LIPOGENESIS FROM CARBOHYDRATE IN MALE AND FEMALE RATS

A Thesis submitted by

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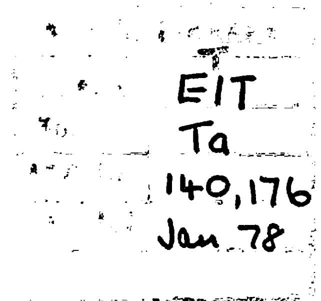

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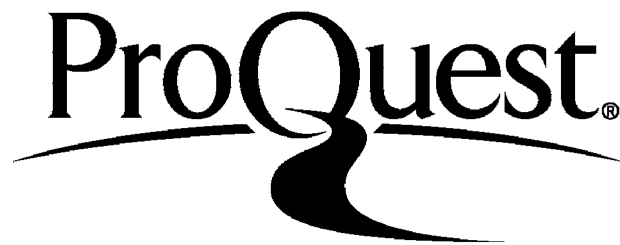

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ABSTRACT

Epidemiological surveys have shown that premenopausal women are less susceptible to atherosclerosis than men. There is also a positive correlation between the levels of serum triglyceride and the incidence of atherosclerosis.

In this study the rat has been used as an experimental model to examine the effects of the prolonged ingestion of 5% sucrose and 5% glucose solutions, in addition to the normal diet. In general, both male and female animals on the sucrose diet showed the greatest increases in body weight and also in the weights of liver, small intestine, heart, kidney and adipose tissue.

Male rats had significantly higher levels of serum triglycerides than females and the sucrose diet increased the levels of serum triglyceride to a greater extent than the glucose diet in both sexes. No significant differences in serum phospholipid were observed but, in most cases, serum cholesterol levels were higher in females than in males. The levels of all three lipids in the heart and liver were generally higher in females than in males, but the effect of various diets on these factors were minimal.

A study on the effect of the diets on various carbohydrate metabolites demonstrated that hepatic α-glycerophosphate, dihydroxyacetone phosphate and fructose 1,6-diphosphate levels were lower in females than in males. There was, however, no significant sex difference in the level of hepatic fructose 1-phosphate. No dramatic sex differences in these metabolites, were found in heart and kidney.

Following the short term gastric intubation of 70% fructose no sex differences in fructose 1-phosphate levels were observed either in the liver or in the small intestine.

Isolated hepatocytes, which responded to physiological levels of glucagon with regard to lipogenesis from glucose and to gluconeogenesis from lactate, were prepared. No sex differences in the capacity of hepatocytes to synthesize either total lipid or triglyceride, from fructose, glycerol or glucose, were observed. However, the incubation of hepatocytes with glycerol raised the α-glycerophosphate levels to a greater extent in the male than in the female. In all cases, hepatocytes synthesized lipids at a faster rate from glycerol than from fructose. Glucose was incorporated into lipid at a slower rate than either of the other substrates.

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ABBREVIATIONS

AMP	Adenosine 5'-monophosphate
ADP	Adenosine 5'-diphosphate
ATP	Adenosine 5'-triphosphate
CAMP	Adenosine 3':5'-phosphate
CHD	Coronary heart disease
DHAP	Dihydroxyacetone phosphate
EDTA	Ethylene diaminetetra acetate
F1P	Fructose 1-phosphate
F16P	Fructose 1,6-diphosphate
GDH	Glycerol 3-phosphate dehydrogenase
G3P	Glyceraldehyde 3-phosphate
αGP	sn-glycerol 3-phosphate
hr	Hour
KRB	Krebs-Ringer Bicarbonate buffer
IMP	Inosine 5'-phosphate
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
Min	Minute
NAD	β-nicotinamide adenine dinucleotide (oxidized)
NADH	β-nicotinamide adenine dinucleotide (reduced)
NADP	β-nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	β-nicotinamide adenine dinucleotide phosphate (reduced)
ND	Not detected
PHLA	Post-heparin lipolytic activity
Sec	Second
TIM	Triose phosphate isomerase
TRIS	Trihydroxymethyl methylamine
TSH	Thyroid stimulating hormone
VLDL	Very low density lipoprotein

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

There is some evidence to suggest a positive correlation between the ingestion of sucrose or fructose and the development of atherosclerosis (18). The onset of this disease is associated with a number of different factors including raised fasting serum triglyceride levels (10). Studies on humans and on experimental animals suggest that fructose or sucrose produce hypertriglyceridaemia to a much greater extent than isocaloric amounts of glucose or starch (47). In addition, the hypertriglyceridemic effort is more apparent in males than in females and this has been correlated to the higher incidence of atherosclerosis in the former compared to the latter (47, 48,51). This thesis reports an investigation of the effects of dietary sucrose and glucose on carbohydrate and lipid metabolism in male and female rats.

I. ATHEROSCLEROSIS

A. PATHOGENESIS OF ATHEROSCLEROSIS

Coronary heart disease (CHD), which is one of the complications of the atherosclerotic process, is the leading cause of death in developed countries at present. Atherosclerosis is a term used to describe a form of the hardening of the arteries which is characterized by the accumulation of soft, amorphous, fatty material in the subendothelial space of some arteries. The pathogenesis of atherosclerosis is attributed to lipid infiltration, mechanical injury, thrombosis and vascular anoxia (12). It was demonstrated at the beginning of the century that the chronic administration of cholesterol in the diet of rabbits produced fat-containing arterial lesions and since then the lipid theory has overshadowed all other theories regarding the aetiology of atherosclerosis. Although the disease is currently considered to be multifactorial in aetiology most investigators are convinced that excessive dietary lipid, leading to metabolic disturbances and raised serum lipid levels, is a primary cause of the disease.

Stehben (1) has recently studied the role of haemodynamics, wear and tear, and age on the development of atherosclerosis and he concluded that dietary factors are not prime factors in the pathogenesis of the disease. He argued that diet-induced atherosclerosis in experimental animal is distinct from the human disease in the following respects: (i) there are histological differences between the two types of disease;

(ii) the occurrence of extravascular lesions which are produced only in experimental animals; (iii) an absence of complications such as intimal tears, ulceration and thrombosis in experimental animals. In addition he claimed that the experimental procedure is not physiological and the injurious nature of cholesterol is possibly compounded by toxic impurities in the cholesterol preparations.

The presence of fibrin-like material in atherosclerotic plaques, has been put forward as evidence for the thrombogenic theory of atherogenesis (2). The lipids present in the plaques are thought to come from the secondary enmeshment of circulating lipids following fibrin deposition, but evidence has now been obtained that this material is an insoluble form of fibrinogen differing from true fibrin as produced during blood coagulation, and therefore not thrombus-derived (3). The possibilities that lipemia, especially that occurring following a meal of fats, can enhance platelet agglomeration or impair fibrinolysis has received much attention but are unproved (4). However, Iacono et al have demonstrated a decreased susceptibility to thrombin and collagen platelet aggregation in man fed a low fat diet (11). Hueper (5) has drawn attention to the possible influence of hypoxia on the local metabolic processes of the intima as a cause of atherosclerosis. Although this theory has now assumed a less important role, nevertheless it has contributed to a more comprehensive understanding and knowledge on atherosclerosis.

Anitschkow and Chalatov (6) in 1913 showed that feeding large amounts of cholesterol to rabbits produced lesions of the aorta and ever since this time the lipid theory has almost monopolised the field of atherosclerosis. Epidemiological surveys (7,8,9,10,13,14,35) have confirmed that there is a strong link between CHD occurring as a result of atherosclerosis and hyperlipidemia. Other causes of CHD implicated following epidemiological surveys include hypertension, diabetes mellitus, cigarette smoking, obesity, hyperuricemia, occupational stress, hereditary factors, sex, personality, exercise and diet (7,8,9,13,14). In a study of 80 patients with CHD, Nash et al (15) were able to show a positive correlation between the progression of the disease and hyperlipidemia. Other factors studied were sex, age, smoking habits, diabetes, hypertension, and family history of CHD. No correlation could be found between these factors and the progression of CHD, emphasizing the importance of hyperlipidemia as a risk factor. However, a study of the histologic and gross appearance, total lipid content and relative lipid composition of

normal intima and intima with atherosclerotic lesions has shown that there is no correlation between the severity of the lesion and either the total lipid content or the changes in relative lipid composition of the intima.(16)

B. SUGAR AND CARDIOVASCULAR DISEASE

Atherosclerosis may have its genesis in childhood (17) and, therefore, although there is currently no conclusive evidence that the restoration of serum cholesterol and triglyceride levels to normal in children will prevent accelerated development of atherosclerosis, dietary control appears to be a possible approach to the prevention of atherosclerosis. A report on the medical aspects of food policy in relation to CHD has been published by the Department of Health and Social Security (18). The dietary characteristics considered as possible risk factors for CHD were (a) over consumption of food; (b) an excess of dietary fat; (c) a low ratio of polyunsaturated to saturated fatty acids in the diet; (d) an excess of dietary cholesterol; (e) an excess of dietary sucrose; (f) a high consumption of common salt; (g) a deficiency of dietary fibre and (h) softness of the water supply (18).

There is no evidence that the average food energy intake by the population of the United Kingdom is increasing but there is a widespread belief that obesity is becoming more prevalent among both adults and children. A possible reason for this could be the greater availability of labour-saving devices in the home and in the factory and in the field. The possible relationship between the increased consumption of fat and sugar and obesity is yet to be explored.

In the United Kingdom the total consumption of all types of carbohydrate is now less than it was a hundred years ago. However, the consumption of sucrose has increased rapidly from the beginning of this period. Epidemiological studies have shown that populations with high sucrose intakes have a high death rate from CHD, and that there is a negative correlation between the starch content of the diet and CHD mortality. The correlation between sucrose consumption and the incidence of CHD is better when the diet is also rich in saturated fatty acids. Costa Rica, Cuba, Jamaica, Ecuador and Columbia are countries in which the diet has a low content of saturated fatty acids and a high proportion of sucrose but in these countries there appears to be no relationship between the incidence of CHD and the sucrose content in the diet (18).

In some experiments (18) in which part of the starch in the diet was replaced by maltose, glucose, or fructose, a raised serum triglyceride was observed only when fructose was ingested which suggested that it is the fructose moiety of the sucrose molecule which promotes hypertriglyceridaemia and enhances fat deposition. It was recommended in the report on Diet and CHD (18) that "the substitution of sucrose by fructose or by sorbitol should not, in the light of present knowledge, be encouraged", and "that the consumption of sucrose, as such or in foods and drinks, should be reduced, if only to diminish the risk of obesity and its possible sequelae".

Keys (19) and Grande (20) have both criticised the theory that sucrose in the diet is a major factor in the development of CHD as proposed by Yudkin (21). Yudkin bases his conclusions on four arguments, which may be summarised as follows:

(i) There is a correlation between the per capita sucrose consumption and the mortality attributed to CHD in several countries.

(ii) The increase of sucrose consumption is associated with a parallel increase in the incidence of CHD in the various countries studied.

(iii) There is an unusually high intake in the diet of men afflicted with CHD.

(iv) Dietary sucrose increases serum lipid levels.

Keys (19) and Grande (20) have pointed to exceptions to Yudkin's first point, citing the countries with high sugar consumption but low incidence of CHD. In countries with similar sugar consumption such as Sweden and Finland the incidence of CHD is higher in the latter. In Britain the correlation between death rates and fat consumption is 0.64 and the correlation between death rates and sugar consumption is 0.55 (22). A better correlation can be obtained between CHD death rates and time (r=0.78). Grande (20) has suggested that the increased mortality attributed to CHD and increased sugar consumption were coincidental and that the increased mortality from CHD would be attributed to changes in medical diagnosis of the cause of the death.

Yudkin reported a higher sugar intake in coronary patients (23,24) than in controls but Grande and Keys were unable to confirm these findings (19,20). Burns-Cox _et al._ (25) determined the sugar intake of 80 patients and 160 controls. The average intake of the patients was marginally higher than that of the controls but this difference was not

statistically significant ($P > 0.05$). The conclusion reached in a survey, by a working party of the Medical Research Council, of groups of 150 coronary patients and 275 controls, was that the average sugar consumption was slightly greater in the patients with myocardial infarction than among the controls, but the differences were not statistically significant (26).

The effect of dietary sucrose on human serum lipid has been the subject of many investigations. Grande (20) after reviewing this field concluded that the hypertriglyceridaemic effect of sucrose, relative to starch, cannot be demonstrated in normolipaemic men under normal dietary conditions. In addition, however, he stated that the evidence indicates that sucrose causes higher fasting serum lipid levels than starch in some hyperlipidaemic patients. It is clear, however, that not all hyperlipidaemic patients show this response, and that the effect of substituting sugar for starch may be affected by the composition of the dietary fat.

It would seem reasonable to conclude that even the most ardent opponent of the sugar theory has to admit that there is a relationship between sucrose and hypertriglyceridaemia if only in abnormal humans. As there is insufficient evidence to be dogmatic about the role of dietary sucrose in atherosclerosis, Macdonald (27) has recently suggested that patients seeking advice on the prevention or treatment of atherosclerosis should be individually screened for "carbohydrate sensitivity".

C. LIPIDS AND ATHEROSCLEROSIS

In the atherosclerotic plaque there is a subendothelial accumulation of cholesterol, triglyceride and phospholipids (4). The cholesterol and triglyceride components are derived mainly from plasma lipids, whereas the vessel wall at least partially synthesises the phospholipid (30,34). In general, plasma cholesterol, phospholipid, triglyceride and free fatty acids are bound to transport proteins which impart solubility on otherwise insoluble lipids (see II.C.1.). There is a positive correlation between the levels of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherosclerosis (8,36,37). However, high density lipoprotein (HDL) may slow the atherogenic process (28,75). Epidemiological surveys have shown a negative correlation between the plasma concentration of HDL and the incidence of CHD. However, Fuller *et al.* (31) have shown that there is a statistically significant positive correlation

between HDL-cholesterol and total cholesterol concentration in both men and women. It has been postulated that the role of HDL may be in the transport of cholesterol out of the cell (29).

One of the most pressing problems in the study of atherosclerosis, is the mechanism by which elevated serum lipids accelerate the progression of this disease. Numerous reviews have been published concerning the role of lipoprotein in atherosclerosis (32,33,34), however, the mechanism of the deposition of pathological amounts of lipids in the arterial tissue from plasma lipoprotein is not known.

In the normal subendothelial intima there is a very slow increase in lipid levels in the first two decades of life (34). There is also very little increase in all serum lipids and lipoprotein levels in school children from the ages of 5-16 years (38). However, the rate of cholesterol ester deposition in the intima increases dramatically from about 20 years upwards. At the age of 40 it becomes the major lipid component in the normal intima.

In the development of atherosclerosis the proportion of free cholesterol in the atheroma is increased with a concomitant decrease in the esterified cholesterol (34). Triglycerides in the lesions generally increase less than the other lipid components and therefore have not stimulated much interest. Nevertheless, frequently in cases of CHD plasma triglyceride appears to be elevated (10). This is not surprising in view of the hepatic secretion of cholesterol and triglyceride in the form of VLDL.[39] VLDL can be degraded to LDL by the enzyme lipoprotein lipase, which breaks down the triglycerides in the VLDL and the LDL can be taken up by the intima (36).

Chylomicron level increases after a fatty meal and this lipoprotein fraction is rich in exogenous triglycerides. There is also evidence (40) that some patients with CHD have prolonged postprandial lipaemia which is associated with an inability to clear triglyceride. The administration of heparin has been shown to improve this condition. Hulsmann and Jansen (41) have recently proposed a mechanism whereby the high myocardial and low hepatic lipoprotein lipase activities are responsible for the initiation of atherosclerosis. This theory, first put forward by Zilversmit (43), claims that VLDL and chylomicrons are absorbed by the arterial wall in proportion to the local concentration of heparin or a heparin-like material. This arterial lipoprotein lipase degrades VLDL and chylomicrons to cholesterol-rich LDL and

chylomicron remnants respectively. Some of the LDL and chylomicron remnants stay bound to the intimal surface long enough to be incorporated into the arterial intima, although much of the LDL is released into the blood.

Free fatty acids are released from the lipoproteins by lipoprotein lipase in the endothelial tissue. A high local concentration of fatty acids could contribute to the formation of endothelial gaps in the vascular inner lining, thus allowing LDL-like particles to enter (41).

Epidemiological surveys (42) have shown that saturated fatty acids in the diet increase serum cholesterol, whereas polyunsaturated fatty acids have an opposite effect. Of the saturated fatty acids, lauric, palmitic and myristic acids have the most significant effects. Linoleic acid, a polyunsaturated fatty acid, is a hypocholesterolemic agent. The mechanisms of these actions remain to be fully established.

D. SEX DIFFERENCES AND THE SUSCEPTIBILITY TO CHD

Numerous studies have shown that premenopausal women are less susceptible to atherosclerosis than men (45,46,47). Hagerup (45) and Kannel (48) have scrutinised the risk factors associated with atherosclerosis. Kannel, in his Framingham study, concluded that the rate of arterial atherogenesis is linked to age, serum lipid level, blood pressure and carbohydrate intolerance in both sexes. Hagerup in his extensive report on the serum cholesterol levels in men and women has found that the sterol levels are lower in the women than in the men. After the age of 50 the reverse was true. It is generally accepted that 50 year old men are more susceptible to CHD than 50 year old women. In an analysis of the genetic and environmental sources of variation in serum cholesterol, Orr _et al._(49) have shown there is a relationship between socioeconomic status and cholesterol in males but not in females. In young subjects (18-22yr) with type II hyperlipoproteinaemia, Kocher _et al._ (50) found that the only sex differences in serum lipids were in the total cholesterol and LDL-cholesterol levels. Women had lower levels than men, and no sex differences in VLDL and triglyceride levels were observed.

In type IV hyperlipoproteinaemia the serum levels of the triglyceride-rich lipoprotein VLDL are raised. VLDL is considered by some researchers (51) to be the most prevalent lipoprotein disorder associated with CHD, even more widespread amongst patients with atherosclerosis than type II lipoproteinaemia. Type IV hyperlipoproteinaemia is 2.7 times more

common in men than in women. This evidence suggests that the type IV disorder, which is accompanied by a raised VLDL and serum triglyceride levels, is responsible for the observed sex difference in the incidence of CHD. Olsson (36,37) found that males with hyperlipoproteinaemia (moderate elevations of LDL and VLDL) are more susceptible to atherosclerosis of the peripheral vessels than females. There is a better statistical correlation between the advent of atherosclerosis and an increase in both LDL and VLDL levels than with singular elevations of either lipoproteins. Elevated LDL alone is less frequently associated with the disease than other types of hyperlipidaemia.

Although there is strong evidence that there is a higher incidence of atherosclerosis in women with either bilateral oophorectomy or with oestrogen deficiency (47), Engel et al. (52) were not able to find any relationship between the incidence of pregnancies, menstruations, oophorectomies and estrogen therapy, and the development of precocious CHD in young women. Neither the administration of oestrogens nor castration inhibited the progressive atherogenic process in pigs (53). Thus the role of oestrogens in the development of CHD is ambiguous. Administration of oestrogens has been shown to lower blood cholesterol by Robinson et al. (54) and this hormone has been used clinically in an attempt to prevent atherosclerosis. Glueck (55) found that oestrogen therapy increased the plasma VLDL-triglyceride levels by enhanced VLDL-triglyceride production in women. Nikkila and Kikki (56) believe that the human population does not have uniform plasma triglyceride kinetics and that the use of oral contraceptives increase the rate of triglyceride production and also increase the efficiency of triglyceride removal. In women of fertile age there is a more efficient rate of clearing of plasma triglycerides than in men.

In male and female rats subjected to endothelial trauma of the abdominal aorta, Uzunova et al. (57) were able to increase the mortality rate by more than fourfold in both cases by the administration of testosterone over a 4 week period prior to the experiment. Oestradiol treatment on the other hand decreased the mortality rate in both sexes. They claim that this is the first significant experimental evidence for an association between sex hormones and the development of arterial thrombosis. Recently Jolly et al. (58) were able to demonstrate that ethynylestradiol increased the plasma prothrombin levels but methyltestosterone had an opposite effect in Vitamin K deficient castrate male rats.

E. ARTERIAL METABOLISM AND ATHEROSCLEROSIS

Of the many factors involved in the pathogenesis of atherosclerosis changes in the arterial metabolism have recently received considerable attention. Ciobanian and Manzur (59) have demonstrated an increased incorporation of $[^{14}C]$acetate into lipids in fatty streak compared to the normal human intima. They also established that the predominant fatty acids are derived from chain elongation and desaturation of aortic linoleic and linolenic acids. The major labelled product was a long-chain polyenoic fatty acid with chromatographic properties similar to $C_{22:4}$ acid. These authors suggest that the lipid in the normal intima is derived primarily from plasma lipoproteins whereas lipids in fatty plaques are locally synthesised. Vost (60) found that intact tri-glyceride was taken up from $[^{3}H]$ labelled chylomicrons by perfused rabbit aorta and that this uptake was independent of triglyceride hydrolysis by aortic lipoprotein lipase unlike the situation in adipose tissue. Thus arterial triglyceride can be produced by intracellular synthesis from glycerol 3-phosphate and fatty acid and also by the uptake of lipoprotein. Local metabolic factors may also be involved in the accumulation of cholesterol from the serum.

Female rat aortas have a greater in vitro capacity to synthesise lipids from glucose than male aortas and oestradiol administration increases the synthesis of total lipids (61). Similarly there is evidence that in the atherosclerosis-susceptible White Carneau (WC) pigeons there are more prominent primary lipid depositions in the thoracic aortas of females than in male pigeons (62). Zemplenyi and Rosentein (62,63) observed higher activities of phosphofructokinase (E.C.2.7.1.11), aldolase (E.C.4.1.2.13), isocitrate dehydrogenase (E.C.1.1.1.42), glycerol kinase (E.C.2.7.1.30), ATPase (E.C.3.6.1.3) and creatine phosphokinase (E.C.2.7.3.2) in the arteries of female pigeon compared to the male. Phosphofructokinase, aldolase and glycerol kinase are important enzymes in the synthesis of lipids from carbohydrate. Phosphofructokinase is also a key enzyme in the regulation of glycolysis and is subjected to regulation by ATP and citrate (63). A fall in ATP/ADP ratio or a fall in citrate concentration which can both occur in hypoxia leads to an increase in phosphofructokinase since this enzyme is inhibited at high concentrations of citrate and ATP. Resistant Show Racer (SR) pigeons have a lower phosphofructokinase level than atherosclerosis-susceptible WC pigeons. The activities of malate

dehydrogenases (E.C.1.1.1.37) and lipoamide dehydrogenase (E.C.1.6.4.3) are significantly lower in the WC pigeons than in the SR pigeons. The reduced enzyme activities can lead to decreased Krebs cycle operation, which, in turn, leads to low citrate and ATP production and this to an increase in phosphofructokinase activity. The increase in phosphofructokinase together with a reduction of Krebs cycle activity is similar to a situation observed in cases of tissue hypoxia when the glycerol 3-phosphate concentration and the ratio of $NADH/NAD^+$ are increased and there is a concomitant decrease in ATP/ADP ratio (133).

