHT LOSS AND CONSTIPATION 6 MONTHS. CONSTRICTIVE CARCINOMA COLON	SUBTOTAL COLECTOMY AND COLOSTOMY	138	-	PROTEIN 6.8/4.2 ALK.PHOS. 2.1 BILIRUBIN 0.5 THYMOL TURB 0.8 S.G.O.T. 27	SLIGHT PORTAL FIBROSIS AND MONONUCLEAR INFILTRATION. NO EVIDENCE OF MALIGNANCY.
*20 GLYCEROL	J.L. MALE 58 PERFORATED D.U. 12 MONTHS BEFORE. OVERSEWN. INCISIONAL HERNIA.	REPAIR OF INCISIONAL HERNIA.	100	-	PROTEIN 7.1/3.8 BILIRUBIN 0.8 ALK.PHOS. 1.7	MODERATE FATTY CHANGE
*21 ALANINE	B.R. FEMALE 58 10 YEARS INCREASING FATTY DYSPEPSIA. MULTIPLE GALL STONES.	ELECTIVE CHOLECYSTECTOMY.	64	-	-	NORMAL
*22	B.S. FEMALE 59 GRADUALLY PROGRESSIVE DYSPEPSIA NON-FUNCTIONING GALL BLADDER.	ELECTIVE CHOLECYSTECTOMY.	112	-	BILIRUBIN 1.0 ALK.PHOS. 1.5	MILD MONONUCLEAR CELL INFILTRATION.
*23 ALANINE	M.L. FEMALE 70 MASS IN RIGHT UPPER QUADRANT. NO WEIGHT LOSS OR JAUNDICE. NON-FUNCTIONING GALL BLADDER.	ELECTIVE CHOLECYSTECTOMY.	93	-	BILIRUBIN 0.7 ALK.PHOS; 1.7 AMYLASE 62 B.S.P. 1.3	MINIMAL PORTAL FIBROSIS SLIGHT MONONUCLEAR INFILTRATION

APPENDIX I (CONTD)

CLINICAL, EXPERIMENTAL AND BIOCHEMICAL DETAILS.

EXPT. NO * SUBSTRATE	CLINICAL DETAILS	PROCEDURE	B.S.L. MG%	N.E.F.A. M EQ/l	L.F.T.'S	HISTOLOGY
*24	L.L. FEMALE 70 RECURRENT EPI-GASTRIC DISTRESS AND FLATULENCE 20 YEARS. NON-FUNCTIONING GALL BLADDER.	ELECTIVE CHOLECYST-ECTOMY.	97	-	BILIRUBIN 0.4 ALK.PHOS. 1.5 AMYLASE 129	-
*25 LACTATE	V.D. FEMALE 42 CHRONIC CHOLE-LITHIASIS. POSITIVE W.R.	ELECTIVE CHOLECYST-ECTOMY.	80	-	BILIRUBIN 0.7 PROTEIN 7.7/4.1 ALK.PHOS 3.1 CEPH.FLOCC 2.2	MILD ROUND CELL IN-FILTRATE & FATTY CHANGE
*26 & *27	J.R. MALE 70 RECURRENT CHOLE-CYSTITIS	ELECTIVE CHOLECYST-ECTOMY.	-	-	-	NORMAL

TABLE 7

THE EFFECT OF ETHYL ALCOHOL ON THE METABOLISM OF ALANINE-U-C^{14} IN HUMAN LIVER SLICES*

DISPOSITION OF ALANINE-U-C^{14} (%TOTAL COUNTS/GM. INITIAL WEIGHT)

EXPT.	"UPTAKE"	$C^{14}O_2$	GLUCOSE C^{14}	LACTIC ACID-C^{14}	GLYCOGEN C^{14}	S.A.** GLUCOSE	S.A.** GLYCOGEN	ASPARTIC-C^{14}
CONTROL:								
1	121.6	17.3	12.0	26.3	3.38	11060	1371	17.9
2	255.3	21.0	4.1	34.6	0.137	2492	49.7	8.7
3	223.7	69.8	26.6	42.3	3.91	22735	1984	-
11	349.1	115.4	55.4	45.2	3.57	49026	4667	15.1
13	-	30.9	15.4	47.1	1.57	10621	445	9.74
21	216.4	36.4	11.9	36.7	1.87	8351	507	-
22	199.3	52.2	34.7	58.4	1.34	18962	430	12.27
23	145.5	54.4	11.8	43.7	0.902	6378	307	25.81
MEAN	215.84	49.7	21.489	41.85	2.085	16203	1220.1	14.92
±S.E.M.	28.23	12.08	5.92	3.318	0.487	5329	541.6	2.58
ALCOHOL:								
1	99.6	10.0	7.1	23.4	1.47	6892	590	19.9
2	198.1	3.7	1.1	32.1	0.061	6145	16.7	6.1
3	130.6	9.0	3.6	56.8	0.472	3380	357	-
11	239.7	24.3	5.7	102.7	0.409	5757	624	30.0
13	-	7.2	2.9	50.8	0.212	1555	43.9	14.55
21	139.6	7.5	2.4	40.7	0.213	1411	58.9	-
22	177.1	12.6	2.9	57.1	0.098	1312	29.4	21.89
23	54.4	14.0	4.3	42.2	0.360	2810	108	29.95
MEAN	148.44	11.034	3.746	50.725	0.4128	3658	228.5	20.40
±S.E.M.	23.52	2.21	0.677	8.499	0.159	811	91.2	3.76
T	5.12	4.087	3.103	1.236	4.061	2.446	2.115	2.203
P	.01	.01	.05	N.S.	.01	.05	N.S.	N.S.
ACETATE:								
11	342.2	113.9	51.8	48.5	4.61	50784	5488	23.20
13	-	-	14.0	42.1	2.91	10409	739	16.55
21	235.7	45.7	13.1	35.8	2.27	7988	495	-
22	292.1	45.9	22.6	45.1	0.82	12483	311	12.96
23	159.4	42.8	10.8	40.9	0.366	7883	107	32.27
MEAN	257.35	62.1	22.46	42.5	2.195	17909	1428	21.24
±S.E.M.	39.24	17.29	7.46	2.13	0.77	8263	1020	4.24
T	1.368	0.567	1.463	1.358	0.89	0.507	0.843	3.349
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	.05

*INCUBATION WAS PERFORMED FOR 3 HOURS IN KRB EXCEPT IN EXPERIMENTS 21 & 23 WHERE 2 HOURS WERE EMPLOYED. ALL VESSELS CONTAINED 10 MM ALANINE. ALCOHOL VESSELS WERE SUPPLEMENTED WITH 10 MM ETHYL ALCOHOL (I.E. 46 MG%). ACETATE VESSELS WITH 10MM SODIUM ACETATE.

** S.A. = SPECIFIC ACTIVITY = COUNTS GLUCOSE - C^{14} PER ML MEDIUM/UG GLUCOSE PER ML MEDIUM.

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