In aortas the rates of incorporation of $[1-^{14}C]$acetate into triglyceride and fatty acids are increased five fold, when this experiment is conducted in anoxic conditions (64). Similarly, Howard (65) observed that hypoxic incubation of atherosclerotic aortas with $[2-^{14}C]$glucose will increase the incorporation of label into the glycerol moieties of the glycerides and phospholipid. Recently, Albers and Bierman (66) were able to show that hypoxia also has an effect on the uptake and degradation of LDL by the human arterial smooth muscle cells.

Kirk (67) in a review on atherosclerosis and arterial metabolism has made an extensive survey of the enzymes of the aortic walls. This tissue, unlike other tissues, exhibits a high rate of glycolysis, low respiratory rate and a low Pasteur effect. Sex differences in the levels of enzymes in human aorta and coronary arteries were observed. The NADP-dependent enzymes, glucose 6-phosphate dehydrogenase (E.C.1.1.1.49), 6-phosphogluconate dehydrogenase (E.C.1.1.1.43), isocitric dehydrogenase and NADP-malic enzyme (E.C.1.1.1.40) are present in lower activities in both the aorta and the coronary artery of the female but no sex differences were found in the pulmonary artery. This may explain the lower susceptibility to atherosclerosis of premenopausal women.

Lipoprotein lipase in the aorta from the rat and rabbit is raised in atherosclerosis (67). In humans the level of phosphofructokinase is raised in aorta atherosclerosis, although lower values were obtained for this enzyme in the atherosclerosis of the coronary artery tissue. Because of the high activities of phosphofructokinase, glycerol 3-phosphate dehydrogenase (E.C.1.1.1.8), triosephosphate isomerase (E.C.5.3.1.1) and the low respiration rate in the human arterial tissue the formation of glycerol 3-phosphate is favoured (61). Thus, it is possible that *in situ* synthesis of triglycerides from the readily available glycerol 3-phosphate may be partly responsible for the accumulation of lipids in the atherosclerotic wall.

The importance of arterial metabolism in the development of atherosclerosis is at present ill defined. Although there is extensive work done on the role of hormones in atherosclerosis in experimental animals the effects of the hormones on the enzymes in human vascular tissue have not been thoroughly examined.

F. EXPERIMENTAL ATHEROSCLEROSIS

Species difference is a constant problem in the study of experimental atherosclerosis. The blood lipid levels in the pigeon do not seem to be important in the development of spontaneous atherosclerosis although these are important factors in human atherosclerosis. Both the SR and WC pigeons have similar lipoprotein levels, although the WC pigeon is more sensitive to atherosclerosis (63). The rat, unlike humans, primates, birds and swine, does not usually develop spontaneous atherosclerosis. Charles River Breeding laboratories have recently bred a strain of rat that will develop spontaneous hypertension, hyperglycaemia, hyperlipidaemia and intima hyalization and fibrosis (69). The arterial lesions are found only in the male rats. Variation between related species has also been found in primates. The squirrel monkey and the rhesus monkey respond to dietary cholesterol in different ways (70). In the former plasma cholesterol level is inversely related to the ability to increase bile acid secretion, whereas in the latter it is related to the ability to absorb cholesterol. Nevertheless experimental atherosclerosis induced in primates bears a close resemblance to human atherosclerosis.

Rats are small, cheap and easily maintained in large numbers, and consequently this animal, which develops only minimal arterial lesions, is a common experimental model in the study of atherosclerosis. Although there are no reports of spontaneous myocardial infarcts in the Wistar or Sprague-Dawley strains of rat, lesions in the rat can appear if these animals are fed a basic atherogenic diet of 40% fat, 20% casein, 5% cholesterol, 0.3% propylthiouracil, and 19.7% sucrose. A more detailed composition of this diet is given by Thomas _et al._ (71). Thiouracil acts as an antithyroid agent because it inhibits the production of thyroxine by preventing the gland from incorporating inorganic iodide into the organic form. Such a diet is not physiological but nevertheless short-term feeding experiments on the effect of unsaturated fat compared to saturated fat on the development of atherosclerosis has produced interesting findings (72). A peanut oil-containing diet produced lesions

similar in many respects to human atherosclerotic lesions whereas the lesions produced by a butter-containing diet did not resemble that of human atherosclerosis.

More recently, Testa et al. (73) found that rats fed a diet supplemented with Vitamin D_2 had higher levels of cholesterol and phospholipid in the blood but only rats on a diet supplemented with cholesterol and Vitamin D_2 had increased levels of cholesterol, phospholipids, and triglycerides in the aorta. Altman (74) treated rats on a diet of Vitamin D_2 with cholesterol for five days and found that the animals developed atherosclerotic lesions which were calcified as well as lipidic.

II. CARBOHYDRATE AND LIPID METABOLISM

A. INTESTINAL ABSORPTION AND METABOLISM

1. Carbohydrate Absorption

Food consumed by animals has to be digested and absorbed before it can be metabolised by the body. The major carbohydrate constituents of the human diet are starch, glycogen, sucrose and lactose which must be hydrolysed to monosaccharide form before being absorbed. The resultant monosaccharides are glucose, fructose and galactose. In humans, the normal carbohydrate absorption is remarkably efficient and the majority of carbohydrates are almost completely absorbed in the jejunum (68). The ileum does contain disaccharidases and can absorb monosaccharides, although at a slower rate than the jejumun. Carbohydrates are not normally absorbed in the colon or large intestine. The rate of intestinal absorption of carbohydrates depends on the rate of absorption of the component monosaccharides across the brush border of the mucosal cells and on the rate at which the carbohydrate is emptied from the stomach (76).

(a) Sucrose

Sucrose is not absorbed unchanged into the blood stream but is hydrolysed by intestinal sucrase (E.C.3.2.1.26) (77). The activity of this enzyme has been reported to be greatest in the duodenum and to rapidly decrease with distance from the pylorus (78). Dahlquist and Borgstrom (79) reported that the major sites of absorption of sucrose in man are the distal jejumun and the ileum. Sucrase located in the brush border of the small intestine subsequently hydrolyses this disaccharide into glucose and fructose. Gray and Ingelfinger further

examined the site of absorption and hydrolysis of sucrose in man and came to the conclusion that sucrose, like maltose and lactose, was principally absorbed and hydrolysed in the jejumun and not in the ileum (80).

The sucrase level in the rat small intestine shows clear circadian fluctuations. A remarkable rise of this enzyme activity can be observed just before feeding (81,82) and it is postulated that this circadian rhythm is closely related to the food intake and not to the light-dark cycle. Both the total and specific activities of intestinal sucrase increase after the administration of a carbohydrate-containing diet for 24 hr to rats which have been previously starved for three days (83). These authors found that in fasted rats glucose derived from sucrose hydrolysis was almost completely absorbed whereas only 25% of fructose was absorbed. The increase in sucrase activity was paralleled by an increase in the capacity of the intestine to hydrolyse sucrose and absorb the component monosaccharides.

Intestinal sucrase activity is low in the newborn rat and increases with age. The reverse is true for lactase (E.C.3.2.1.23) which is high at birth and decreases with age. The hormones of the adrenal cortex and ACTA can stimulate sucrase activity. The administration of hydrocortisone to 16 day old rats increases the activity of sucrase (84). Blair _et_ _al._ (101) have also found an age dependent increase in intestinal sucrase activity of rats, but no sex difference was observed. High levels of sucrose (70%) in the diet produced highly significant increases.

(b) _Glucose_

There is an active transport mechanism involved in the absorbtion of glucose by the small intestine and numerous reviews have been written on this topic (77,78,84,85). Galactose and glucose are absorbed at a faster rate than fructose (85). In the rat 97% of the radioactivity from [^{14}C]glucose which disappeared from the intestine loop appears in the blood as glucose, lactate and alanine, and of this, 80-90% was glucose.

The mean rates of water absorption, _in_ _vitro_, of the intestine from female rats is 20% faster than for males. This sex difference in water absorption is reflected in the glucose absorption rate which is also higher in the females than in males (78). There appears to be a linear relationship between the rate of water absorption and the rate of absorption of sugars, a phenomenon known as solvent drag. On the other hand Fenton (86) showed a lower absorption rate for female rats

when compared with males. However, when the weights of the animals were taken into account this difference was diminished.

(c) Fructose

It is now clear that there are species variations in fructose absorption. This is largely due to the differences in fructose metabolism by the intestinal mucosa of various species. Fructose is absorbed across the intestinal epithelial cells of man and rat by a transport mechanism which may not involve active transport (85,78). However, this is not a simple, passive diffusion of the hexose since D-fructose transport in rat small intestine is a saturable, rate-limiting process (87). Guy and Deren (88) have also shown that the epithelial cell of the small intestine of rat and rabbit possess a specialised membrane which allows the rapid entry of fructose. Support for the existence of an active transport mechanism for D-fructose in the rat has come from Gracey et al. (89) and Macrae and Neudoerffer (90). Both these groups of workers found that the small intestine could accumulate D-fructose against a concentration gradient by an energy- and Na^+- dependent process. The former workers found that at a high concentration of phlorizin, a compound which inhibits the transport of glucose and galactose, also inhibits D-fructose absorption. However, the latter workers could not demonstrate this inhibition.

Evidence for a distinct transport system of D-fructose in humans have come from studies on patients with malabsorption symptons. Patients with glucose-galactose malabsorption disease have poor absorption of glucose and galactose but normal rates of fructose absorption (91,92).

In the guinea pig (93,94,95), hamster (96) and dog (97) fructose is reported to be converted to glucose and lactate during absorption. In rat the amount of fructose transformed into glucose is low (85,93, 78,94), only 10% of the $[^{14}C]$fructose absorbed is converted to glucose, 60% is converted to lactate and the remaining 30% passes into the portal blood unchanged (94,78,85). Mavrias and Mayer (98), however, observed that fructose was poorly metabolised in the rat small intestine and only very small conversion to lactate and glucose occurred. More recently Crouzoulon (100) has provided some evidence for the conversion of fructose to glucose in small intestine of rats fed high fructose diets.

In man, up to 80-90% of fructose is absorbed from the jejumun as fructose (85). In pigs a sucrose load of 400-800 g caused the level of fructose to rise to 200-400 mg/l in the peripheral blood collected from

the anterior caval vein (99). This means that in the pig, fructose in the blood can circulate in the body and is not fully metabolised in the liver and the intestine.

2. Intestinal Metabolism of Fructose and Glucose

It is generally accepted that rats, unlike guinea pigs, have very little glucose 6-phosphatase (E.C.3.1.3.9) activity in the intestine and thus cannot convert fructose to glucose efficiently (93, 102). However, James and Solomon (103) found glucose 6-phosphatase in the small intestine of the rat but its measurement was complicated by the presence of naturally occurring inhibitor (104). In a study of 750 Swiss ICR male mice, the levels of glucose 6-phosphatase in the duodenal and jejunal epithelium were raised after feeding diets containing either 7.5% or 60% fructose. The stimulation of this enzyme occurred within 6 hr of feeding. Lipoprotein spherules and polymorphic dense bodies were also observed. No such modification was detected with glucose in the diet instead of fructose (105) and this suggests that fructose may have an influence on intestinal lipid metabolism.

Fructose in the intestine can be phosphorylated by fructokinase (E.C.2.7.1.1) and hexokinase (E.C.4.1.2.7) and in the guinea pig and rat there is evidence for another pathway involving the enzyme PEP: monosaccharide phosphotransferase system. The PEP-activated system is independent of ATP supply and can phosphorylate fructose to fructose 1-phosphate (106).

An ATP dependent fructokinase phosphorylates fructose to fructose 1-phosphate. This enzyme is significantly increased in rats fed on a 60% fructose containing diet for three days but this activity fell to normal levels when rats were fed on this diet for 15 days. Fructose 1-phosphate aldolase (E.C.4.1.2.7) activity was similarly increased. The levels of both these enzymes were lower in the intestine than in the liver (98). Heinz and Lamprecht (107) observed very low activities of fructokinase and fructose 1-phosphate aldolase in the rat small intestine. However, Mavrias and Mayer (98) found considerable activities of both enzymes in intestines from mature rats. The activities of both intestinal enzymes increased in response to a 60% fructose diet and similar changes in enzyme levels were observed in the liver. In addition, there was an increase in fructose uptake by intestinal segments in rats fed on a fructose diet for 3 days. There was, however, very little conversion of fructose to glucose and lactate by intestinal epithelium cells.

In the guinea pig, fructose feeding for 3 days resulted in no change in the activities of either fructose 1-phosphate aldolase or fructokinase. There was no difference in the rate of uptake of fructose by segments of intestine from animals fed for 3 days on 60% fructose diet and from that of animals fed normal laboratory chow. However, histological sections of the small intestine taken from animals fed on the fructose diet for 20 days showed a deposition of yellowish material in the sub-epithelial region at the tip of the villi. Preliminary evidence indicates that the material is protein containing a carbohydrate component (108).

Different dietary regimes can affect the levels of the glycolytic enzymes in the small intestine and normally such changes in the jejunal enzymes are also observed in the liver. Shakespeare et al. (109) found that administration of glucose by intubation to starved rats led to an increase in mucosal hexokinase (E.C.2.7.1.1) activity whereas isocaloric amounts of either monoglyceride and fatty acid or a caesin hydrolysate did not affect the enzyme activity. Stifel et al. (110) demonstrated that fructose in the diet of rats had a specific adaptive effect upon fructokinase and fructose 1-phosphate aldolase levels while glucose exerts an effect on hexokinase and glucokinase (E.C.2.7.1.2) activity. The levels of jejunal pyruvate kinase (E.C.2.7.1.40) and phosphofructokinase are low in fasted humans whereas feeding increases the enzyme activities. The increase in activities is greatest in subjects on a fructose diet compared to a glucose diet. On the other hand fructose diphosphatase (E.C.3.1.3.11) an important gluconeogenic enzyme, is at its highest in fasted subjects (111). The adaptive changes in the levels of these jejunal enzyme in response to diet are similar in the rat (113).

Clofibrate has been used to lower the plasma triglyceride levels in man. Administration of this drug to normal human males leads to decreased activities of jejunal glucokinase, glucose 6-phosphate dehydrogenase, fructokinase, fructose 1-phosphate aldolase and fructose 1-6-phosphate aldolase. The effects of clofibrate are specific since hexokinase and 6-phosphogluconate dehydrogenase activities were unchanged (114).

The rat small intestine is subject to the Pasteur effect and in the absence of oxygen the ATP level falls with concomitant increase in the lactate level. In the presence of oxygen the rate of glycolysis

decreases and there is an increase in the ATP concentration in the tissue (115). Lamers and Hulsmann (116) showed that intraluminal fructose loading causes the jejunal fructose 1-phosphate level to rise rapidly in the rat. At the same time the P_i level decreased and creatine phosphate concentration was lowered to 55% of the control values. The level of glycerol 3-phosphate was also raised.

An increase in the level of fructose 1-phosphate was also observed in the liver but no elevation of the level of glycerol 3-phosphate was observed.

Although a great deal is known about the adaptive responses of the jejunum to diet, the mechanism of sugar transport and the metabolism of monosaccharides, there are still a number of unanswered questions. The exact mechanism of fructose and glucose transport are as yet unknown (85) as is the fate of fructose 1-phosphate which accumulates in the small intestine after fructose ingestion.

3. Lipid absorption and Metabolism in the Small Intestine

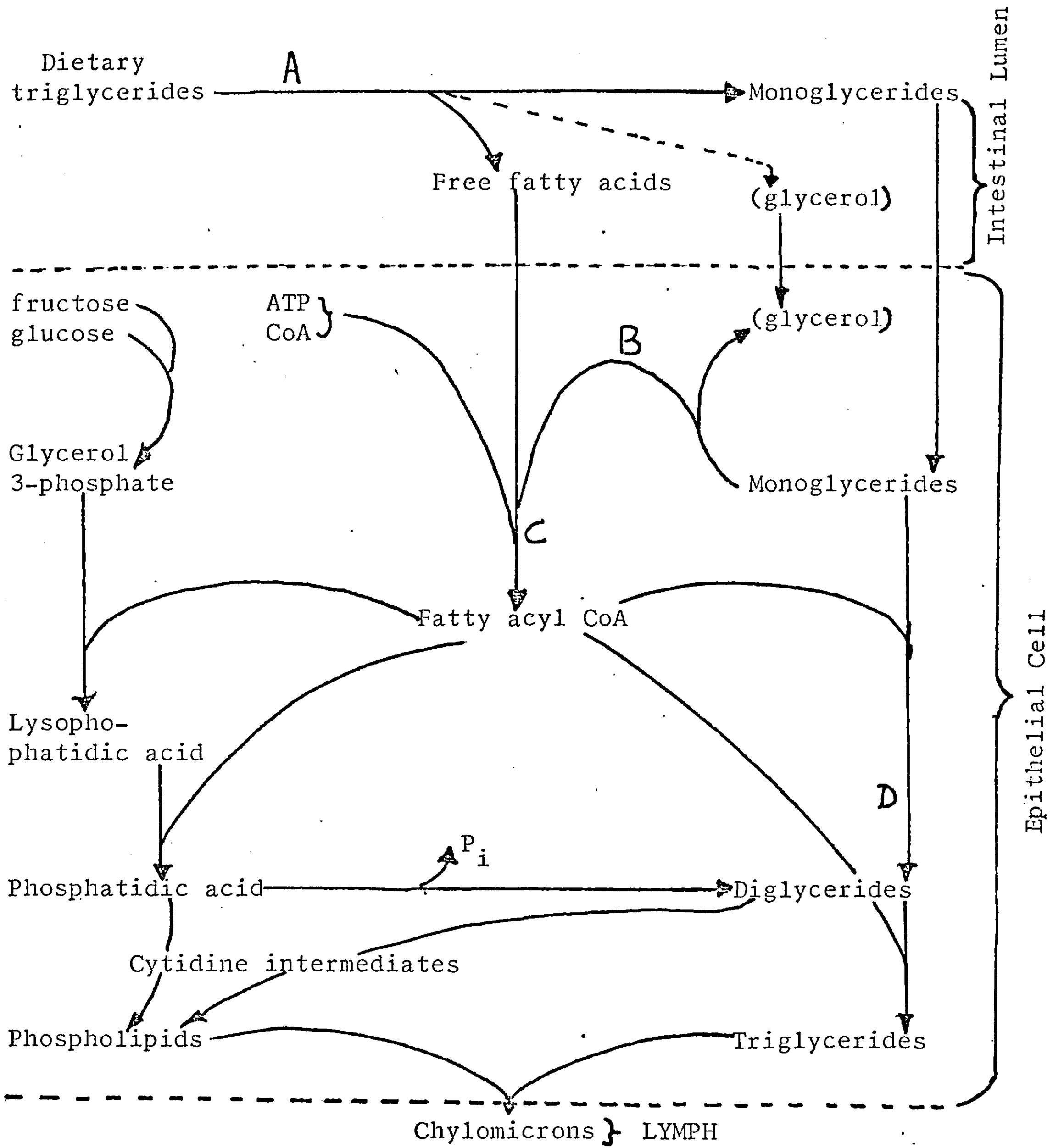
Pancreatic and biliary secretions are important for the digestion and absorption of the lipids. During digestion there is partial or complete hydrolysis leading to the formation of micellar aggregates. Thus, triglyceride is converted to 2-monoacylglycerol, lecithin to lysolecithin and cholesterol esters rehydrolysed to cholesterol. The lipids readily enter the mucosal cell in this micellar state and are re-esterified (117). Pancreatic lipase, which is responsible for the hydrolysis of triglyceride, acts preferentially on the ester linkage at the 1 position of the triglyceride. Bile salts are cofactors for pancreatic lipase and are also important for the formation of micelles containing monoglyceride,fatty acids and other lipids which are taken up by the mucosal cells (118).

Much of the triglyceride absorbed is re-esterified and is incorporated into chylomicrons which then appear in the lymphatic system. Windmueller and Spaeth (119) confirmed that this lipoprotein is synthesized in the rat intestine. More recently, Arreaza-Plaza et al. (120) have shown that the ingestion of a margarine emulsion by rats results in an increase in plasma triglyceride levels. This increase is abolished by pretreatment with colchicine which is known to have an inhibitory effect on the secretion of lipoprotein by the liver. They demonstrated a five fold increase in the triglyceride content of the proximal jejunum in colchicine treated rats. These results suggest that lipoprotein

secretion from the small intestine can be inhibited by colchicine and that the microtubular-microfilamentous system may be involved in the release of chylomicrons from the intestinal cell into the circulation.

The synthesis of triglycerides in most tissues proceeds via the glycerol 3-phosphate pathway. However, in the intestine it is believed that the predominant pathway is the monoglyceride pathway (Fig. 1) (118) which may account for 80-90% of the triglyceride synthesized in the small intestine (121). The products of triglyceride digestion, monoglycerides and fatty acids, are known to enter the mucosal cells. Polheim et al. (122) have shown that the monoglyceride can inhibit triglyceride synthesis via the glycerol 3-phosphate pathway. They showed that 2-octadecenyl glycerol ether can inhibit the synthesis of triglyceride from [U-^{14}C]glycerol 3-phosphate. It has also been suggested that bile salts can specifically activate the monoglyceride pathway (129) and that the K_m for the monoglyceride pathway was lower than that for the glycerol 3-phosphate pathway (112). Intestinal microsomes and intestinal slices from male golden hamsters were used in this study. Breckenbridge and Kuksis (123) found that the composition of sn-2,3 diacylglycerol corresponded to that of the exogenous fatty acid but the sn-1,2 diacylglycerol contained both exogenous and endogenous fatty acids. The pathway involving glycerol 3-phosphate proceeds via phosphatidic acid intermediates yielding exclusively sn-1,2 diacylglycerols (124,125). These workers also found that between 10 and 41% of the total diacylglycerol did not contain the fatty acid in the 2- monoacylglycerol supplied to the rat intestinal mucosa (126). These findings are at odds with the results obtained using isolated intestinal microsomes from hamster which suggest an inhibition of the glycerol 3-phosphate pathway by monoacylglycerol in addition to providing evidence that sn-1,2 diacylglycerol is exclusively synthesized from 2-monoacylglycerol. In vivo (127) and in vitro (128) experiments with everted sacs of the rat intestine mucosa have shown that a minimum of 20% of the total triglyceride synthesized is contributed by the glycerol 3-phosphate pathway. It was further shown that unsaturated fatty acid had a preference for the monoglyceride pathway whereas the saturated fatty acid is utilised predominantly by the glycerol 3-phosphate pathway (128).

It is conceivable that fructose and fat in the diet promotes an environment where there is increased synthesis of triglyceride in the



A = pancreatic lipase   C = thiokinase

B = monoglyceride lipase   D = monoglyceride acylase

Fig. 1. Intestinal lipid metabolism

small intestine via the glycerol 3-phosphate pathway.

B. METABOLISM OF CARBOHYDRATE

Hepatic lipogenesis from carbohydrate involves the breakdown of the latter to Acetyl-CoA and glycerol 3-phosphate which are then utilized for lipid synthesis. The enzymes involved in the process are shown in Fig. 2.

1. Control of Hepatic Glucose Metabolism

Glucose is the major metabolic fuel utilized by most animal tissues. Normal blood glucose levels in fasting human adults range between 3 and 6mM and there is no apparent sex related difference in the mean values (130). The blood glucose level is regulated by dietary and hormonal factors. Excess glucose can be stored as glycogen in the liver or as triglyceride in the adipose tissue (132) and these reserves can be utilized when required. The relative constancy of blood glucose levels, despite various disturbing factors, is an example of homeostatic regulation.

(a) Control of glycogen metabolism

Hepatic glycogen levels are controlled by the relative activities of phosphorylase (E.C.2.4.1.1) and glycogen synthetase (E.C.2.4.1.11). Both enzymes can exist in active and inactive forms which are interconvertible by a system of protein kinases (E.C.2.7.1.37) and phosphatases (Fig. 3) (Hers, Ann.Rev.Biochem., 45, (1976)) (167). The activation of phosphorylase can be brought about by the CAMP dependent activation of a protein kinase which, in turn, leads to an activation of phosphorylase kinase (E.C.2.7.1.38) and the consequent activation of phosphorylase. Conversely, the CAMP dependent protein kinase can phosphorylate, and thus inactivate, glycogen synthetase. Therefore glucagon can stimulate the utilization of glycogen by raising intracellular CAMP levels whereas insulin is thought to exert an opposite effect. In addition changes in the physiological concentration of glucose can regulate glycogen levels. The binding of glucose to phosphorylase 'a' results in an inhibition of the enzyme and in the stimulation of the rate of conversion of phosphorylase 'a' to 'b' by phosphorylase phosphatase. A secondary effect is that the inhibition of synthetase phosphatase by phosphorylase 'a' is reversed and this results in the activation of glycogen synthetase (136).

Whitton and Hems (147) have found impaired glycogen accumulation by the perfused livers from diabetic rats. This can be reversed by pretreatment of the animal with either insulin or glucose and fructose,

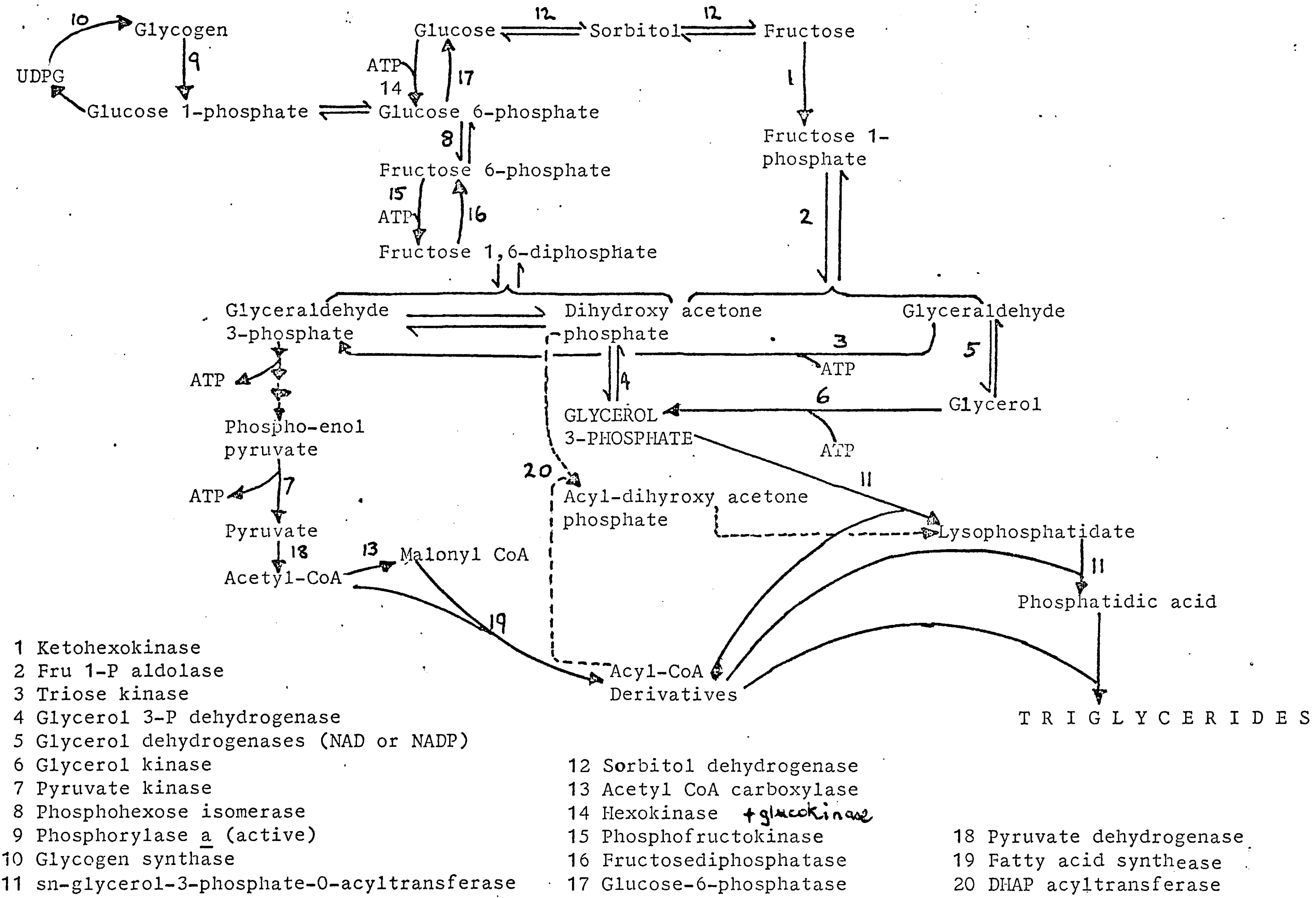


Fig. 2. Conversion of hexoses to triglycerides by the liver.

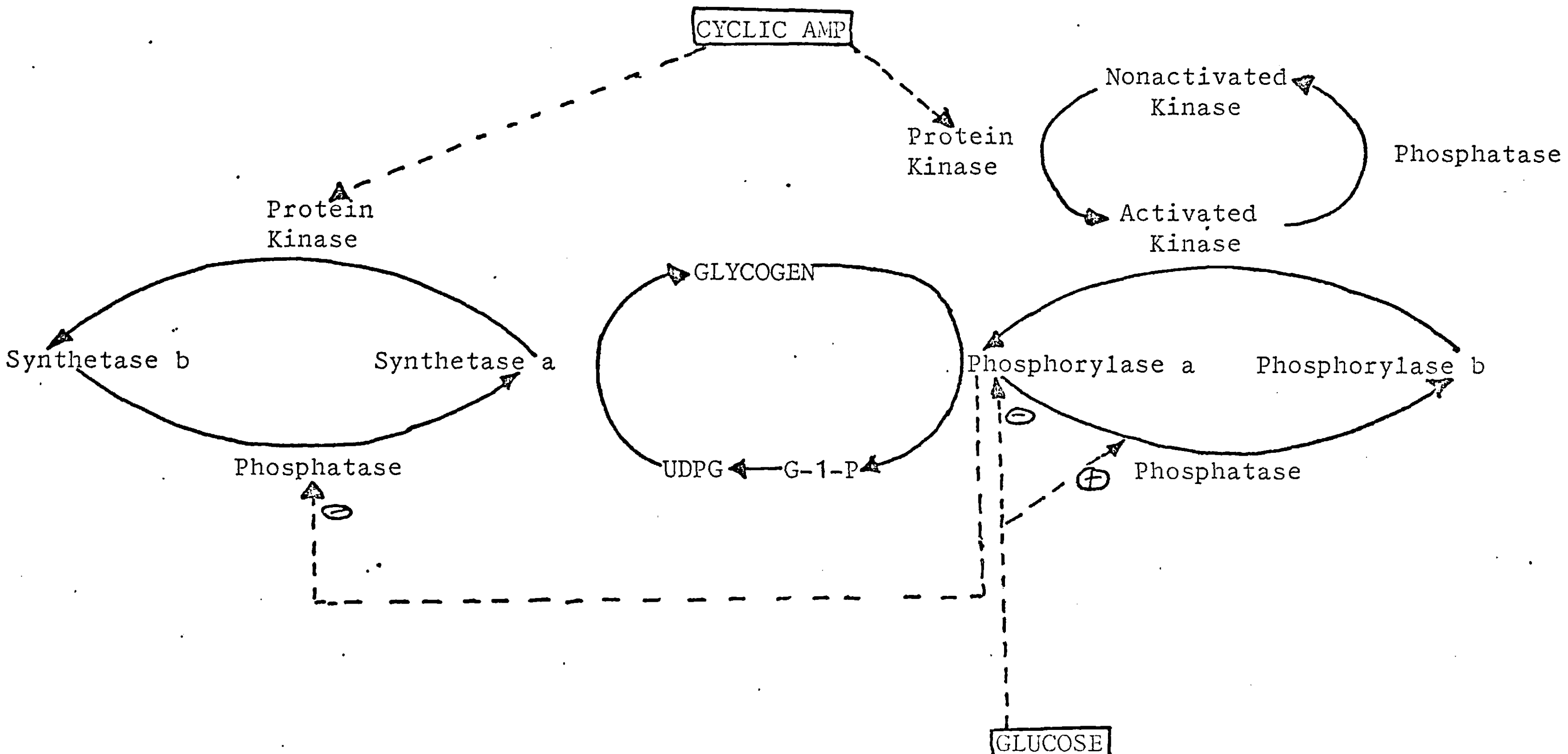


Fig. 3. The control of glycogen metabolism in the liver

although the authors were unable to explain the restorative effect of fructose.

Fructose is a good precursor of glycogen and the intravenous administration of the hexose to rats is followed by an inactivation of the phosphorylase and an activation of glycogen synthetase but the mechanism of the effect is unknown (134).

(b) Gluconeogenesis

Classically, gluconeogenesis is considered to be the synthesis of glucose from non-carbohydrate precursors such as pyruvate, lactate, certain amino acids and intermediates of the tricarboxylic acid cycle (138). However, some authors (139,140,155) have expanded the meaning of the term to include glucose synthesis from fructose. Glucose is more readily formed from fructose or dihydroxyacetone than from either lactate or pyruvate by perfused rat liver (139). This suggests that the rate limiting step in gluconeogenesis from lactate occurs before the formation of triose phosphate. Glycerol, D-glyceraldehyde, L-alanine and serine are less effective than L-lactate as gluconeogenic substrates.

Glucocorticoids, insulin, glucagon and the nutritional state of the animal can affect the rate of gluconeogenesis. Gluconeogenesis from lactate is enhanced in perfused livers treated with glucagon, or in perfused livers from fasted or diabetic rats. The enhanced gluconeogenesis associated with diabetes is reversed by insulin treatment (135).

There is evidence that hormonal and dietary factors can influence the activity of the key gluconeogenic enzymes, glucose 6-phosphatase, fructose 1,6-phosphatase, pyruvate carboxylase (E.C.6.4.1.1) and phosphoenol-pyruvate (PEP) carboxykinase (E.C.4.1.1.32). In the diabetic rat the levels of these enzymes are raised and insulin treatment restores the activities to normal (137). Clark *et al.* (142) have provided evidence that glucagon can act, via CAMP, to enhance gluconeogenesis and inhibit glycolysis at the site of fructose 1,6-phosphatase and phosphofructokinase. Recently, it has been shown that CAMP can induce the synthesis of PEP carboxykinase by a mechanism which involves an increase in the level of a functional mRNA which codes for the enzyme (143).

The gluconeogenic responses to fasting, diabetes or glucagon treatment are reduced or abolished in livers from adrenalectomized rats and it has been shown that glucocorticoids are required for the normal hormonal

activation of gluconeogenesis (366). Seits et al. (144) have found that the gluconeogenic response of rats to fasting consists of an increase in plasma glucagon, an increase in hepatic CAMP, a co-ordinated elevation of the activities of the key gluconeogenic enzymes, an increase in the NADH/NAD^+ ratio and a decrease in the intrahepatic gluconeogenic substrates. However, they found that adrenalectomy did not affect the nature and degree of these responses.

Friedmann (375) believes that glucagon or CAMP cause a large scale redistribution of ions and hyperpolarization of the liver cell membrane which results in an outward movement of glucose from the cell. This does not apparently involve the activation of a protein kinase (145).

(c) Glycolysis

The key glycolytic enzymes are glucokinase hexokinase, phosphofructokinase and pyruvate kinase (E.C.2.7.1.40). Thus the control of glycolysis at these points can regulate lipogenesis from glucose. Two other enzymes believed to be important in the transformation of carbohydrates to lipids are pyruvate dehydrogenase (E.C.1.2.4.1) and glycerol 3-phosphate dehydrogenase (E.C.1.1.1.8). Pyruvate dehydrogenase is the enzyme involved in the conversion of pyruvate to acetyl-CoA and is thus an obligatory step in the conversion of carbohydrate to fatty acids. Glycerol 3-phosphate dehydrogenase is involved in the conversion of carbohydrate to glycerol 3-phosphate, which in turn is incorporated into the glycerol moieties of lipid (146).

In the starved or diabetic animal the fatty acid levels are elevated, glycolysis is inhibited and gluconeogenesis takes predominance. This is due to an inhibition of glucokinase, phosphofructokinase and pyruvate kinase by free fatty acids. Under these conditions hepatic acetyl-CoA levels are also increased which also results in the inhibition of hepatic pyruvate kinase and glucokinase (148). Cyclic AMP and bovine serum albumin can protect phosphofructokinase from inactivation by fatty acids (149). Batenburg and Olson (150) have found that pyruvate dehydrogenase kinase, an enzyme which phosphorylates and then inactivates the pyruvate dehydrogenase complex is stimulated strongly by acetyl-CoA and is inhibited by NAD^+ and CoASH. This forms the alternative mechanism to the theory that the products of the pyruvate dehydrogenase reactions, NADH and acetyl-CoA are competitive feedback inhibitors of the reaction.

(d) Glucose metabolism in isolated hepatocytes

The liver is a composite organ composed of several types of cells. In the normal adult liver these cells include parenchymal cells, kupffer cells, endothelial cells, smooth muscle cells, fibroblasts, bile duct epithelium and blood cells. Parenchymal cells are the most abundant of these cells, comprising 60-70% of all cells (156). Berry and Friend (157) were the first workers to prepare isolated parenchymal cells by perfusing rat liver with a medium containing collagenase and hyaluronidase. Krebs *et al.* (158) reported that isolated liver cells are superior to liver slices in terms of metabolic competence. The rates of oxygen consumption, of gluconeogenesis, of urea synthesis, of ketone body production and the adenine nucleotide concentrations, are identical in both perfused liver and in isolated cells. Isolated liver cells respond to polypeptide hormones (158) and to glucocorticoids (161) and in general the effects of these hormones do not vary greatly from those found using intact perfused liver. The gluconeogenic response of hepatocytes to glucagon is a good system for testing the viability of the isolated cells (158). One drawback of the method is that the metabolic activities of the parenchymal cell may not be a true reflection of the situation in the whole liver. For example, Sapag-Hagar *et al.* (159) have found that the hepatocyte is virtually free of hexokinase whereas glucokinase is predominantly present in the hepatocytes.

Seglan (162) has shown that rat hepatocytes have a capacity for glucose stimulated glycogen synthesis which is independent of protein synthesis. The glycogenic effect of insulin on rat foetal hepatocytes is dependent on the presence of cortisol (163,164). The glucagon stimulation of glycogenolysis in the same system is not cortisol dependent (163). Glucagon stimulates glycogenolysis in hepatocytes by a mechanism which involves the activation of phosphorylase kinase and phosphorylase (165,166). Insulin can inhibit this glucagon stimulated glycogenolysis (170).

Gluconeogenesis in hepatocytes have also been studied (167,168,169, 171,172,173,174). Krebs *et al.* (173) found that in this system there is a lag in the gluconeogenesis from lactate which is abolished in the presence of lysine or NH_4Cl. Claus *et al.* (174) found that following the preincubation of the cells without any added substrates for 20-40 min there was a linear rate of synthesis of glucose from lactate. According to these authors preincubation restored the ratio of ATP to ADP to normal.

increasing glucose concentration in the medium resulted in a progressive inhibition of gluconeogenesis from lactate. Glucagon also enhances glucose synthesis from dihydroxyacetone, fructose, or xylitol. Insulin had no effect on gluconeogenesis from pyruvate either in the presence or absence of glucagon (175). Incubation of hepatocytes with glucagon results in an increase in the K_m of pyruvate kinase for phosphoenol pyruvate. A similar result is obtained when partially purified rat liver pyruvate kinase is incubated with protein kinase and ATP. Riou et al. (176) have used this evidence to suggest that the inhibition of hepatocyte pyruvate kinase by glucagon may be mediated at least in part by a phosphorylation-dephosphorylation mechanism.

Seglen (167,177) found that hepatocytes, isolated from 16 hr fasted rats, exhibit high rates of gluconeogenesis and of glycolysis from fructose. Gluconeogenesis from fructose is not inhibited by glucose whereas glycolysis from fructose is subject to end product inhibition by lactate (177). Glucose at low concentrations is a poor glycolytic and glycogenic substrate for these cells (167).

The effects of insulin on hepatocytes are more variable than the effect of glucagon. Muller et al. (172) found that the exposure of cells to collagenase during preparation must be minimal in order to demonstrate the inhibitory effect of insulin on glucagon induced glycogenolysis. A large number of microvilli are present on the surface of hepatocytes and Wagle (170) has postulated that these structures may be important to obtain hormonal responses at physiological concentration. Furthermore, he suggested that a high concentration of collagenase or a prolonged exposure to the enzyme will destroy the microvilli. Mayo-Johnson (169) found that short term treatment of isolated liver cells with trypsin abolished the effects of both insulin and glucagon. In contrast the response to dibutyryl cyclic AMP was not altered in the trypsin-treated cells. This implies that the plasma membrane hormone receptors are destroyed by this treatment. Claus and Pilkis (168,171) were able to show that insulin had no effect on the basal rate of gluconeogenesis from lactate but care must be taken in the interpretation of such negative results.

In conclusion, it can be said that the metabolic response to glucagon by hepatocytes is easier to demonstrate in hepatocytes but the effects of insulin on the regulation of glycogen synthesis, glycolysis and gluconeogenesis may be complicated by cellular damage to these cells

during isolation. The insulin receptors on the hepatocytes appear to be more fragile than those of glucagon (172).

2. Fructose Metabolism

In 1874, Kulz (178) reported that fructose, unlike glucose, is tolerated by human diabetics. Consequently, numerous workers have undertaken to distinguish the differences between the metabolic pathways of these two carbohydrates.

(a) Sites of fructose metabolism

The liver has long been considered to be intimately concerned with the metabolism of fructose. Liver function can be tested in clinical laboratories by a fructose tolerance test. When an oral load of fructose (50g) is given to the fasting patient, there is little or no rise in the blood sugar if the hepatic function is normal (179).

There are species differences in utilization of fructose and Bollman and Mann have found that hepatectomised dogs are able to convert fructose into glucose (180). The injection of fructose at any time before the extreme stage of hypoglycaemia is reached, relieves the symptoms of hypoglycaemia. This conversion was demonstrated following the removal of the liver, spleen, pancreas and kidneys but did not occur when the stomach and intestines were removed with the liver. In the eviscerated-nephrectomised rabbit Wick et al. (181) observed a very low rate of oxidation and utilization of fructose by the extrahepatic tissues. Insulin administration to these animals increased the metabolism of glucose but not of fructose and these authors concluded that in the rabbit the utilization of fructose by intact animals must be due to metabolism in the liver.

(b) Metabolic changes after fructose administration

There are many reports that the administration of fructose, particularly by the parenteral route, to animals and to man is followed by profound metabolic changes. Maenpaa et al. (184) were able to show that the intravenous administration of fructose to rats rapidly depletes liver ATP and inorganic phosphate. Uric acid and allantoin in plasma were significantly raised and there was a concomitant decrease in liver protein synthesis from (^{14}C)leucine. Liver polysomes were extensively disaggregated after the administration of fructose. Changes in the hepatic levels of free Mg^{2+} may be involved in the alteration of the polysome profiles (185).

Pereira and Jangaard (186) studied the rate of conversion of [^{14}C]-

fructose and $[^{14}C]$glucose into various intermediates of carbohydrate and lipid metabolism in liver slices. They found that more lactic acid, pyruvic acid, carbon dioxide, fatty acids and glyceride-glycerol were synthesised from fructose than from glucose. Fructose can be more readily converted to glycerol 3-phosphate in the liver and this may promote lipogenesis. However, it is not possible to completely eliminate the effect of differential isotope dilution by the intracellular pools of glucose and fructose in this experiment.

The oxidation of fructose, sorbitol and xylitol is similar in the normal fasted and the streptozotocin-diabetic rat. Keller and Froesch (193) concluded that the similarities in metabolism are due to the rapid conversion of these monosaccharides to glucose. Within 10 min after the intravenous injection of the sugars more than 70% of label in the plasma is present as $[C^{14}]$glucose in each case (194).

In the intact animal sorbitol is converted to fructose by sorbitol dehydrogenase (195) and xylitol is oxidised to D-xylulose by the same enzyme (196). D-xylulose is phosphorylated and is further metabolised via the pentose phosphate cycle to glyceraldehyde and fructose 6-phosphate. More than 80% of each of these sugars is metabolised in the liver and the main product, glucose, is subsequently released to the circulation (196).

The concentration of lactate and the lactate-pyruvate ratio is increased when fructose, sorbitol or xylitol is given intravenously (196,197,198). The other metabolic consequences of the metabolism of these carbohydrates include uric acid production and a raised serum bilirubin. Serum uric acid can also be raised if the sucrose intake is 200g/day. This is considered to be the normal sugar consumption in North America (196).

Cori found that the liver glycogen increased more rapidly following fructose ingestion than after the ingestion of glucose or galactose (199). In the diabetic animal xylitol, sorbitol or fructose administration caused glycogen storage in the liver of diabetic animals whereas glucose does not (196). Hers (200) has shown that glycogen formed in the liver and in the muscle from $[1\text{-}C^{14}]$glucose contains most of the isotope in position 1 and provides a strong evidence that glucose is incorporated into the glycogen molecule essentially as an intact 6-carbon unit. In contrast $[1\text{-}C^{14}]$fructose and $[1\text{-}C^{14}]$sorbitol are incorporated by the intact animal into glycogen labelled at both C-1 and C-6 of the glucose

moieties. This indicates that the first step in the metabolism of sorbitol is the oxidation to fructose and that fructose incorporation into the liver and muscle glycogen is dependent on the breakdown of the molecule into two 3 carbon units.

Hereditary fructose intolerance and essential fructosuria are inborn errors of fructose metabolism. Liver fructokinase is absent (201) in essential fructosuria. As a result the ingested fructose is not metabolised, accumulates in the blood and is excreted in the urine. This shows the importance of fructokinase in the metabolism of fructose. Hers and Joassin (202) found that in cases of hereditary fructose intolerance, the fructose 1-phosphate aldolase level is greatly depressed. The administration of fructose to these patients leads to a fall in blood glucose and P_i levels. In addition the liver ATP levels are lower. This form of hypoglycaemia is unresponsive to glucagon (203,204). It is clear that in patients with fructose intolerance the massive build up of fructose 1-phosphate is at the expense of ATP. This leads to a fall in the cyclic AMP level and a decrease in the phosphorylation of the enzymes involved in the glycogenolysis (see Fig. 3). The accumulated fructose 1-phosphate is also a competitive inhibitor of phosphorylase.

(c) Fructose and the insulin response

Foster (196) and Froesch *et al.* (194,193) have found fructose metabolism is not controlled by insulin. Phillips *et al.* (205) and Samols and Dormandy (206) found that fructose does not produce an insulin response. However, Brodanova *et al.* (207) were able to demonstrate an increase in blood insulin in healthy subjects when fructose was administered intravenously. This discrepancy probably reflects the different amounts of fructose administered. The rate at which fructose is given and the mode of administration can effect the insulin response. Glucose stimulates the *in vitro* release of insulin from rat pancreas at concentrations above 50mg/100ml whereas fructose can only stimulate this release at very high concentrations above 500mg/100ml (209).

Glucose, mannose and fructose which are known to stimulate insulin secretion *in vitro* can form complexes with solubilized components of β cell membranes (211). In a study of the electrical activity of superfused mouse pancreatic islets, Pace and Price (212) found that glucose, mannose and fructose caused changes in membrane potential. Fructose is a weak secretory stimulus and a higher concentration is needed to elicit a half-maximal electrical response.

More recently, Jain et al. (213) were able to stimulate insulin secretion from isolated rat pancreatic islets in static incubation and perifusion systems by the addition of D-glyceraldehyde. D-Glyceraldehyde, an intermediate in the metabolism of fructose, was found to be a more potent insulin releasing agent than glucose at low concentrations (2-4mM).

(d) Hepatic fructose metabolism

The pathway of fructose metabolism by the liver is shown in Fig. 2.

(i) Uptake of fructose into the liver. The uptake of fructose into the liver is thought to be by free diffusion (214), The rate of entry of fructose into the liver cells is dependent on the rate of phosphorylation of the hexose. Glucose does not significantly inhibit the rate of fructose uptake presumably because hexokinase is not a major factor in the phosphorylation of fructose (215).

(ii) Fructose phosphorylation. Fructose is phosphorylated in the liver by an ATP dependent fructokinase yielding fructose 1-phosphate. This enzyme is also found in both small intestine and kidneys but not in adipose tissue or skeletal muscle (133). In the absence of glucose, fructose is phosphorylated in the peripheral tissues by means of hexokinase to fructose 6-phosphate but when glucose is present, glucose is preferentially utilized by this enzyme (216).

The level of fructokinase in rat liver is two times that of glucokinase and hexokinase. According to Heinz et al. (216) this can partially explain the well known observation that fructose is metabolised faster than glucose.

Fructokinase can be distinguished from the other hexokinases by an absolute requirement for K^+. This enzyme is strongly inhibited by ADP but this inhibition can be partially reversed by K^+ (217)

(iii) Fructose 1-phosphate aldolase. Fructose 1-phosphate is converted to D-glyceraldehyde and dihydroxyacetone-phosphate by a liver aldolase. This enzyme is specific for both fructose 1-phosphate and fructose 1,6-diphosphate (218).

(iv) Enzymes involved in the D-glyceraldehyde metabolic crossroads. The metabolism of D-glyceraldehyde is at present a controversial subject. There are three pathways, involving four enzymes, which could utilise this metabolite. These involve the phosphorylation, reduction and oxidation of D-glyceraldehyde.

An ATP dependent triokinase (E.C.2.7.3.10) can phosphorylate D-glyceraldehyde to D-glyceraldehyde 3-phosphate. Various authors believe

that this enzyme could account for as much as 90% of the phosphorylation of the triose (133,219). This is because the enzyme has a low K_m for D-glyceraldehyde. Frandsen and Grunnet (220), however, found that the enzyme has a relatively high K_m value for ATP-Mg^{2+}. They also found a lack of physiological inhibitors other than ADP which suggests that the enzyme is controlled by the ATP/ADP ratio in the cytoplasm. When the ATP concentration is high, the triokinase pathway will predominate and gluconeogenesis from fructose can proceed. When the ATP concentration is low, such as in the case of an intravenous load of fructose (221), this enzyme is less active, and hence the reduction of glyceraldehyde to glycerol or the oxidation to glycerate may occur.

When [1-C^{14}]fructose (200) or [6-C^{14}]fructose (222) are administered to rats the radioactive distribution of label in the glycogen formed is primarily in the 1 and 6 positions of the glucose moieties. If there is reduction of glyceraldehyde to glycerol, glucose should be labelled not only in the 1 and 6 position but also on the 3 and 4 positions. However, Laudau and Merlevede (222) found that the direct administration of D-[3-C^{14}]glyceraldehyde gave rise to labelling of the 3 and 4 positions of glucose units in the glycogen molecule. This could be due to the reduction of the triose to glycerol by alcohol dehydrogenase. At the high level of glyceraldehyde administered it was possible that there was conversion by this pathway (219).

There are two forms of hepatic alcohol dehydrogenases, an NADH dependent alcohol dehydrogenase (E.C.1.1.1.1), which has an extremely high K_m for D-glyceraldehyde, and an NADPH dependent enzyme (E.C.1.1.1.2) with a low K_m for the triose.

D-Glyceraldehyde can also be oxidised to glyceric acid (227) and phosphorylated to yield 2-phosphoglycerate. The enzymes involved are aldehyde dehydrogenase (E.C.1.2.1.3) and D-glycerate kinase (E.C.2.7.1.31) (146,204). After a fructose load there is accumulation of D-glycerate in the liver and this observation has been used to support the existence of this pathway.

In summary, it is possible to conclude that there is evidence to show that triokinase is the major enzyme involved in the metabolism of D-glyceraldehyde. Kinetic studies (219,220), dietary fructose induced activation of triokinase (223,224) and radioisotope studies have been used to corroborate this hypothesis. Hue and Hers (225) have shown that when [4-^3H, 6-^{14}C]fructose is administered to mice, ^3H is not lost in

the conversion of fructose into glycogen. If the pathway proceeds via glyceric acid there should be a loss of ^3H during the oxidation of D-glyceraldehyde (see Fig. 4). In another study, Rognstad *et al.* (226) have found that glucose formed from [5-^3H]fructose by rat hepatocytes contains about 10-30% ^3H. The breakdown of [5-^3H]fructose phosphate should yield D-[2-^3H]glyceraldehyde and dihydroxyacetone phosphate. The D-[2-^3H]glyceraldehyde formed can be phosphorylated and the ^3H can be readily exchanged with water in the triose-phosphate isomerase reaction. Because of this it has been considered that glucose formed from [5-T]fructose should have lost all the ^3H. These authors have shown that this assumption is not valid. This finding highlights the problems associated with the use of radioactive tracers in the study of the metabolic pathway of glyceraldehyde.

(e) Species differences in fructose metabolism

Heinz *et al.* (216) in a study on the levels of enzymes of fructose metabolism in the human and rat livers found that the activities of alcohol dehydrogenase (NAD and NADP) in the human liver are higher than in the rat. The level of sorbitol dehydrogenase is again higher in the human than in the rat liver but the reverse is true for the levels of fructokinase and fructose 1,6-diphosphatase. Only trace amounts of glycerate kinase can be found in human liver in contrast to rat liver. The human liver contains more triokinase and less glycerokinase than rat liver. These results suggest that in man the most likely fate of D-glyceraldehyde is conversion to glyceraldehyde 3-phosphate whereas the situation in the rat is less clear.

(f) Glycerol metabolism

Glycerol represents a junction of sugar and fat metabolism. The importance of the pathway by which D-glyceraldehyde is reduced to glycerol is debatable (see Section II.B.2.d.iv). However, there is no doubt that during lipolysis, glycerol is released from glycerides. Glycerol has been shown to be a gluconeogenic substrate *in vivo* (229) and *in vitro* (228). The two initial enzymes involved in this process are glycerol kinase and glycerol 3-phosphate dehydrogenase.

(i) Glycerol kinase. Robinson and Newsholme (230) found that this enzyme is inhibited by high levels of glycerol when the concentration of ATP is low. ADP and AMP are also inhibitors of this enzyme and this inhibition is augmented by Mg^{2+}. The physiological significance of this inhibition may be to prevent phosphorylation when the hepatic ATP

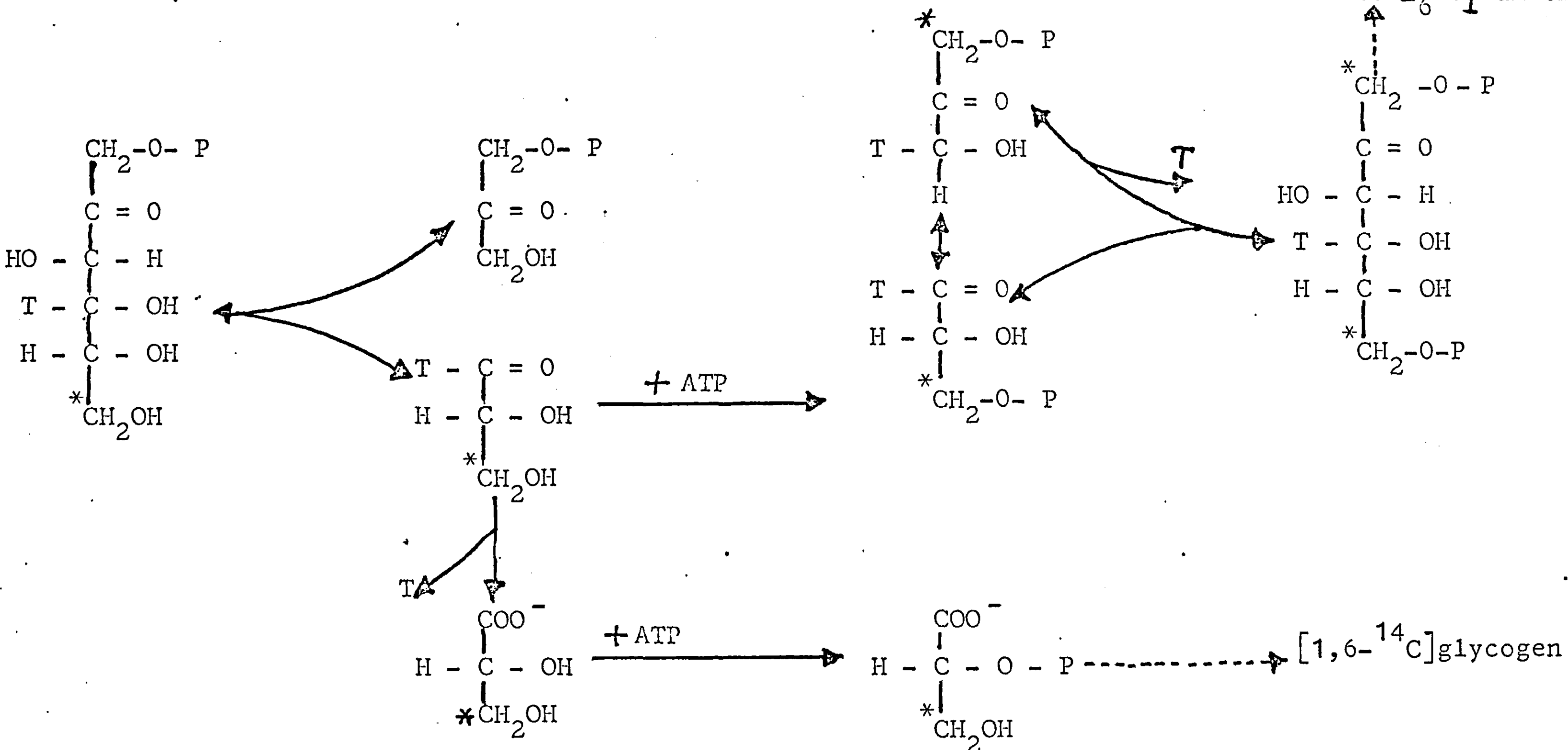


Fig. 4. Labilization of tritium (T) and randomization of carbon in the conversion of [4-H, 6-^{14}C]fructose into fructose diphosphate.

concentration is low (eg. in ischaemia, and following a fructose load). L-glycerol 3-phosphate is a competitive inhibitor of glycerol kinase when glycerol is used as a substrate. In rats fed glycerol there is an increase in the glycerol 3-phosphate dehydrogenase activity which may reduce the hepatic levels of glycerol 3-phosphate. This can then lead to an increase glycerol uptake by the liver.

Hepatic glycerol kinase level is decreased during starvation, in alloxan diabetes, after adrenalectomy and after three days of fat feeding. This decrease can be reversed by insulin treatment in alloxan diabetes, cortisol administration to adrenalectomised rats and feeding rats on a fat diet for more than three days (231). There is no plausible explanation for the decrease in glycerol kinase activity after starvation or during the first three days on a fat diet. However, Kampf _et al._ (231) have shown that the hormonal induction of this enzyme can be inhibited by actinomycin D. Kida _et al._ (232) were able to show that when rats were fed a fat diet an increase in the levels of glycerol kinase resulted. Glycerol feeding did not increase this enzyme but the feeding of fatty acids, especially the unsaturated fatty acid such as oleic and linoleic acids, enhanced liver glycerol kinase activity.

(ii) _Glycerol 3-phosphate dehydrogenase_. There are two forms of glycerol 3-phosphate dehydrogenase. One of these forms is associated with the outer part of the inner membrane of the mitochondria (233) whilst the other form is found in the cytoplasm (234). This soluble glycerol 3-phosphate dehydrogenase, at physiological pH, favours the production of glycerol 3-phosphate from dihydroxyacetone phosphate (146). The glycerol 3-phosphate shuttle involves these two enzymes. During glycolysis NADH is produced and reoxidation of this compound is necessary for the maintenance of glycolysis. As pyridine nucleotides cannot cross the mitochondrial membrane, cytoplasmic NADH generated cannot be directly oxidized. This can be partially overcome by the glycerophosphate shuttle. Glycerol 3-phosphate dehydrogenase in the cytoplasm can reduce dihydroxy-acetone phosphate to glycerol 3-phosphate and oxidise NADH to NAD. This glyerol 3-phosphate can then move into the mitochondria (235).

Heinz (146) believes that the level of soluble glycerol 3-phosphate in the liver is not rate limiting in lipid synthesis (146). Fatty acids (236,237) can inhibit glycerol 3-phosphate dehydrogenase but this may be a general detergent effect on the enzyme. Tepperman _et al._ (238) found that a high glucose or fructose diet elevates glycerol 3-phosphate

dehydrogenase. The activity of this enzyme is lowered by feeding high fat diets (146).

The mitochondrial glycerol 3-phosphate dehydrogenase is induced by feeding rats dessicated thyroid powder (249, 250). Thyroidectomized rats have lower levels of mitochondrial and cytoplasmic glycerol 3-phosphate dehydrogenase than control (250, 252). However, Carnicero et al. (254) do not support the concept of a glycerol 3-phosphate cycle in the liver regulated by thyroid hormones. Their results indicate that the pathway for the oxidation of extramitochondrial hydrogen is through respiratory chain-linked NADH dehydrogenase which is inhibited by rotenone. Mitochondrial glycerol 3-phosphate dehydrogenase is a flavin linked enzyme and is insensitive to rotenone inhibition. In hepatocyte studies, Berry et al. (268) found that glycerol 3-phosphate shuttle is more important than the rotenone-sensitive path. The latter accounts for only 20-30% of the total flux of reducing equivalents from the cytoplasm to mitochondria.

Birch et al. (253) found that in rats fed a riboflavin-deficient diet there is a decreased level of the mitochondrial enzyme which can be related to an increase in the level of glycerol 3-phosphate.

3. Metabolites Involved in Carbohydrate Metabolism

In the study of the control of metabolic pathways it is useful to study the changes in the appropriate metabolite levels after imposing a sudden change to metabolic situation. For instance, during the metabolism of monosaccharides the concentrations of the glycolytic intermediates are altered, some to a greater extent than others, and a study of these metabolites will reveal some information about the regulation of the pathway.

(a) The problems associated with the measurement of metabolites

It is now known that anoxia, stress and narcosis can affect the levels of metabolites. Nikkila and Ojala (239) in 1963 reported that the data concerning glycerol 3-phosphate levels obtained following extraction of tissue which had been frozen with liquid air, was incorrect. They recommended a method that involved heat precipitation of protein and did not involve the freezing of the tissue. As a result of this procedure very high levels of glycerol 3-phosphate were obtained. Since that time Lowry et al. (240) found evidence to show that ischemia effected the levels of the substrates and cofactors of the glycolytic pathway in brain. It is now generally accepted that the concentrations of metabolites in

animal tissues which have been freeze-clamped (241) or immersed in a refrigerant are more likely to reflect the *in vivo* situation (242).

Ischaemia can affect the hepatic and renal metabolite levels (243) in the following ways:-

i) increased levels of AMP, P_i, lactate, glycerol 3-phosphate, dihydroxyacetone phosphate, fructose 1,6-diphosphate and triose phosphate;

ii) decreased levels of ATP and other phosphorylated C_3 intermediates;

iii) the rate of increase of glycerol 3-phosphate in the liver was linear during the first 120 sec and that for lactate was linear for at least 300 sec.

Faupel, Seitz and Tarnowski (244) studied the effect of anoxia, stress and narcosis on the concentration of glycolytic intermediates and related compounds in rat liver. They came to the conclusion that the double hatchet method of killing the rat and removing the liver for freeze clamping gave the most reliable result. The time course of the alteration of metabolite concentrations during anoxia was investigated and found to be linear for the first 13.6 sec in all cases studied.

Seitz *et al.* (245) have found that anaesthetics can affect the glycolytic metabolite levels. For example, lactate levels were dramatically changed by all anaesthetics studied. However, Kral (246) observed that rats killed by blows to the head before ex-sanguination had higher levels of free fatty acid and glycerol in the blood than anaesthetised rats. This increase in fatty acid and glycerol is most probably due to stress which induces release of noradrenaline. Boime *et al.* (247) have shown an increase in mitochondrial free fatty acids when the organelles were prepared from ischaemic liver.

Rats treated for up to 96 hr with phenobarbital showed an increase in levels of both phospholipids and acetyl-CoA carboxylase in the liver. There was no significant change in hepatic glycerol 3-phosphate and glycerol 3-phosphate dehydrogenase levels during this time (248).

(b) Changes in liver metabolites following administration of various substrates

The levels of fructose 1-phosphate in the liver are increased following fructose feeding (225) and following fructose infusion through the tail vein of rats (256). Sorbitol infusion into the tail vein of rats can also increase the levels of fructose 1-phosphate (257). In the

liver, fructose administration leads to an increase in fructose 1-phosphate level (258,259) and this is accompanied by lactate production (260) and a fall in ATP concentration (261). This has been shown to occur in man (262), pig (263) and rat (264). Woods, Eggleston and Krebs (221) have shown that the accumulation of fructose 1-phosphate after fructose loading is due to the inhibition of the breakdown of the hexose phosphate by IMP which arises from the degradation of AMP.

Dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, fructose 1,6-diphosphate, lactate and pyruvate levels have been shown to increase after fructose or sorbitol administration (221,264,262,257,265,266). However, Exton and Park (266) could not detect a fall in ATP levels when rat liver was perfused with 20mM fructose for 1 hr. Perfusion of rat liver with a higher concentration of fructose (40mM) caused a decrease of 40% in the total liver adenine nucleotides but did not alter the ATP-ADP or ATP-AMP ratio.

Intraperitoneal injection of dihydroxyacetone can also produce similar changes in hepatic metabolite levels. There is more deposition of glycogen from dihydroxyacetone than from fructose. This is probably because dihydroxyacetone does not give rise to a high level of fructose 1-phosphate and thus does not cause a profound drop in ATP level and thus a subsequent drop in UTP and UDPG levels (265).

Injection of nicotinamide increases the hepatic NAD^+ level and also the NAD^+/NADH ratio. This is followed by a 50-70% decrease in lipogenesis from [^{14}C]glucose. The levels of glycerol 3-phosphate, glucose 6-phosphate, citrate and malate are also decreased. The inhibition of lipogenesis could be due to several factors: a shortage of glycerol 3-phosphate, accummulation of fatty acyl CoA, decreased citrate stimulation of acetyl-CoA carboxylase and a limitation of reducing power in the form of NADPH (267).

Changes in the levels of hepatic glycerol 3-phosphate are more dramatic when glycerol is injected intraperitoneally into rats than when fructose is injected (253). The dihydroxyacetone phosphate level remained unchanged. The rate limiting step in the utilization of glycerol by the liver is the oxidation of the glycerol 3-phosphate. This is presumably because the equilibrium for the reaction does not favour the synthesis of dihydroxyacetone phosphate.

Berry _et al._ (268) also found that incubation of hepatocytes with glycerol led to an accumulation of glycerol 3-phosphate whereas sorbitol caused a smaller accumulation of the metabolite.

(c) Hepatic levels of glycerol 3-phosphate following fructose administration

Glycerol 3-phosphate has been shown to stimulate fatty acid synthesis in rat microsomes (269). The incorporation of acetate into fatty acids by the yeast extracts was also stimulated by glycerol 3-phosphate (270). Wakil et al. (271) found that glycerol 3-phosphate can enhance the synthesis of palmitic acid from acetyl CoA and malonyl CoA but of the phosphorylated sugars tested fructose 1,6-diphosphate caused the greatest stimulation of lipid synthesis.

It has been reported by a number of laboratories that fructose is converted rapidly to glyceride-glycerol in liver whereas glucose produced substantially less glyceride-glycerol (272). It is thought that this is due to the fact that fructose administration increases the hepatic glycerol 3-phosphate levels. However, there are apparently conflicting reports on the ability of fructose to increase the level of this metabolite. These findings are summarized in Table I. On closer analysis of these results it can be seen that the authors showing increases in glycerol 3-phosphate levels have, i) studied the effect of fructose administration in perfused livers or, ii) injected high concentrations of fructose. Apparently dietary fructose does not affect the levels of glycerol 3-phosphate and, on the contrary, the metabolite level may be lowered by high fructose or glucose diets.

Zakim et al. (276) found that a transient increase in glycerol 3-phosphate level occurred 5 min after the injection of fructose but the level decreased to its original value after 20 min.

In conclusion it can be stated that fructose may affect the level of hepatic glycerol 3-phosphate but only under some conditions. Dietary fructose may not raise the levels of glycerol 3-phosphate to a great extent because under physiological conditions the levels of fructose in the portal blood and consequently the concentrations of the hexose in the liver are insufficient to cause any change in the level of the metabolite.

C. LIPID METABOLISM

The body reserves of carbohydrate, i.e. glucose and glycogen, are limited and, except during the intervals immediately following meals, stored fats are the major source of calories in the body.

1. Lipoproteins

Lipids are generally transported in the blood in the form of lipoproteins

TABLE I. Effect of fructose on hepatic glycerol 3-phosphate levels

Mode of fructose administration	Species	Period of administration	Glycerol 3-phosphate levels nmole/g liver		Reference
			initial	final	
Infusion of 500ml of 10% fructose	Man	30 min	164 ± 23	497 ± 49	Bode *et al.* 1973 (263)
Infusion via tail vein (75mg fructose/ 100g body wt.)	Rat	5 min	300	1600	Heinz *et al.* 1969 (264)
Infusion via tail vein (75mg sorbitol/ 100g body wt.)	Rat	10 min	110	3290	Heinz *et al.* 1970 (257)
Perfused liver 10mM fructose	Rat	10 min	130 ± 110	1060 ± 230	Wood *et al.* 1970 (221)
Intraperitoneal injection 40mmoles fructose /Kg	Rat	60 min	164 ± 20	5 fold increase	Burch *et al.* 1969 (265)
Perfused liver (20mM fructose)	Rat	60 min	480 ± 34	2400 ± 450	Exton & Park, 1969 (266)
Intraperitoneal injection of 40mmole fructose/Kg	Rat	60 min	164 ± 22	4 fold increase	Burch *et al.* 1970 (252)
Dietary fructose (70%)	Rat	48 hr	700 ±60.6	539 ± 35.9	Zakim *et al.* 1967 (273)
Dietary fructose (75%)	Rat	4 days	293 ± 19	315 ± 41	Waddell & Fallon 1973 (274)
Intravenous injection (fructose 1mmole)	Rat	5 min	606	478	Lamers & Hulsman 1973 (116)
Dietary fructose (70%)	Rat	3 wks	874 ± 125	487 ± 40.7	Zakim *et al.* 1967 (275)
Intravenous injection (fructose 400mg)	Rat	5 min	427 ±39.8	1479 ± 172	Zakim et al. 1968 (276)
		20 min	427 ±39.8	461 ± 122	
Perfused liver (200mg fructose, followed by 90mg/hour)	Rat	120 min	75	150	Wieland & Matschinsky 1964 (277)
Intraportal fructose infusion (150mg)	Rat	10 min	399 ± 84	323 ± 123	Kupke & Lamprecht 1967 (278)

The percentage of lipids in the lipoproteins varies between 98% in the chylomicrons and less than 50% in the high density lipoproteins (280).

Lipoproteins are classified according to their floatation properties into chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoproteins (HDL) fractions. The floatation properties are directly related to the lipid composition and content. VLDL and chylomicrons are the main triglyceride carrying lipoproteins. Chylomicrons transport exogenous lipids and are synthesised in the small intestine (282). VLDL is formed in the rough and smooth endoplasmic reticulum of liver (281) and small intestine cells and this lipoprotein is important in the transport of newly synthesised endogenous triglyceride. Although the predominant lipid in these two lipoproteins is triglyceride, variable amounts of cholesterol and phospholipids are also present.

HDL and LDL have significantly greater cholesterol contents than either VLDL or chylomicrons. LDL consists of about 50% cholesterol. The roles of HDL and LDL in the transport of cholesterol are unclear, however there is some evidence to support a possible role for HDL in the excretion of cholesterol by the body (280). In patients with familial HDL deficiency (Tangier disease), there is accumulation of cholesterol ester in the reticuloendothelial cells throughout the body (283). There is also good evidence to suggest LDL is formed by the breakdown of both VLDL and chylomicrons. This process occurs when the triglyceride components of the lipoproteins are removed by the action of lipoprotein lipase (280).

Plasma LDL and VLDL levels have been shown to increase with age and are higher in men than in women. HDL on the other hand is 30-60% higher in females than in the males of comparable age (284). The reasons for these observations are, as yet, unknown. Species differences in the lipoprotein concentration in animals have also been shown. The dog and rat have low VLDL and LDL levels and a high HDL level when compared to men (280).

The proteins that are present in lipoproteins are known as apolipoproteins (285). They constitute from as little as 1% of the chylomicron mass to as much as 50% of the mass of HDL. A-I and A-II are the major apoproteins in HDL and they are synthesized in both the small intestine and liver. Apoprotein B is the major protein of LDL and is also present

in chylomicrons and VLDL. If all the apoprotein in VLDL in circulation was transferred from VLDL to LDL it could account for the total production of the latter lipoprotein. In both VLDL and HDL from human plasma there are small molecular weight proteins called apoprotein C. This group can be separated chromatographically into C-I, C-II, and C-III (280). C-II has been shown to activate adipose tissue lipoprotein lipase (286) but the mechanism of this activation is unknown. There are two forms of post heparin plasma lipoprotein lipase, one form originating in adipose tissue and the other from the liver. In familial type I hyperlipoproteinaemia, lipoprotein lipase of extrahepatic origin is absent (280).

In atherosclerosis changes in plasma lipoprotein and apoprotein levels have been recorded in pigs (287). Salmon and Hems (288) have shown that genetically obese mice exhibit hyperlipoproteinaemia but the plasma concentrations of triglyceride and phospholipid remain normal. It appears from this brief survey that the levels of lipoproteins in the blood may be just as important as the lipid levels in affecting the rate of lipid deposition in arteries.

2. Lipoprotein Lipase

In cases of Type III or Type IV hyperlipoproteinaemia, plasma triglyceride levels are raised. Eaton (324) found that in these patients the synthesis of triglyceride from [^{14}C]palmitic or oleic acids was only slightly higher than normal and therefore cannot account for the unusually high plasma triglyceride levels. This author suggests that overproduction of triglyceride cannot be implicated as the aetiology of these forms of hyperlipoproteinaemia and thus the rate of 'clearance' of triglyceride may be the cause of these diseases. Dutt (325) has shown that both pre- and post-heparin lipoprotein lipase activities are depressed in CHD. It is possible, therefore, that the 'clearance' of triglyceride by lipoprotein lipase may be an important factor affecting serum triglyceride levels.

Lipoprotein lipase has been shown to occur in adipose tissue (326). The enzyme from this source acts mainly on long chain fatty acid triglyceride combined with proteins in the form of lipoproteins. Addition of heparin to adipose tissue causes release of lipoprotein lipase into the medium and this effect will also occur *in vivo*. The lipase thus released is known as post heparin lipoprotein lipase.

A triglyceride lipase is also released from the liver after heparin treatment (496) but this can be distinguished from adipose lipoprotein lipase in that the former is inactivated by protamine and does not require serum cofactors.

It has been shown that the function of the lipoprotein lipase is in the hydrolysis of plasma lipoproteins during the uptake by the fat cell (326). Lipoprotein lipase is thought to be synthesized in the adipocyte and transported to the luminal surface of the endothelial cells which line the blood capillary of adipose tissue. It has been shown that the enzyme is distinct from triglyceride lipases which also occur in the adipose tissue but the latter enzymes act at water-lipid interfaces, do not require serum cofactors and are stimulated by glucagon and adrenaline.

Starvation of rats results in a decrease of the lipoprotein lipase activity in the adipose tissue whereas insulin or glucose administration have an opposite effect (328). Adrenaline, noradrenaline, ACTH, glucagon and TSH are all known to enhance the activity of the rat adipose tissue triglyceride mobilizing lipase. These hormones have been shown to have an inhibitory effect on lipoprotein lipase. Robinson *et al.* (329,330) have also found that in rats in a variety of nutritional states, the adipose tissue clearing factor lipase activity is positively correlated with the uptake of triglyceride by the fat cells.

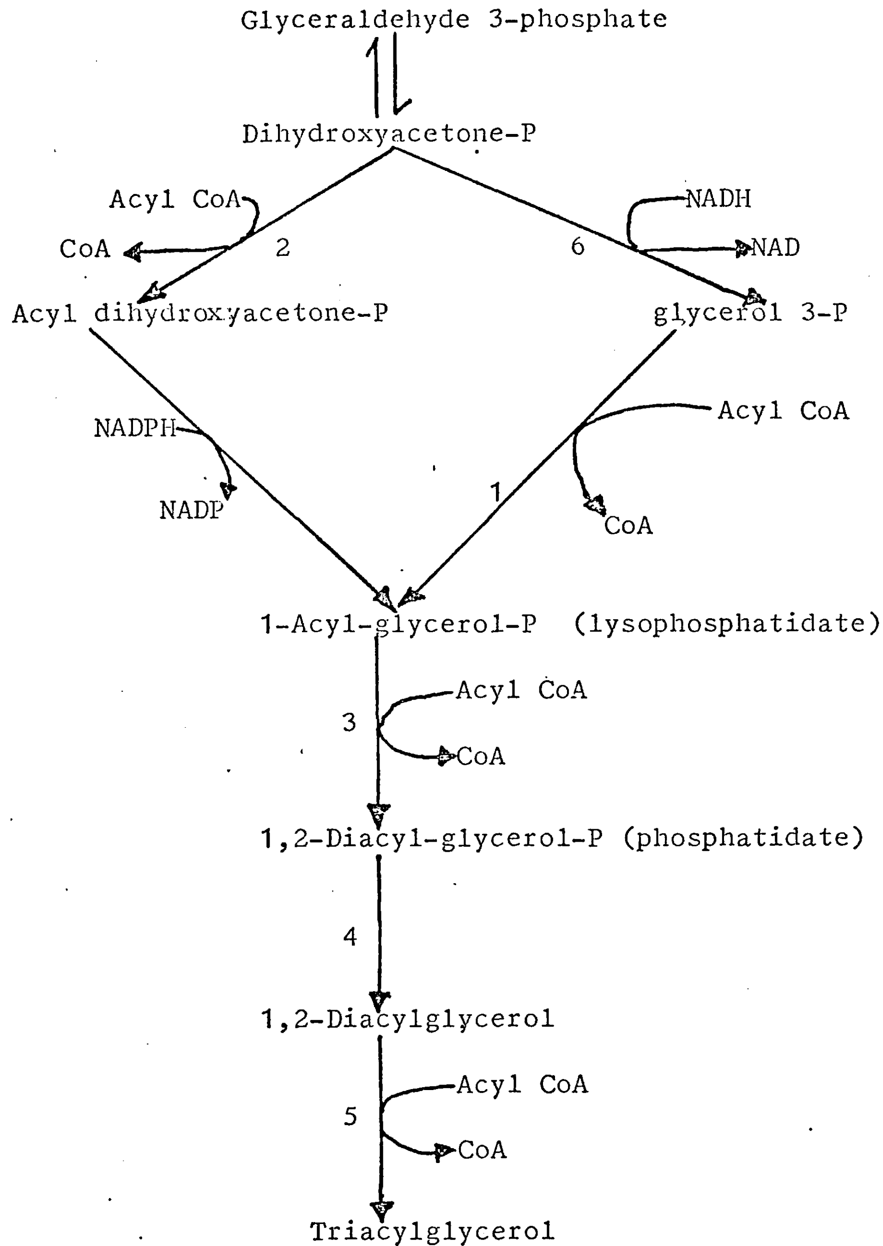
3. Triglyceride Synthesis

(a) Pathways of triglyceride synthesis in the liver

The proposed pathways for the synthesis of triglyceride from glycerol 3-phosphate or dihydroxyacetone phosphate and Acyl-CoA are shown in Fig. 5 (373). Phosphatidic acid, a key intermediate in triglyceride synthesis, can be synthesized by two different routes, one involving the acylation of glycerol 3-phosphate and the other involving the acylation of dihydroxyacetone phosphate. In addition there are at least two other pathways.

(i) Glycerol 3-phosphate pathway. Evidence obtained from work on intact animals, liver slices and microsomal preparations have indicated that there is a high degree of specificity in the formation of phosphatidate from glycerol 3-phosphate. In mammals, saturated fatty acid species appear predominantly in position 1 of phosphatidate and the more unsaturated fatty acids are largely found in position 2 (361).

The first enzyme involved in the conversion of glycerol 3-phosphate is glycerol 3-phosphate acyltransferase. The highest activities of this



Enzymes

1. Glycerol 3-Phosphate acyltransferase (E.C.2.3.1.15)
2. Dihydroxyacetone phosphate : acyl CoA transferase (E.C.2.3.1.42)
3. Acyl-glycerol 3-phosphate acyltransferase
4. Phosphatidate phosphohydrolase
5. Diacylglycerol acyltransferase
6. Glycerol 3-phosphate dehydrogenase

Fig. 5. The *de novo* synthesis of triglyceride

enzyme are found in the adrenal gland, liver and adipose (363). The mitochondrial enzyme has a preference for saturated fatty acids and lysophosphatidic acid is the major product. In the microsomes, both saturated and unsaturated fatty acids are efficient substrates and phosphatidate is formed (362,363). Kinetic studies using intact animals injected with [^{3}H]glycerol have shown that phosphatidate is formed from glycerol before the formation of diglyceride (364).

Lamb and Fallon (365) have shown that the enzyme phosphatidate phosphohydrolase which converts phosphatidate to diglyceride may be rate limiting in the formation of triglyceride. However, in intact liver cells, incubated with radioactive glycerol only a minor amount of phosphatidate accumulates (161). The esterification of fatty acids by microsomes leads to an accumulation of phosphatidic acid which can be diminished by the addition of a factor present in the soluble fraction of the liver (367).

(ii) *Dihydroxyacetone phosphate pathway*. Liver and intestinal microsome systems can utilize dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and glycerol 3-phosphate in the synthesis of phosphatidate and triglyceride (372). 1-Hydroxy-3-chloro-2-propanone phosphate, an irreversible inhibitor of triose phosphate isomerase, can completely inhibit the synthesis of glycerides from glyceraldehyde 3-phosphate whereas the synthesis from dihydroxyacetone phosphate is unaffected (368,369). This is strong evidence for the conversion of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate before its incorporation into glycerides.

Hajra (332) has shown that acyl dihydroxyacetone phosphate can be synthesized from dihydroxyacetone phosphate in the guinea pig liver. Hajra and Agranoff (370) found that NADPH was required for the synthesis of lysophosphatidate from dihydroxyacetone phosphate. The reduction of acyl dihydroxyacetone phosphate to lysophosphatidate was thought to require a NADPH dependent mitochondrial dehydrogenase. The proposed pathway for the synthesis of phosphatidate by this route is shown in Fig. 5. The dihydroxyacetone phosphate pathway has been shown to occur in both mitochondria and microsomes (368,370).

Ananda *et al.* (371) have shown that the conversion of [^{14}C]fructose 1,6-phosphate to glyceride is activated by either NADH or NADPH. Addition of glycerol 3-phosphate had no effect on the incorporation of radioactivity to glycerides indicating that the dihydroxyacetone pathway

plays a significant role in the synthesis of glycerides.

Pollock _et al_. (373) observed that dihydroxyacetone phosphate was a better precursor of glycerides, compared to glycerol 3-phosphate when NADPH was added to cell free homogenates, suggesting that the pathway involving the acylation of dihydroxyacetone phosphate is more important (see Fig. 5). However, these authors also found that with the same concentration of glycerol 3-phosphate, in the presence of NADH, there was approximately nine times the incorporation of this compound into phosphatidic acid compared to dihydroxyacetone phosphate. These authors admit that _in vivo_ the glycerol 3-phosphate pathway to glycerolipids may be more active than the acyl dihydroxyacetone phosphate pathway because of the greater activity of glycerol 3-phosphate acyltransferase and the higher glycerol 3-phosphate concentration in the liver cells. However, there is also a possibility that not all the glycerol 3-phosphate dehydrogenase activity is involved in glyceride synthesis since 20-60% of the enzyme is peroxisomal and is therefore not involved in glycerolipid synthesis (373)

Incubation of isolated liver parenchymal cells with [^{14}C,2-^{3}H]-glycerol produced glycerides with ^{3}H/^{14}C ratios similar to those ratios found in intracellular glycerol 3-phosphate. This suggests that, in this case, the dihydroxyacetone phosphate pathway does not contribute significantly towards the total glyceride synthesis since the conversion of glycerol 3-phosphate to dihydroxyacetone phosphate will result in a loss of ^{3}H (374).

(b) Fructose and triglyceride metabolism

Zakim _et al_. (376) has shown that there is a faster conversion of [^{14}C]fructose into fatty acids by human biopsies than from equimolar amounts of [^{14}C]glucose. In addition, both the incorporation of [^{14}C]-fructose into glyceride-glycerol and CO_2 production are also greater than from [^{14}C]glucose. In contrast to glucose, fructose has also been shown to depress oxidation of fatty acids (377), to increase fatty acid esterification, to increase secretion of VLDL and to increase the rate of VLDL aprprotein synthesis in perfused liver (377,378,380). At high concentrations of fructose (15mM) however, fatty acid synthesis by perfused liver is inhibited. This inhibition may be caused by a decrease in ATP level and the inhibition of acetyl-CoA carboxylase by citrate and acetyl-CoA (379).

The biochemical basis for the unique role of fructose in lipid synthesis is related to at least three different factors:-

(i) Glucose administration to the intact animal results in an increase in the plasma insulin level. Plasma insulin levels are not raised to any great extent in response to fructose administration and as a result lipoprotein lipase is not stimulated and this leads to increase in plasma triglyceride levels (401). Glucose is not as rapidly metabolised as fructose by the liver. An oral load of glucose raises the blood glucose level whereas fructose administration does not (251). A high plasma glucose level favours the removal of plasma triglyceride into the fat cells since insulin increases the activity of lipoprotein lipase and stimulates glucose uptake by fat cells thus providing the glycerol 3-phosphate required for esterification (314,315,401). Nikkila (331) found that the increase in serum triglyceride after a fatty meal can be reduced by glucose.
(ii) The rate of glycolysis of fructose is much faster than that of glucose. This is due to the differences in the metabolic pathways of these sugars (Fig. 2). Fructose is more readily phosphorylated than glucose because of the high levels of fructokinase in the liver compared to glucokinase and hexokinase (216). Unlike fructose metabolism, glucose metabolism is subject to regulation (II.B.1.c) by insulin and glucagon. The concentration of some metabolic intermediates are increased by fructose. Dietary or intravenous injections of fructose can increase the concentration of glucose 6-phosphate, glycerol 3-phosphate, pyruvate, lactate, malate, acetyl-CoA and citrate in the liver (272).

Zakim (272) believes that the effect of fructose on triglyceride synthesis is mediated through an increased synthesis of fatty acids. Dietary fructose increases the level of hepatic acetyl-CoA carboxylase and fatty acid synthetase. Fructose fed rats, when compared with glucose fed rats, have higher 'malic' enzyme, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and citrate cleavage enzyme. Liver slices from rats fed on a stock chow diet can synthesize fatty acid from [^{14}C]fructose at a rate four times greater than from [^{14}C]glucose. In addition, fructose can increase the synthesis of fatty acids from [^{14}C]acetate by liver slices to a greater extent than glucose. However, the administration of glucose _in vivo_ increases the conversion of [^{14}C]acetate to fatty acids by liver slices 100 fold, whereas fructose has only a small stimulating effect. The reason for this apparent discrepancy is not clear (272).

(iii) Fructose administration may effect the availability of substrates for lipid synthesis. When fructose is metabolised dihydroxyacetone phosphate and glyceraldehyde are formed. Glyceraldehyde can be further metabolised to glycerol 3-phosphate either by the triokinase pathway or reduced by alcohol dehydrogenases to glycerol which is subsequently phosphorylated. Although the present evidence available indicates that the triokinase pathway is the predominant pathway in the conversion of glyceraldehyde to glyceraldehyde 3-phosphate, it is possible that under favourable conditions glyceraldehyde may be metabolised by the alcohol dehydrogenase pathway. It is possible that this latter pathway is important in lipid synthesis from fructose.

Some authors claim that fructose administration results in an increase in hepatic glycerol 3-phosphate level (see Table I) whereas glucose administration does not. If the triokinase pathway predominates in the metabolism of D-glyceraldehyde formed by fructose metabolism, then both glucose and fructose metabolism should have the same effect on glycerol 3-phosphate levels. The increase in the level of this metabolite produced by fructose administration may be due simply to an increased conversion of fructose to glycerol 3-phosphate or alternatively as an indirect effect of the depletion of ATP levels by fructose which results in the oxidation of cytoplasmic NADH and a concomitant increase in the level of glycerol 3-phosphate (221,133).

Zakim (272) states that, *in vivo*, there is no correlation between the rates of hepatic esterification of fatty acids and the concentration of glycerol 3-phosphate in the liver. He believes that fructose increases the synthesis of glycerides mainly through an increase in fatty acid synthesis. He explains the observation that the glyceride-glycerol is always more heavily labelled than the fatty acid moieties by stating that there is a greater dilution of label by endogenous intermediates in the synthesis of the fatty acids.

In conclusion, the administration of fructose definitely increases the synthesis of glycerides, predominantly triglycerides, to a greater extent than glucose. The reason for this observation is subject to some controversy. Studies in this field have been complicated by the effects of different diets, species differences, the mode, concentration, and time of administration of fructose and the age, sex and nutritional state of the animal.

(c) Control of triglyceride synthesis in the liver

Hepatic triglyceride synthesis can be controlled by the level of fatty acids in the liver, the pO_2 redox state, hormones, fructose and the uptake of lipoproteins by the liver (347). In addition there are a number of drugs which can influence the synthesis of the triglyceride (343,344,345).

(i) Free fatty acids. Free fatty acids can enhance the synthesis and the secretion of VLDL-triglyceride (347). There appear to be two pools of triglyceride in the liver, A small pool which is located in the smooth endoplasmic reticulum in which newly synthesized fatty acids are incorporated into VLDL-triglyceride and a large pool of cytoplasmic triglyceride (floating fat) which is turned over at a slow rate (306,347, 348). When labelled free fatty acids are taken up by the liver the microsomal pool contains triglyceride of high specific radioactivity compared to the floating fat fraction (347). The function of the large precursor pool appears to be in the storage of the triglyceride which is not exported as VLDL. Hydrolysis of the storage triglyceride and resynthesis in the microsomal pool must occur before secretion of the triglyceride can take place (348) (see Fig. 6).

The output of triglyceride by the liver has been shown to decrease as the degree of unsaturation of C_{18} fatty acid increases but the output increases as the carbon chain length increases. VLDL formed from oleate is larger and contains less cholesterol and phospholipids than the lipoprotein formed from palmitate. Large lipoproteins are cleared from the circulation at a faster rate than smaller lipoproteins and thus the nature of the triglyceride fatty acids may have an effect on the concentration of plasma VLDL-triglyceride (347).

Petit et al. (349) found that, production of VLDL as assessed by $[^{14}C]$leucine and $[^{3}H]$fatty acid incorporation into the VLDL of a liver perfusate, was depressed by glycerol. One possible reason for this observation could be that the depletion of the hepatic pool of free fatty acids by the glycerol 3-phosphate formed from glycerol.

(ii) Effect of pO_2 and redox state of the liver tissue. In anoxia a more reduced intracellular redox state occurs in the liver. This leads to increases in the glycerol 3-phosphate/dihydroxyacetone phosphate, lactate/pyruvate, 3-hydroxybutyrate/acetoacetate and NADH/NAD ratios. Excess NADH will inhibit the formation of acetyl-CoA from pyruvate, catalysed by the enzyme pyruvate dehydrogenase. This will result in a

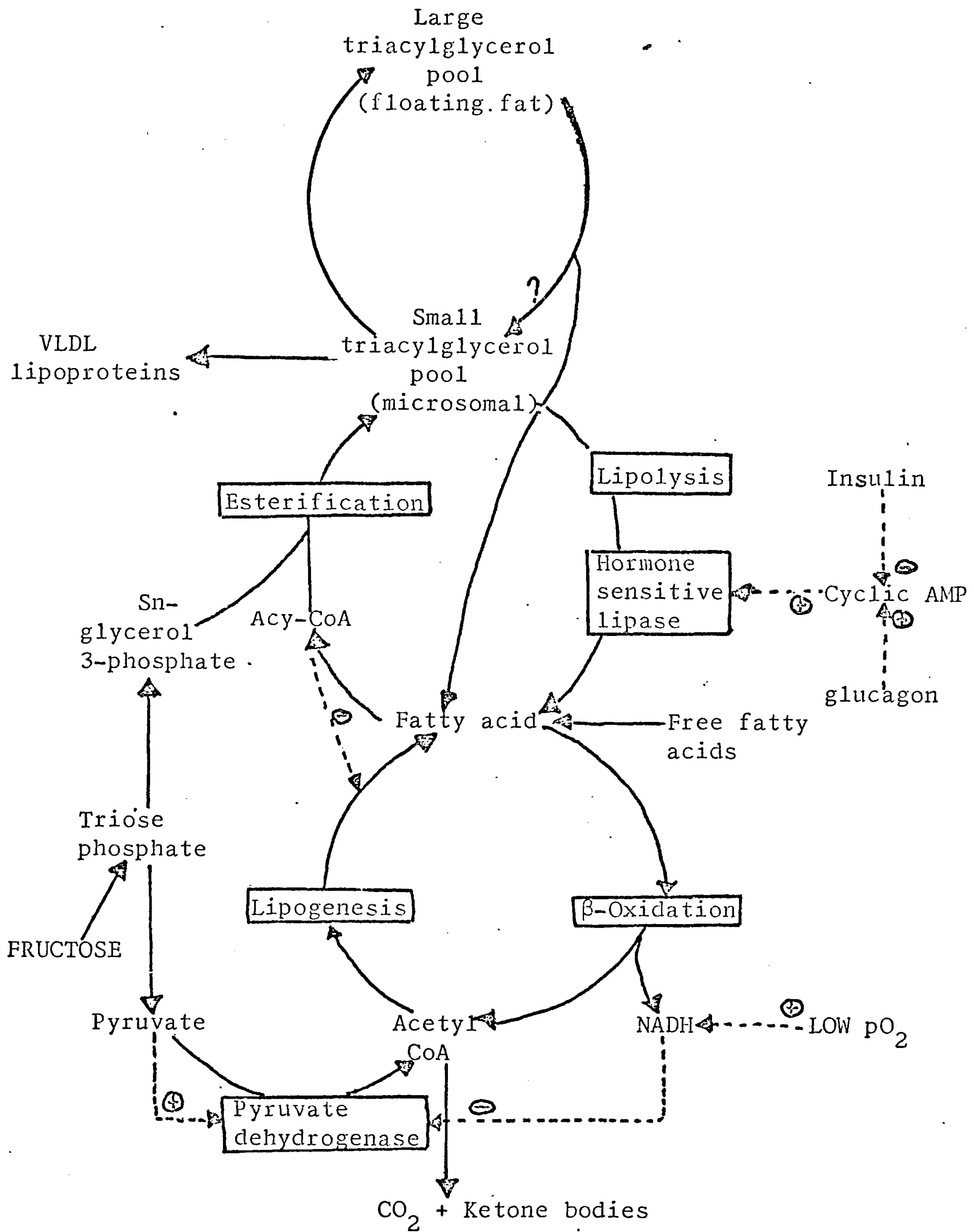


Fig. 6. The effects of free fatty acids, insulin, pO_2 and fructose on the synthesis of triglyceride in the liver. Scheme as proposed by Mayes (347)

decreases in the rate of esterification of fatty acid (347). The availability of both glycerol 3-phosphate and fatty acids appears to be finely controlled by the level of NADH. An increase in the NADH concentration will favour the formation of glycerol 3-phosphate whereas a decrease in NADH will provide more NAD available for the conversion of pyruvate to acetyl—CoA and thus increase the rate of fatty acid synthesis (133,347).

(iii) Effects of hormones on triglyceride secretion. In alloxan diabetes the release of triglyceride by perfused rat liver is inhibited (347). Insulin, glucagon and glucocorticoid can all affect the secretion of VLDL-triglyceride. Perfused livers from female rats have a higher level of triglyceride output than organs from male rats suggesting that sex hormones may be involved in controlling the rate of hepatic triglyceride output. This latter aspect is discussed fully in section II.E.

The effect of insulin on the formation of triglyceride in the liver *in vitro* has variously been reported by authors to be increased, decreased or to remain unchanged. Heimberg *et al.* (308) observed that insulin did not affect the output of triglyceride by perfused rat liver. Salmon and Hems (309) were able to confirm this finding in mice, but these authors found that insulin inhibited the lipolysis of triglyceride in the liver. Penhos *et al.* (310) on the contrary found that insulin reduced the level of triglycerides in the liver perfusate. Alcindor *et al.* (311) showed that insulin did not influence either the uptake or the esterification of $[^3H]$palmitate. Insulin, however, delays the release of newly synthesized triglyceride by intact, fasting rats. In contrast insulin have been shown to raise the rate of secretion of VLDL from oleate in the perfused liver (312). Woodside and Heimberg (313) were able to show that *in vivo* injection of anti-insulin serum causes a fall in the rate of triglyceride secretion. However, perfused liver from rats pretreated with anti-insulin serum did not secrete more triglyceride in the presence of insulin.

Glucagon and dibutyryl cyclic AMP when added to the perfused liver, increased the oxidation of endogenous free fatty acids and decreased the conversion of fatty acid into VLDL-triglycerides. It has been suggested by Topping and Mayes (347) that cyclic AMP activates a hormone sensitive lipase acting on the small precursor pool of VLDL-triglyceride which will decrease the output of triglyceride from the liver, decrease the net esterification and increase the oxidation of fatty acids.

can take up triglycerides after the chylomicrons have been metabolised to the chylomicron 'remnant' particles by extra hepatic tissues.

(d) Development of fatty liver

Abrams and Cooper (352) have proposed that the following mechanisms are involved in the reversible accumulation of hepatic triglyceride: i, increased synthesis of hepatic triglyceride; ii, decreased release of hepatic triglyceride; iii, increased hepatic uptake of unesterified fatty acid from plasma and, iv, hepatic uptake of triglyceride from plasma.

Rats treated with ethionine (353), orotic acid (354), allylisopropylacetamide (355) and ethanol (352) develop fatty liver.

Ethanol has a greater capacity for producing a fatty liver than isocaloric amounts of fat in the diet. Ethanol causes a shift to a more reduced redox state (379) and an increase in the glycerol 3-phosphate concentration with a decrease in the level of long chain acyl-CoA derivatives in the liver (357). This suggests that ethanol stimulates the rate of incorporation of long chain acyl-CoA into triglycerides which leads to a fall in acyl-CoA levels. Ethanol administration can also decrease activities of two key enzymes involved in gluconeogenesis, pyruvate carboxylase and fructose diphosphatase. However, it does not seem likely that this is the major reason for the ethanol induced accumulation of hepatic triglyceride (358). Recently, Abram and Cooper (359) proposed that the effect of ethanol was to increase the hepatic blood flow by 60% and thus to provide more fatty acids for the hepatic esterification of glycerol 3-phosphate to triglyceride.

(e) Triglyceride metabolism in isolated hepatocytes

The metabolism of triglycerides by isolated hepatocytes has many similarities to the metabolism in the intact animal or in perfused liver. The rates of triglyceride cholesterol and VLDL protein synthesis by rat hepatocytes have been studied by Jeejeebhoy et al. (388). The rate of secretion of VLDL protein was comparable to that observed using the isolated perfused liver. However, unlike the perfused liver, most of the hepatocytes were viable, in terms of lipid synthesis, up to 24 hr of incubation.

Hepatocytes, like the perfused liver, cannot take up intact chylomicrons but treatment of this lipoprotein with lipoprotein lipase results in a significant uptake (389). The uptake of the 'remnant particles'

produced can be prevented by colchicine and cycloheximide and this suggest that the uptake of these particles is dependent upon certain cell surface proteins which may be damaged during the isolation procedure (390). Recently, Arnaud and Boyer (391) found that the isolated liver cell when treated with heparin will release lipase into the medium. This must be associated with a change in the plasma membrane since there is a concomitant release of lactate dehydrogenase activity.

Lipid synthesis from acetate by rat hepatocytes is inhibited by human VLDL, LDL and HDL but not by chylomicrons (392). The chain length and degree of unsaturation of free fatty acids can also influence the synthesis of glycerides by isolated liver cells (393).

Insulin increases fatty acid synthesis in rat hepatocytes (394) and in hepatocytes from neonatal chicks (395). Glucagon, on the other hand, inhibits the synthesis of fatty acids in both cases (394,395). Cyclic AMP has been shown to strongly inhibit acetate incorporation into lipids by hepatocytes prepared from chicken livers, but this effect was not observed with cells prepared from rat liver. Dibutyryl cyclic AMP does have some inhibitory effect on this process on chicken hepatocytes but does not have any effect in the rat hepatocytes. It is interesting to note that the major lipid synthesised from acetate by rat hepatocytes is cholesterol whereas in chicken hepatocytes the major lipid is triglyceride (396).

Homcy and Margolis (397) found that dibutyryl cyclic AMP did not affect the rate of palmitate esterification by isolated hepatocytes but the cyclic nucleotide did reduce the rate of oxidation of palmitate and acetate to CO_2 and stimulated palmitate oxidation to soluble products, principally ketone bodies

The effects of monosaccharides and their related metabolic intermediates on lipid synthesis by isolated liver cells have been studied. In hepatocytes prepared from neonatal chicks _de novo_ fatty acid synthesis from [^{14}C]acetate was stimulated by fructose (11mM), glycerol (20mM), dihydroxyacetone (10mM), lactate (10mM) and pyruvate (10mM). The stimulatory effects of fructose and dihydroxyacetone were not observed when $^{3}H_2O$ was used as a substrate for the assay of fatty acid synthesis (395). Clark _et al._ (398), using hepatocytes prepared from livers of _ad libitum_ and meal fed rats, have found that, at concentrations below 10mM, fructose is a better precursor of fatty acids than glucose but the

reverse is true at higher hexose concentrations. There is a linear relationship between glucose or lactate concentrations (up to 100mM) and the rate of synthesis of fatty acid from these precursors. These authors have also found that lactate is a better fatty acid precursor than either glucose or fructose. Fatty acid synthesis is greatly increased in hepatocytes from meal fed rats compared to *ad libitum* fed animals.

The esterification of palmitic acid to glycerides is increased by the addition of fructose (8.3mM) and of glycerol (5mM). Glucose (11.1mM) also increases the rate of esterification but to a lesser extent than either fructose or glycerol (400). Christiansen *et al.* (399) have found that in cells prepared from rats fasted for 24 hr there is increased esterification of [^{14}C]palmitate in the presence of either fructose (7.5mM) or glycerol (5mM) but in hepatocytes prepared from rats fasted for 48 hr and then refed carbohydrate for 48 hr, glycerol and fructose both inhibited fatty acid esterification. However, the oxidation of palmitate was inhibited by these two compounds in both cases.

D. THE EFFECT OF DIET ON LIPOGENESIS FROM CARBOHYDRATE

In humans there are two types of lipaemia, one which is carbohydrate induced and the other induced by fat in the diet (402). A high content of sucrose in the diet has been reported to increase the serum triglyceride in humans (403,404,405,406,407,408,409,410,411) although Keys and his associates were not able to show this (412,413). Several mechanisms have been postulated for the sucrose induced increase in plasma triglyceride; i, increased hepatic synthesis of triglyceride (404,405); ii, increased rate of basal and noradrenaline-stimulated lipolysis in the adipose tissue and a decreased antilipolytic effect of insulin on the same tissue (406); iii, a slow clearance of plasma triglyceride induced by diet due to the insulin response to diet being less than to a diet rich in glucose (403). In rats the substitution of sucrose for starch in the diet results in impaired oral glucose tolerance (414) but in humans this effect is not observed (415).

1. Effect of Dietary Carbohydrate on Tissue Lipid Levels

Miller and Leveille (416) have found that plasma triglyceride levels are elevated in rats, but not in chicks or pigs, fed on diets containing fructose. Bruckdorfer and Yudkin (417) have shown that there is no significant change in plasma cholesterol, triglyceride and phospholipid

in male pigs fed a diet of 50% dry weight of either corn starch or sucrose for 1 year. Fructose or glucose in the diet (10% solution in drinking water) did not alter the serum triglyceride levels in guinea pig. This may be due to the fact that in this species the fructose is converted to glucose in the small intestine before it is absorbed (418). Macdonald (419) has observed that the feeding of sucrose to rabbit causes an increase in the liver lipid levels but a fall in linoleic acid content, when compared to animals on glucose or starch diet.

In adult male rats fed for 26 weeks on diets containing 80% carbohydrate, given as dextrose, fructose, glucose syrup or sucrose, plasma cholesterol levels were significantly increased by fructose and sucrose and to a lesser extent by dextrose. Rats given fructose or sucrose had heavier hearts and livers and the soluble protein content of the liver was significantly lower (420). In a similar experiment, Rozen et al. (421) has confirmed these findings and has also shown that sucrose fed animals show the largest increase in body weight. The levels of triglyceride in the livers and hearts of animals on the sucrose diet are also the highest but fructose in the diet does not have such a marked effect. However, the serum triglyceride level is raised in animals on both these diets. Animals on a glucose diet displayed the lowest values for almost all parameters studied.

The response of serum triglyceride to dietary fructose has been reported to be age dependent. When diets containing 70% carbohydrates in the form of glucose, starch, sucrose or fructose are fed to weanling and mature rats for 3 weeks, serum triglyceride levels in the mature animals on sucrose or on fructose diets are highest (423).

Macdonald (409,419) and Casal and Holman (424) have reported various changes in the triglyceride fatty acid composition in response to dietary carbohydrate. Recently, Macdonald et al. (425) have shown that the triglyceride and cholesterol levels in the liver and serum of rats returned to normal levels 4 weeks after the withdrawal of a high sucrose or a high fructose diet but the fatty acid profiles of liver triglyceride and adipose lipid tended to take a longer time to return to normal.

Laute et al. (426) found that rats fed on sucrose, or a mixture of an equivalent amount of glucose and fructose, showed much greater increases in body fat, liver lipid, serum triglycerides and serum free fatty acid levels compared to rats on a high starch diet. There is no

significant difference between rats fed sucrose diet and those fed glucose/fructose diets suggesting that there is not a specific effect of sucrose on lipid metabolism but rather an effect of the component monosaccharides. However, the values quoted for the serum triglyceride levels in this study are extremely high.

Birch *et al.* (427) found that cholesterol, triglyceride and free fatty acids in the liver and the serum are elevated by the high molecular weight fraction of glucose syrup, this fraction is more effective than sucrose in rising serum triglyceride levels.

Dietary sucrose can also induce hypercholesterolaemia but this effect is dependent on the presence of fat in the diet (428,429).

2. Effect of Dietary Carbohydrate on Hepatic Lipogenesis

Zakim *et al.* (275) state that the incorporation of [^{14}C]fructose into fatty acids by liver slices is always greater than the incorporation of [^{14}C]glucose. In a short term (48 hr) feeding experiment they found that fructose (70%) feeding results in an increased incorporation of both fructose and glucose into fatty acids. Tuovinen and Bender (431) have fed rats on starch (60% w/w), fructose (60% w/w), and carbohydrate free diets for 4 and 12 weeks and shown that lipogenesis from [^{14}C]-glucose by liver slices is reduced by both the fructose diet and the carbohydrate free diet. Chevalier *et al.* (437) observed that fatty acid synthesis from glucose is more rapid than that from fructose in liver slices from both glucose and fructose fed animals. Romson and Leveille (440) found that more fructose than glucose is incorporated into fatty acid at a substrate concentration of 10mM but at higher concentrations the latter is incorporated at a faster rate. This may explain some of the discrepancies in the results obtained from *in vitro* studies of hepatic fatty acid synthesis on rats fed carbohydrate enriched diets.

Fatty acid synthesis from [^{14}C]acetate by liver slices is increased by feeding a high carbohydrate diet to rats for 21 days (275). Mukherjee *et al.* (432) found that fatty acid synthesis by liver slices was increased by feeding glucose or fructose supplemented high starch diets. Bar-on and Stein (418) and Cohen and Teitelbaum (433) found that when rats were fed 10% (w/w) and 72% (w/w) carbohydrate enriched diets respectively there was no difference in lipogenesis from acetate between fructose and glucose fed animals. However, fructose feeding did not enhance the esterification of oleic acid by liver slices from rats fed a 10% carbohydrate supplemented diet (418) in contrast to the results of Mukherjee (432).

Fallon and Kemp (279) could not find any differences between the incorporation of glycerol 3-phosphate into triglycerides by liver microsomes in rats fed on either a high sucrose or a high glucose diet. Later this group showed that the rate of liver triglyceride synthesis in fructose and glucose fed rats depends on the length of time that the animals are fed on a high carbohydrate diet. A high glucose diet results in a three to four fold increase in hepatic triglyceride formation but then declined to the control value after 11 days on this diet. The high fructose diet also increases triglyceride formation by liver homogenates but the total increase is greater (five to ten fold) and is not maximal until 11 days on this diet (274).

3. The Effect of Dietary Carbohydrate on Enzyme Activities.

In a comprehensive review (435) on the effect of diet on the activity of the enzymes involved in fatty acid and cholesterol synthesis it has been reported that the activities of glucokinase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphofructokinase, fructose 1-phosphate aldolase, pyruvate kinase, pyruvate dehydrogenase, citrate cleavage enzyme, 'malic' enzyme, acetyl-CoA carboxylase and fatty acid synthetase can be raised by a high carbohydrate diet. Walker (436) has shown that glucokinase is an adaptive enzyme and is elevated by a high glucose diet. The increase in enzyme activity is greatest when glucose, rather than fructose, galactose or mannose, is refed to fasted animals (435).

The activity of pentose-phosphate pathway enzymes and the rate of fatty acid synthesis in rat adipose tissue are closely related (435). Fructose or sucrose feeding has been shown to increase the activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (432,437,438,439).

Phosphofructokinase, fructose 1-phosphate aldolase, fructose 1,6-phosphate aldolase (432,435), triokinase (437) and fructokinase (441, 437) have also been reported to be raised by a carbohydrate rich diet and in general a fructose diet caused the greatest increase in enzyme activities. However, Zakim *et al.* (273) found that the activity of fructokinase, although significantly raised by a carbohydrate rich diet, is increased to a greater extent by glucose than by fructose.

Middleton and Walker (442) were able to characterize two forms of pyruvate kinase in rat liver. The L-form is stimulated by fructose 1,6-phosphate and is increased two fold in response to either a high fructose

or a high glycerol diet (443).

High carbohydrate diets which include fructose have also been shown to increase the activities of gluconeogenic enzymes, glucose 6-phosphatase (431,444,438), fructose 1,6-diphosphatase (445) and to slightly decrease the activity of phosphoenolpyruvate carboxylase (444).

Generally, fatty acid synthetase (437,447,448), pyruvate dehydrogenase (435), 'malic' enzyme (444,435,437) and citrate cleavage enzyme (435,437) and acetyl-CoA carboxylase (435,444,273) activities are elevated to a greater extent by diets rich in fructose or sucrose.

A high fat diet on the other hand can depress the activities of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and 'malic' enzyme and offset the action of fructose in diet (439). Glycerol 3-phosphate dehydrogenase has been reported to be increased by dietary fructose or sucrose (432,439) and this increase has been correlated to a decrease in glycerol 3-phosphate levels in the liver (432).

Bruckdorfer, Khan, and Yudkin (447,448) and Chevalier and Leveille (437) found that some of the enzyme activities in the liver and in the adipose tissues are inversely related. Fatty acid synthetase in the liver is stimulated by a diet high in sucrose or fructose but these activities were depressed in the adipose tissues. However, starch, maltose and glucose did not stimulate the hepatic enzyme to the same extent but the enzyme from adipose tissue was activated (447,448). Similarly, Chevalier and Leveille (437) have found that fructose feeding raised the hepatic 'malic' enzyme and citrate cleavage enzyme levels but reduces the activities of these two enzymes and also that of hexokinase in the adipose tissue.

Exercise can affect the serum triglyceride or lipoprotein levels (449,450). Winder _et al._ (451) found that the activity of the liver lipogenic enzymes which are raised by fructose feeding can be lowered by exercise.

Chang _et al._ (438) found that differences in the ages and strains of animals did not have any effect on the increase in glucose 6-phosphatase, glucose 6-phosphate dehydrogenase and fructose 1,6-diphosphate aldolase activities which occur following sucrose feeding. However, Bartley _et al._ (445) found strain differences in Wistar rats with respect to dietary activation of lipogenic enzymes. A high sucrose

diet supplemented with corn oil fed to one strain of Wistar rats (obtained from Tucks and Sons) led to smaller increases in the activities of hepatic pyruvate kinase, glucose 6-phosphate dehydrogenase and fructose 1,6-diphosphatase than in another strain of Wistar rats (obtained from Shell Research Ltd.). Omission of corn oil in the diet led to a 3 fold increase in the activity of glucose 6-phosphate dehydrogenase in rats obtained from the former breeder whereas this was not observed in the latter strain.

Finally, Fallon et al. (279,446,452) have shown that diets rich in fructose or glucose increase the activities of glycerol 3-phosphate acyltransferase and phosphatidate phosphohydrolase and also increase the rate of esterification of dihydroxyacetone phosphate in the presence of NADH.

In conclusion, fructose or sucrose in the diet has been shown by most authors to raise the serum triglyceride levels but the exact mechanism of this increase is not known but many control enzymes have been implicated.

E. SEX RELATED DIFFERENCES AND THE EFFECT OF SEX STEROIDS ON CARBOHYDRATE AND LIPID METABOLISM

In Section I.D. sex differences in the development of atherosclerosis were discussed. Men have a higher serum triglyceride level than pre-menopausal women, and this may be related to the greater incidence of atherosclerosis in males (47).

1. Carbohydrate Metabolism

Sex differences in the carbohydrate metabolizing enzymes have been reported. Fathipour and Pridham (453) have shown that male rat liver has a higher glycerol kinase activity than the female liver and when castrated males are treated with testosterone, there is a marked rise in the activity of the enzyme. These workers could not find any sex difference in the activity of either NADP or NAD-dependent glycerol dehydrogenase.

The activities of fructokinase and fructose 1-phosphate aldolase in the adult male rat liver have been reported to be greater than in the female rat liver (454). However, Pridham and Mahmoud (506) and Pridham and Pollard (507) have not been able to find a sex difference in the level of fructokinase. Walker (455) also found that gravid female and male rat livers had the same level of fructokinase. The latter author did not find any sex differences in the levels of either

glucokinase (456,457) or hexokinase (456). Stifel *et al.* (460) have found sex differences (♀>♂) in the activities of phosphofructokinase and pyruvate kinase in rat jejunum and the activities of several of the enzymes of glycolysis and gluconeogenesis are changed in response to the administration of sex hormones. Oral administration of either testosterone to male rats or of β-oestradiol to female rats increased the pyruvate kinase and phosphofructokinase levels, decreased fructose diphosphatase levels, but had no effect on fructose 1,6-diphosphate aldolase or hexokinase activities (460,461). The stimulation of pyruvate kinase is due to increased synthesis of the enzyme (463). Intramuscular administration of sex hormones produced no demonstratable effect on the activities of the jejunum carbohydrate-metabolizing enzymes in either male or female rats (462).

Kerr and Frankel(466) have found that the levels of glutamate and malate dehydrogenases and aspartate and malate levels are consistently higher in the female rat liver than in males between the ages of 3 and 24 months. No consistent sex differences were obtained in the levels of NAD, NADH, NADP, citrate, glutamate or α-ketoglutamate or in the activity of lactate dehydrogenase. However, a sex difference was observed in the level of NADH, female liver had a higher level of this coenzyme compared to male liver in rats aged between 12 and 18 months.

Macdonald (467) has found variations in glucose tolerance of women at different times during the menstrual cycle. Selmi *et al.* (468) have shown that the plasma glucose concentration is lower in premenopausal women than in men following a 48 hr fast period. In postmenopausal women, no such sex difference is observed.

2. Plasma and Hepatic Lipid Levels

Lunell (469) has observed that oral contraceptives increased serum triglyceride levels but had no effect on glucose, free fatty acids, glycerol or D-β-hydroxybutyrate. Serum glycerol levels have been shown to be higher in women than in men but no changes in serum glucose, glycerol or cholesterol levels have been observed during the menstrual cycle (470).

Curtis-Prior *et al.* (470) have shown an increase in serum cholesterol in women using hormonal contraception. Oliver and Boyd (471) have reported changes in plasma cholesterol and phospholipid ratios during the menstrual cycle.

There is no doubt that men have a higher serum triglyceride level than women (see I.D.). Macdonald and Nowakowska (472) found that a high sucrose diet will raise the serum triglyceride and cholesterol levels in men but not in premenopausal women. More recently, Poggi et al. (473) have observed that male patients with myocardial infarction had a higher mean cholesterol, triglyceride and total lipid than controls but surprisingly the mean plasma testosterone is significantly lower in myocardial infarction patients.

Coltart and Macdonald (474) found that sucrose feeding to male and female baboons increases the incorporation of [^{14}C]sucrose into serum triglyceride in both sexes but only in the males is there an increase in the fasting serum triglyceride levels. Oestrogen given parenterally to males reduces the serum fasting triglyceride levels but does not affect the incorporation of sucrose into serum triglyceride. Testosterone given to female baboons does not affect either the incorporation of sucrose into triglyceride or the fasting serum triglyceride concentration. These authors suggest that the sex differences in the pattern of serum triglyceride in the baboon is due to the fact that oestrogen may enhance the removal of triglyceride from the plasma of the female animal. Macdonald (467) has shown that sex differences in the baboon in the conversion of fructose to serum triglyceride is not due to differences in the absorption of the hexose.

Hill (475) has shown that in rats fed diets enriched with 10% fructose for 10 days, an increase in the serum triglyceride occurs in mature male and female animals but not in young females. Bruckdorfer, Kang and Yudkin (477) have found that plasma triglyceride levels are higher in male rats than in female rats but there are no sex differences in plasma cholesterol levels or in the activities of either hepatic fatty acid synthetase or lipoprotein lipase from adipose tissue. In both male and female rats, sucrose feeding produces an increase in plasma triglyceride level and increases in the activity of fatty acid synthetase and a decrease in lipoprotein lipase activity. According to these authors, the results indicate that the lower plasma triglyceride levels in females cannot be fully explained by differences in the levels of the enzymes studied.

Takemoto (476) found that feeding rats for 30 days on fat-free diets containing 77% (w/w) carbohydrate can cause changes in liver and serum lipid levels. He found that in male rats fed a high fructose diet

there was a high serum lipid level but a low hepatic lipid content in comparison with animals fed on other high carbohydrate diets. A starch diet fed to male rats resulted in a high hepatic lipid content but the serum lipid level was not markedly changed. In the female rats, the diet rich in sucrose gave the highest serum lipid level whereas the hepatic lipid content was increased most by a commercial diet.

However, Tuovinen and Bender (478) have shown that the liver lipid levels of both male and female rats increase as the levels of dietary sucrose increases. Most of the increase in hepatic triglyceride can be accounted for by changes in the triglyceride content.

Morton and Homer (479) have found that in rats fed on a high sucrose diet supplemented with linoleic acid female rat livers have a higher level of liver triglyceride than livers from male rats.

Jefferys and White (480) have suggested that oestradiol and progesterone appear to have a synergistic action on lipid metabolism. Progesterone or oestradiol administration to male rats decreases triglyceride levels in liver but has no effect on the serum triglyceride concentration. However, when these hormones are administered together to rats fed on a high sucrose diet the liver triglyceride levels are raised. Groener and Macdonald (481,467) have also shown that when oestradiol and progesterone are administered to female rats on a fructose diet the hepatic triglyceride level is raised. Fasting serum triglyceride concentrations have been shown by some authors to be increased following oestrogen administration (467) and also in pregnant rats (482). Overproduction of triglyceride, a decreased clearance rate or a combination of both factors can affect the level of serum triglyceride. Knoop _et al_. (482) found that the hypertriglyceridaemia which occurs in pregnancy is largely due to overproduction of the lipid.

Heimberg and his co-workers (433) and Weinstein _et al_. (434) have shown that the output of triglyceride by isolated perfused liver from female rats exceeds that from livers obtained from male rats. Ovariectomy reduces this secretion but it can be restored by the administration of oestrogen to the ovariectomised animal (483). Orchidectomy has no effect upon the triglyceride output from livers of male animals but this surgical treatment abolishes the inhibitory effect of glucagon on triglyceride secretion. Weinstein _et al_. (484) found that glucagon at 10^{-6}, inhibits hepatic triglyceride secretion to a greater extent in

the male than in the female rat. Recently, Heimberg's group (485, 486) have found that the female perfused rat liver secretes more VLDL-triglyceride than the male perfused rat liver and that the VLDL produced by female livers are larger and contain fewer moles of phospholipid and sterol per mole triglyceride than VLDL from male livers. They postulate that uptake (aided by lipoprotein lipase) and metabolism of VLDL-triglyceride by extrahepatic tissues is also more rapid in the female rat than in the male (485,486). They have also shown that the female rat liver takes up and esterifies fatty acid to triglyceride at a more rapid rate than male liver. There is a slower rate of fatty acid oxidation in the female rat liver. Therefore, when provided with equal quantities of free fatty acid, the female will secrete and synthesize more triglyceride than the male (487). Oestrogen has also been shown to induce hypertriglyceridaemia in chickens which is brought about by the overproduction of triglyceride (488). Recently, Afolabi et al. (489) found that oestrogen administration to the intact rats increases plasma triglyceride levels and also enhances hepatic acetyl-CoA carboxylase and fatty acid synthetase activities. Young (490) has found that oestradiol administration decreases diglyceride acyltransferase in both castrated and non-castrated male rats whereas testosterone administration increases the activity of the enzyme.

Hosotani and Yoshida (491) have shown that the accumulation of fat in the liver, induced by a protein deficient diet, was significantly greater in female rats than in male rats. Ethionine treatment leads to the accumulation of fat in the liver only in female rats, and administration of testosterone propionate to females stimulates the resistance to the ethionine-induced development of fatty liver (458).

Cycloheximide treatment leads to an accumulation of liver triglyceride in rats. This accumulation is more pronounced in livers of female rats compared to males (492). Stein and co-workers (493) found that colchicine, which inhibits the release of proteins by the liver into the plasma, also reduces the secretion of triglyceride. However, the effects of colchicine on serum triglyceride levels are similar in male and female rats and they postulate that the secretion of triglyceride, via vesicular transport, is essentially the same for both sexes.

In brief, the evidence given shows that the female liver has a greater capacity to synthesize triglyceride and oestrogen stimulates

the secretion and synthesis of triglyceride. However, males have a higherserum triglyceride level than females. In order to account for a lower level of serum triglyceride in the female animals, there must be a faster removal of triglyceride from the plasma of female animals than from male plasma.

3. Plasma Triglyceride Clearance

Glueck and Fallat (494) found that oestrogen induces elevated human plasma triglyceride levels either by increasing the rate of hepatic triglyceride synthesis or through the suppression of post-heparin lipolytic activity (PHLA). In general, progestational and anabolic-androgenic agents lower plasma lipid levels. In cases where progestational compounds lower plasma triglyceride a simultaneous increase in PHLA is observed (494). More recently, Glueck et al. (495) have shown that both the extrahepatic and the hepatic PHLA in women are depressed by oestrogen. This can be correlated to an increase in serum triglyceride levels. Oxandrolone, an anabolic-androgenic steroid decreases the serum triglyceride levels in some patients with familial hypertriglyceridaemia and in some female patients both the extrahepatic PHLA and hepatic PHLA are increased by this steroid. In male patients there are cases where the treatment by this steroid increases the activity of hepatic PHLA but there is no effect on extrahepatic PHLA. Huttunen et al. (496) have recently used an immunochemical method to distinguish between extrahepatic and hepatic PHLA and they have shown that the former enzyme is significantly higher in normal women than in men whereas the converse is true for the latter enzyme. The activity of the extrahepatic PHLA decreases with age in both men and women and a highly significant negative correlation exists between the activity of this enzyme and the serum triglyceride levels.

Kim and Kalkhoff (497) found that oestradiol decreases and progesterone increases both hepatic and extrahepatic PHLA in rats. However, there is no correlation between PHLA activity and plasma triglyceride levels. The plasma triglyceride level is significantly correlated to both the hepatic synthesis and the release of this lipid by the liver.

4. Plasma and Hepatic Cholesterol Levels

There are sex differences in the liver and blood cholesterol levels of the rat. However, according to the review by Jungmam and Schweppe (498) "the observed experimental results are often contradictory and

no clear unifying concept of the hormonal regulation of sterol metabolism has emerged".

Generally, the free and esterified cholesterol levels in female livers are lower than in males although the female rat has a higher rate of cholesterol biosynthesis. Oestrogen administration increases the free and esterified cholesterol levels in the liver (498).

The concentration of cholesterol in the plasma of female rats has generally been reported greater than in the male (483). A similar sex difference has recently been reported for chickens (499). However Boyd (500) has shown that human and rat ovariectomy results in an increase in the plasma cholesterol concentration. The administration of oestrogens to women results in lowering of plasma cholesterol levels. Oestrogen administration increases the liver cholesterol and decreases serum cholesterol levels in male rats (501). Uchida et al. (502,503) have shown that when male rats are injected with oestrogen for 3 days there is a decrease in the serum cholesterol levels, a decrease in the rate of hepatic cholesterol synthesis and an increase in hepatic cholesterol levels. Rats treated for 3 weeks with oestrogen showed an increase in the serum cholesterol, an increase in the rate of hepatic cholesterol synthesis and the liver cholesterol level was normal. Macdonald (467) has also shown that the fall in serum cholesterol levels which occurs in women on high glucose diet can be reversed by administration of oral contraceptives.(467).

Recently, Cole and Margolis (504) found that there was no difference in the basal rate of lipid synthesis from [^{14}C]acetate by hepatocytes isolated from male and female rats. Lipid biosynthesis was however stimulated to a greater extent by testosterone, progesterone, diethylstibesterol, and 17-β-estradiol in cells prepared from female rat livers when compared to those from male rats. According to these authors, these rapid responses to sex hormones suggest that there are sex differences in the enzymes involved in the pathway of lipid metabolism.

The effect of sex hormones on lipid and carbohydrate metabolism is further complicated by the observation that plasma testosterone levels in the adult male rat can fluctuate between 2 to 5 fold, in samples collected from the same animal at different times, and variations of up to 30 fold have been observed between animals (505).

RESULTS AND DISCUSSION

III LIPOGENESIS FROM CARBOHYDRATES IN RATS

Sucrose and fructose in the diet of humans can increase the plasma triglyceride levels and this increase is more significant in men than in premenopausal women (see sections I.D. and II.E.). This thesis reports a study on the possible sex differences in the metabolism of lipids in animals fed on diets enriched with glucose or sucrose. The rat has been used as an experimental animal in this study because it has been shown that male rats fed a normal diet supplemented with either fructose or sucrose showed raised liver and serum triglyceride levels in comparison with animals fed on glucose or starch supplemented diets (see II.D.1).

There have been reports indicating that mature male rats had higher serum triglyceride levels than females and that a diet rich in fructose or sucrose produces a hypertriglyceridaemic response in both sexes (418,475,476,477,478). It is evident that the composition of the diet, the length of the feeding period and the age and strain of the animal can influence the level of serum triglyceride and the rate of synthesis of the lipid (see II.D).

A. LONG TERM FEEDING EXPERIMENTS

In this study the levels of fructose and glucose metabolites in the livers of male and female rats fed on a normal diet were examined and the results compared to similar studies on animals fed on diets enriched with glucose or sucrose. Metabolite levels in the heart, adipose tissue, small intestine and kidneys were also studied. An attempt was made to relate these results to the lipid levels in the liver, blood and heart in rats fed under identical conditions. The lipid levels in the heart were measured because this organ is involved in blood circulation and may be implicated in atherosclerosis. The gross effect of the diets were examined by measuring the weight gain of various organs and the overall body weight changes.

1. Effect of Diet on Body and Organ Weights

The effect of diet on the growth of the animals is shown in Table II.a. The results indicate that the body weights and the weights of organs are generally higher in male animals than in females fed on a commercial diet (Dixon CDD[R]). Nevertheless, these sex differences were not as evident in rats fed on another commercial diet (Dixon .86). However, the animals fed on the latter diet were bred in our own laboratory whereas those on Dixon CDD[R] diet were obtained from a

commercial source. A statistical examination of the data shows that there are significant differences between male animals fed on CDD[R] supplemented with glucose and male animals fed on CDD[R] supplemented with sucrose. The mean weight of the liver, small intestine, heart and kidneys of the latter group of animals were consistently higher than in rats on other diets. A similar trend was observed in female animals fed on the various diets although the differences were less marked. Rozen et al. (421) has recently reported similar results in male rats fed high fructose or high sucrose diets (72% w/w) for 7 months.

Sex differences in the deposition of fat in the peritoneal cavity were not evident when the mean weight of the adipose tissue is considered but there was a considerably larger ratio of adipose tissue to body weight in the females on all diets (Table II.b).

There is no doubt that the diet supplemented with sucrose had a more pronounced effect on gross changes in the animals compared to the other diets.

2. The Effect of Diet on Metabolite Levels

In this study some of the metabolites involved in the metabolism of glucose and fructose and in the synthesis of triglycerides were assayed. These metabolites were fructose 1-phosphate (F1P), fructose 1,6-phosphate (F16P), Glyceraldehyde 3-phosphate (G3P), dihydroxyacetone phosphate (DHAP), glycerol 3-phosphate (αGP), glycerol, lactate and pyruvate. Evidence has already been presented for the importance of these metabolites in the regulation of carbohydrate metabolism and lipid synthesis (see II.B.2 and II.C.3).

(a) Problems in the analysis of tissue metabolite levels

Before starting the analysis of carbohydrate metabolites in the liver some care was taken to check each procedure and to determine whether standard amounts of metabolites added to liver preparation could be accurately accounted for. Recoveries of between 90 and 100% were obtained for all the metabolites studied. Linear relationships between absorbance and concentrations were found for each metabolite studied. Abnormally high values of glycerol were found initially in liver extracts but this was traced to an impurity in the hydrazine hydrate reagent supplied by Koch-Light.

The use of a stainless steel container as a mortar, as recommended by Zakim (357) was found to be unsatisfactory. When liver tissue was frozen in nitrogen, powdered in a steel mortar and extracted with

TABLE IIa. The effect of dietary carbohydrate on the weights of rats and of various organs

Tissue / Diet	Body weights (g)	Liver weight (g)	Heart weight (g)	Weight of small intestine (g)	Weight of adipose tissue (g)	Weight of kidneys (g)
♂ Dixon .86 (n=8)	331 (± 35)	10.7 (± 1.8)	1.23 (±0.19)	7.2 (± 1.1)	3.5 (± 2.6)	2.55 (± 0.32)
♀ Dixon .86 (n=8)	305 (± 40)	8.4 (± 3.7)	1.22 (±0.16)	8.0 (± 1.1)	5.9 (± 5.1)	2.40 (± 0.23)
♂ Dixon CDD[R] (n=8)	418 (± 40)	11.9 (± 2.4)	1.43 (±0.34)	6.0 (± 0.9)	12.8 (± 5.4)	2.71 (± 0.35)
♀ Dixon CDD[R] (n=9)	287 (± 29)	8.8 (± 1.2)	0.97 (±0.35)	5.1 (± 0.7)	14.9 (± 4.9)	1.82 (± 0.19)
♂ Dixon CDD[R] + sucrose (n=25) ⍀	451 (± 55)	15.6 (± 2.4)	1.48 (±0.22)	9.3 (± 1.5)	14.5 (± 7.1)	3.07 (± 0.57)
♀ Dixon CDD[R] + sucrose (n=25) ⍀	302 (± 35)	10.5 (± 1.0)	1.04 (±0.21)	7.7 (± 1.1)	12.0 (± 4.5)	2.01 (± 0.25)
♂ Dixon CDD[R] + glucose (n=15) ⍀⍀	421 (± 33)	12.3 (± 1.5)	1.33 (±0.24)	8.0 (± 1.4)	11.0 (± 2.6)	2.73 (± 0.36)
♀ Dixon CDD[R] + glucose (n=15) ⍀⍀	265 (± 20)	8.9 (± 1.0)	1.02 (±0.08)	6.5 (± 0.9)	8.9 (± 2.8)	1.83 (± 0.20)

Rats (initial wt 50g) were fed ad lib for 110 days on Dixon .86 diet, CDD[R]diet, CDD[R] supplemented with 5% glucose in the drinking water or CDD[R]supplemented with 5% sucrose in the drinking water. After this time the rats were weighed and then killed and the wet wt of various organs determined. Rats fed on Dixon.86 were bred in our laboratories. The other animals were obtained from a commercial source. ⍀ Represents the results from 4 separate experiments; ⍀⍀ represents the results from 3 separate experiments - refer to appendices 1a and 1b for detailed results of individual experiments. Body weight: ♂ CDD[R] + sucrose vs ♂ CDD[R] ($0.1 > P > 0.05$, t-test)
♂ CDD[R] + glucose vs ♂ CDD[R] + sucrose ($P < 0.05$, t-test)

TABLE IIb. Effect of dietary carbohydrate on the organs of rats - expressed as % body weight

Tissue (% body weight) / Diet	Livers	Hearts	Intestines (small)	Peritoneal adipose tissue	Kidneys
♂ Dixon .86	3.23	.383	2.18	1.06	0.770
♀ Dixon .86	2.75	.400	2.62	1.93	0.736
♂ Dixon CDD[R]	2.85	.342	1.44	3.06	0.648
♀ Dixon CDD[R]	3.07	.338	1.78	5.19	0.634
♂ Dixon CDD [R] + sucrose †	3.46	.328	2.06	3.21	0.681
♀ Dixon CDD [R] + sucrose †	3.47	.345	2.54	3.98	0.666
♂ Dixon CDD [R] + glucose ††	2.93	.316	1.91	2.62	0.647
♀ Dixon CDD [R] + glucose ††	3.38	.385	2.47	3.36	0.691

Rats (initial weight 50g) were fed ad lib for 110 days on Dixon .86 diet, CDD[R] diet, CDD[R] supplemented with 5% glucose in the drinking water or CDD[R] supplemented with 5% sucrose in the drinking water. After this time the rats were weighed and then killed and the various organs determined. Rats fed on Dixon .86 were bred in our laboratories. The other animals were obtained from a commercial source.

† Represents the results from 4 separate experiments.
†† Represents the results from 3 seperate experiments.

perchloric acid a contaminant was produced which affected the determination of αGP. However, treatment of the extract with EDTA followed by the addition of $MgCl_2$ resulted in the detection of higher levels of αGP. For example, the values obtained for two untreated liver extracts were 576 and 460 nmoles/g liver. This level increased to 3080 and 2330 respectively after the addition of EDTA and $MgCl_2$ to the extract. Later this anomaly was traced to the effect of ferrous ions which can react with the hydrazine hydrate. Fe^{2+} (250μM) can cause a 50% error in the determination of αGP by this method (Fig. 7). The effects of a number of different ions on the assay were examined and it was found that only $FeCl_2$ and $FeSO_4$ reacted with hydrazine hydrate with a resultant change in the optical density at 340nm whereas $CaCl_2$ and $MgSO_4$ had no such effect. Fe^{3+} did not interfere with the determination of αGP by this method. From these studies it was concluded that the use of a steel mortar in the preparation of a tissue extract can affect the results of αGP determinations. Therefore, in all subsequent experiments a porcelain mortar was used for metabolite extraction.

In the initial stages of this study, rats were killed by cervical fracture and the liver removed before the extraction of metabolites. The freeze-clamping technique was not used at this stage. Table IIIa shows the metabolite levels in the animals fed on a normal Dixon .86 diet.

These results indicated that rats fed a Dixon .86 diet had very high levels of αGP and glycerol in the liver. No apparent sex or age differences were observed in this preliminary study.

The effects of supplementation of the CDD[R] diet of young rats with sucrose and glucose for six weeks on the hepatic metabolite levels were examined. These rats were killed after the administration of pentobarbitone, the liver removed and immersed in liquid nitrogen. Table IIIb shows the values obtained.

From the Table IIIb it can be seen that there may have been differences in the αGP levels in male and female rats fed on Dixon CDD[R] diet supplemented with carbohydrates. However, the values of αGP and glycerol reported in Table IIIa and b are very much higher than reported values (242) except those obtained by Nikkila (239). It has been demonstrated that ischaemia can affect the levels of metabolites in various tissues (see II.B.3.a). Fig. 8 and Table IIIc show the effect of anoxia on αGP and glycerol levels in the livers of female rats. A freeze clamping

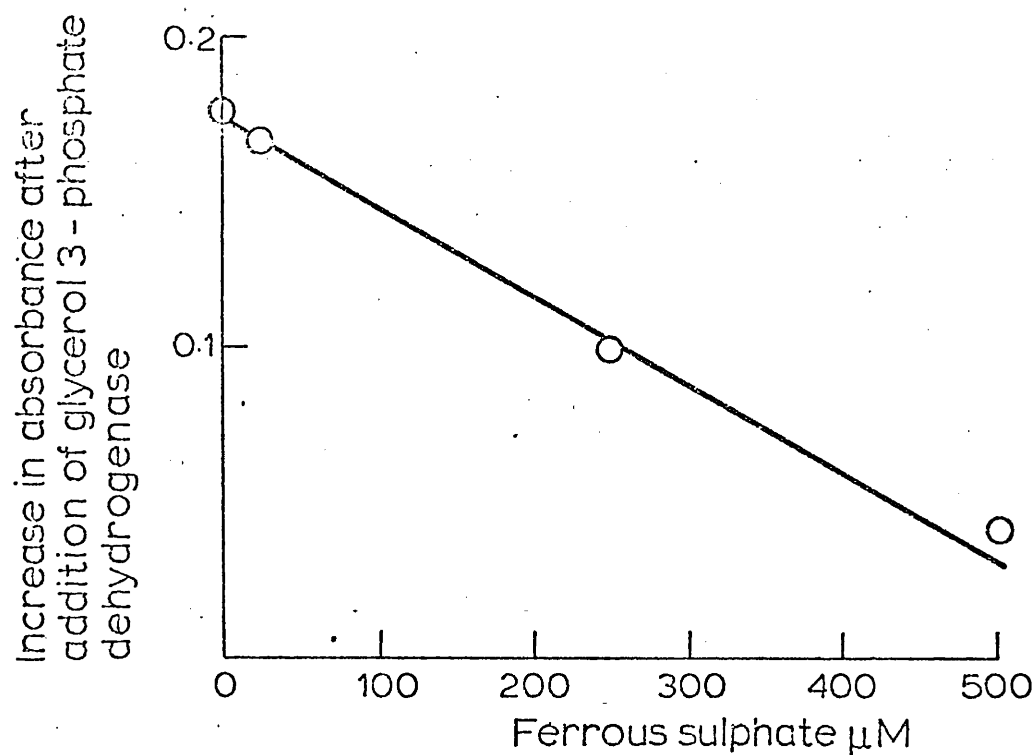


Fig. 7. Effect of ferrous sulphate on the enzymic estimation of glycerol 3-phosphate.

For assay conditions refer to Materials and Methods (Section IV.B.1).

The assay medium (2.02ml) contained 58.4nmoles of glycerol 3-phosphate.

TABLE IIIa. Hepatic metabolite levels[✓] in rats fed on normal diets

		Metabolite levels nmoles/g liver			
	Glycerol	αGP	F16P	DHAP	G3P
Adult ♂ rats	1. NOT DETERMINED	1580	33	43	11
	2. 105.	2470	87	112	4
Adult ♀ rat	5	1530	30	60	- (ND)
Young ♂ rat	158	1780	58	78	72
Young ♀ rat	131	1780	36	56	108

Rats were fed *ad libitum* on Dixon .86 diet. The details of the extraction and assay of the metabolites are given in Section IV.B.1.a. In this case the liver was not freeze-clamped before extraction.

[✓]Metabolite levels are expressed in nmoles/g liver (wet weight)

TABLE IIIb. Level of hepatic metabolites in rats fed diets supplemented with sucrose and glucose

	Metabolites (nmole/g liver)				
Animal	αGP	Glycerol	F16P	DHAP	G3P
♂ rat on sucrose	2890	1180	57	72	36
♂ rat on sucrose	3080	565	84	107	12
♂ rat on glucose	2100	368	130	41	9
♂ rat on glucose	3140	1000	26	12	ND
♀ rat on sucrose	1970	1270	51	60	48
♀ rat on sucrose	2330	1100	54	107	17
♀ rat on glucose	1780	210	50	50	ND
♀ rat on glucose	1760	262	35	18	ND

Rats were fed on CDD[R] diet supplemented with either 5% glucose or 5% sucrose in the drinking water. The animals were killed by bleeding, after the administration of pentobarbitone. The livers were quickly removed and immersed in liquid N_2. Metabolites were extracted and assayed as described in Section IV.B.1.a.

TABLE IIIc. The effect of ischaemia on liver metabolite levels

Time after death (sec)	Metabolites (nmole/g)				
	αGP	Glycerol	DHAP	G3P	F16P
20	374	19	27	10	29
60	560	32	34	8	31
120	1214	58	46	12	34
300	1921	71	61	15	53

Rats were fed ad libitum on Dixon ·86 diet.

Two female rats (240g each) were killed by cervical fracture and the liver freeze-clamped *in situ* after different time intervals (see IV.B.1.a.). Metabolite levels were determined as described in IV.B.1.a.

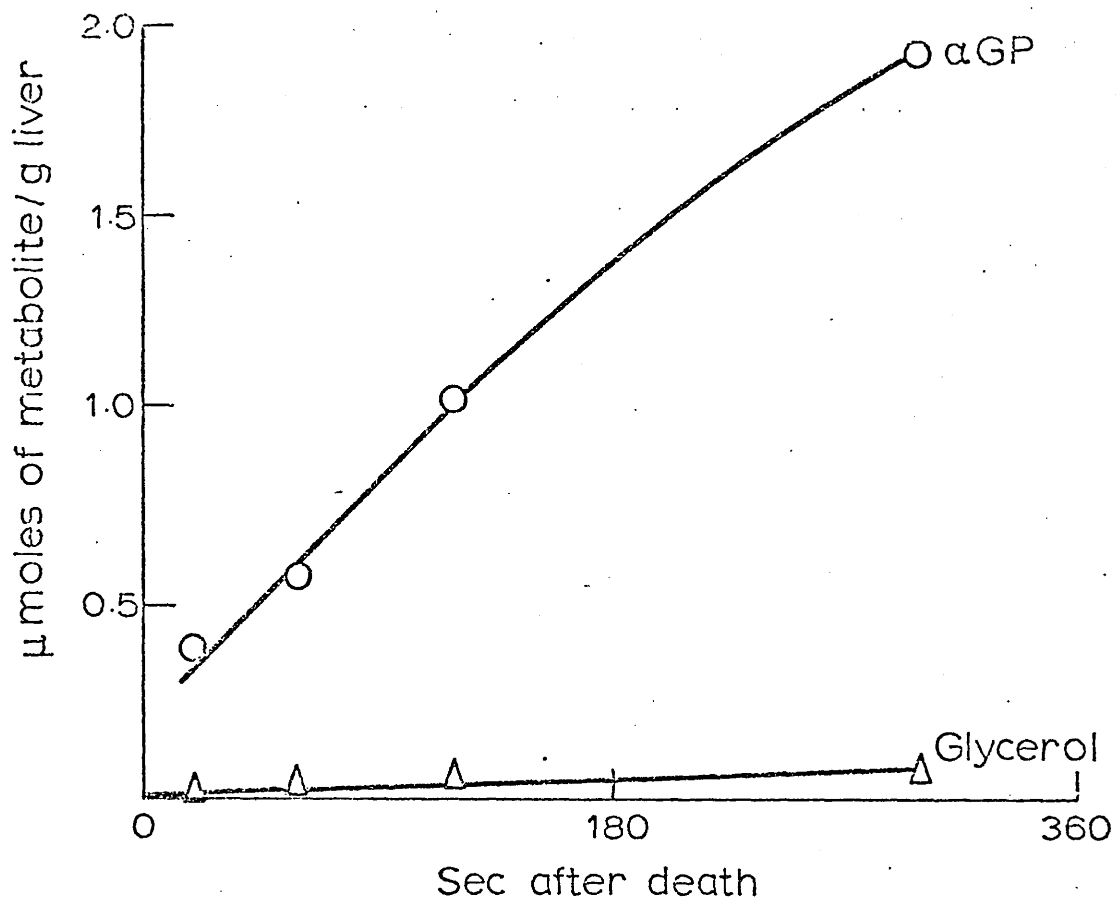


Fig. 8. Effect of anoxia on levels of glycerol 3-phosphate and glycerol in the livers of ♀ rats.

See legend to Table IIIc.

technique (241) was used to combat the effects of anoxia on hepatic metabolite levels. A very dramatic increase in the level of αGP was observed after the animal was killed. This increase was linear for a least 2 min. The level of glycerol also increased butnot as rapidly as αGP.

It was evident from this study that the effects of ischaemia set in very rapidly following death. The levels of all the metabolites examined appeared to increase.

As a result of this study it was decided to freeze-clamp liver 45 sec after the death of the animal. A shorter period was found to be impracticable. From the results shown in Fig. 8 and Table IIIc it was concluded that 45 sec was an adequate time for routing work. Liver samples were also freeze-clamped after 2 min and the metabolite levels in this extract were compared to those obtained from liver freeze-clamped after 45 sec. It was shown that storage of the liver samples at -70°C for periods of up to one month did not affect the levels of the metabolites studied.

In the extraction of metabolites a step where the powdered frozen tissue was homogenized with a Potter-Elvejham homogenizer was included but it was shown that there was very little difference in the metabolite levels obtained when the tissue was stirred with a glass rod, homogenized or ultrasonicated.

The metabolite levels in the rat liver slices incubated in KRB at 37°C were also assayed at various times. The results in Fig. 9 demonstrate the effects of ischaemia on this tissue. There was a very rapid increase in αGP level which reached a maximum after 30 min incubation. This is comparable to the rapid increase observed in intact ischaemic tissue (see Fig. 8). In both cases it is assumed that the rapid increase in αGP levels observed is due to αGP derived from DHAP because of the high concentration of reduced pyridine nucleotide present in ischaemic tissues where the lack of oxygen prevents NADH oxidation (see II.B.2.f.ii and ref. 133). It can also be seen that there was an increase in glycerol levels with time. The glycerol may be derived from the action of hepatic lipase on endogenous triglycerides (see 327 and II.B.3.a). The results in Fig. 9 also indicate a fall in the level of F16P. The levels of DHAP and G3P remained basically unchanged. Quite clearly these results show that it is not possible to study the effect of fructose on the levels of metabolites using liver slices under these conditions because of the complicating factors of ischaemia and lipolysis.

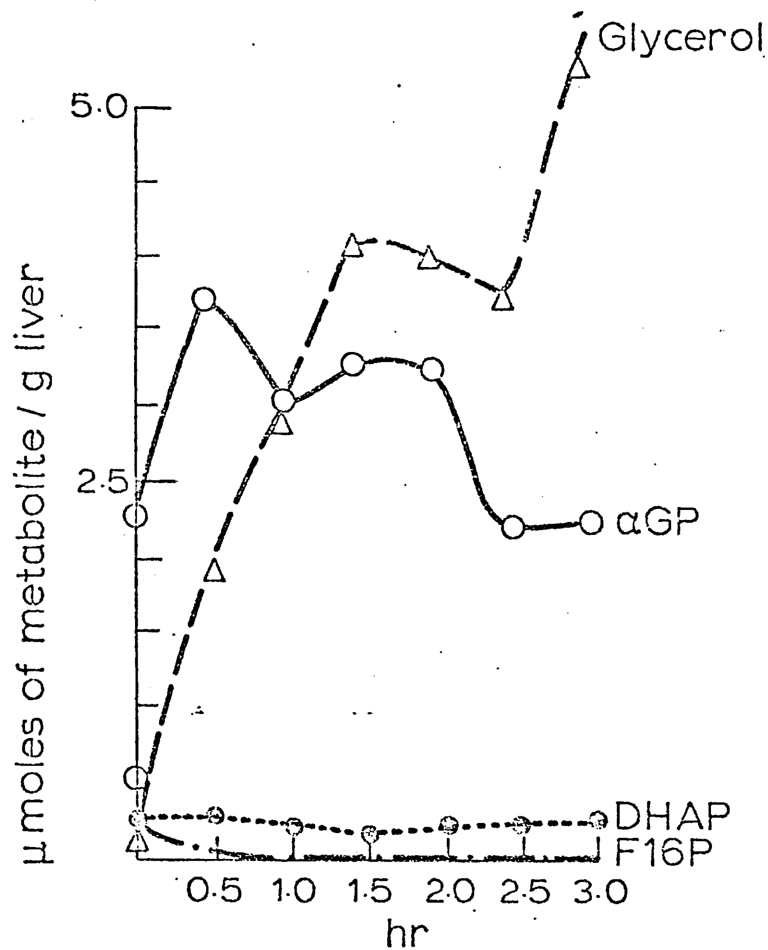


Fig. 9. The effect of ischaemia on metabolite levels in liver slices

NB Glyceraldehyde 3-phosphate (not shown on the graph) was only present in trace amounts and no detectable changes in the level of this compound occurred during incubation period.

Liver slices were prepared from a ♂ rat weighing 320g. Slices 0.2mm thick were prepared and kept on ice until ready for use. Approximately 0.5g of tissue was placed in a pre-weighed quick-fit flask containing 3.8ml of KRB gassed with 95% O_2 and 5% CO_2. These flasks were quickly re-weighed and incubated at 37°. After the appropriate time perchloric acid (0.2ml, 70% w/w) was added. The mixture was homogenized in an Ultraturrax homogenizer and the metabolites assayed as described in Materials and Methods (IV.B.1.a)

b. Metabolite levels in rat livers

The concentrations of liver metabolites were assayed in male and female rats fed for 110 days on laboratory chow (CDD[R] or Dixon .86) or on sucrose or glucose supplemented diets. The rats were fasted for approximately 4 hr before killing and the livers were freeze-clamped 45 sec after death to minimise the effect of ischaemia. The results of two separate experiments are summarized in Tables IVa and IVb. These results show that male rats fed on all diets containing CDD[R] generally have higher levels of αGP than female rats but a statistically significant difference was only observed in the αGP contents of liver from male and female rats on a sucrose supplemented diet. If the levels are based on DNA content, there is approximately 80% more αGP in the male liver than in the female liver in the animals fed on sucrose supplemented diet (see Table IVa). Rats fed Dixon .86 diet showed very little sex difference in any of the metabolites studied, including αGP. Statistical examination of the results in Tables IVa and IVb also reveal that liver DHAP, F16P and lactate levels are lower in female rats fed a sucrose supplemented diet when compared to male animals on a similar diet. This is not the case in animals on the other diets examined.

No significant sex differences in F1P levels in livers freeze-clamped 45 sec after death were found in animals fed on either CDD[R] or CDD[R] supplemented with sucrose. However, sucrose feeding leads to a statistically significant increase ($P < 0.05$) in F1P levels in the male (Table IV.b). This increased level of F1P is presumably due to extra fructose in the diet. Pridham and Mahmoud (506) and Pridham and Pollard (507) using different assay methods have found that there are no sex differences in fructokinase levels but that sucrose feeding for 110 days results in a significant increase in hepatic fructokinase activities in both sexes. There may be a correlation between the level of this enzyme and F1P levels.

There are some problems in the assay of F1P levels in liver. Although a linear relationship can be shown between concentration of F1P and the change in optical density in the analytical method, if the concentration of F1P in the tissue is low, an unknown substance can cause a slow additional removal of NADH at a steady rate of ΔE/min of ca 0.001-0.002. However, a correction can be made for this drift and

TABLE IVa. Metabolite levels in livers of rats fed on diets of Dixon .86 or Dixon CDD[R] supplemented with carbohydrate

Diet	DNA	DHAP		F16P		G3P		Glycerol		αGP	
	mg/g liver	nmol/g liver	nmol/mg DNA	nmol/g liver	nmol/mg DNA	nmol/g liver	nmol/mg DNA	nmol/g liver	nmol/g DNA	nmol/g liver	nmol/mg DNA
♂ Dixon .86 (n=8)	2.88 (±.22)	57(±9)	20(±2)	29(±7)	10(±2)	6(±3)	2(±1)	ND	ND	470 (±139)	162 (±47)
♀ Dixon .86 (n=8)	3.26 (±.20)	61(±19)	19(± 6)	37(±11)	11(±4)	8(±7)	3(±2)	ND	ND	468 (±161)	143 (±50)
♂ CDD[R] + sucrose (n=5)	2.59 (±.18)	58(±9)	23(±4)	44(±13)	17(±5)	10(±4)	4(±2)	ND	ND	627 (±109)	244 (±49)
♀ CDD[R] + sucrose (n=5)	3.40 (±.23)	49(±12)	** 15(±4)	* 29(±8)	** 9(±3)	8(±7)	2(±2)	ND	ND	** 454 (± 54)	*** 137 (±22)
♂ CDD[R] + glucose (n=5)	2.96 (±.30)	43(±13)	15(±5)	25(±7)	9(±3)	8(±6)	3(±2)	ND	ND	545 (±189)	189 (±82)
♀ CDD[R] + glucose (n=5)	3.50 (±.12)	35(±11)	10(±3)	18(±8)	5(±2)	14(±6)	4(±2)	ND	ND	401 (±119)	114 (±33)

Rats were fed for 110 days on diets of Dixon .86 chow, or Dixons CDD[R] and 5% sucrose in drinking water, or on Dixons CDD[R] and 5% glucose in drinking water. The liver was freeze-clamped 45 sec after death. The metabolites in a perchloric acid extract were determined as described in Section IV.B.1.a.

Deviations quoted are S.D. values.
* These values differ significantly ($P < 0.05$, t-test) when ♂ rats are compared with ♀ rats in each group
** These values differ significantly ($P < 0.02$, t-test) when ♂ rats are compared with ♀ rats in each group
*** These values differ significantly ($P < 0.01$, t-test) when ♂ rats are compared with ♀ rats in each group

TABLE IVb. <u>Metabolite levels in livers of rats fed on diet of Dixon CDD[R] or Dixon CDD[R] plus 5% sucrose in drinking water</u>

Diet	DHAP	F16P	G3P	Glycerol	αGP	F1P	lactate	pyruvate
	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver
♂CDD[R] (n=8)	66 (±10) ***	34 (±7)	13 (±12)	ND	651 (±187)	308 (±43)	1701 (±520)	94 (±21)
♀CDD[R] (n=9)	36 (±14)	31 (±9)	10 (±8)	ND	491 (±234)	310 (±49)	1962 (±1066)	72 (±38)
♂CDD[R] + sucrose (n=10)	39 (±11) **	28 (±8) ***	9 (±4)	ND	699 (±214) ***	402 (±114)	1883 (±582) ***	48 (±11)
♀CDD[R] + sucrose (n=10)	27 (±8)	17 (±4)	10 (±4)	ND	271 (±103)	359 (±142)	1154 (±420)	45 (±19)

Rats were fed for 110 days on diets of Dixons .86 chow, or Dixons CDD[R] and 5% sucrose in drinking water, or on Dixons CDD[R] and 5% glucose in drinking water. <u>The liver was freeze-clamped 45 sec after death.</u> The metabolites in a perchloric acid extract were determined as described in Section IV.B.1.a.

Deviations quoted are S.D. values.
** These values differ significantly ($P < 0.02$, t-test, when ♂ rats are compared with ♀ rats in each group
*** These values differ significantly ($P < 0.01$, t-test, when ♂ rats are compared with ♀ rats in each group

TABLE IVc. Metabolite levels in livers of rats fed on normal and carbohydrate diets

	DHAP	F16P	G3P	Glycerol	αGP
Diet	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver
♂ Dixon .86 (n=8)	51 (±10)	50 (±9)	5 (±3)	ND	1222 (± 165)
♀ Dixon .86 (n=8)	55 (±13)	63 (±29)	5 (±3)	ND	1229 (± 167)
♂ CDD[R] + sucrose (n=5)	90 (±27)	74 (±35)	10 (±6)	65 (±29)	1489 (± 124)
				*	*
♀ CDD[R] + sucrose (n=5)	77 (±25)	43 (± 9)	9 (±10)	25 (±25)	1300 (± 163)
♂ CDD[R] + glucose (n=5)	57 (±11)	45 (± 4)	12 (±12)	45 (±30)	1405 (± 195)
♀ CDD[R] + glucose (n=5)	54 (± 4)	42 (± 7)	9 (± 6)	21 (±20)	1229 (± 167)

Rats were fed for 110 days on diets of Dixon .86 chow, or Dixons CDD[R] and 5% sucrose in drinking water, or on Dixons CDD[R] and 5% glucose in drinking water. The liver was freeze-clamped 2 min after death. The metabolites in a perchloric acid extract were determined as described in Section IV.B.1.a.

Deviations quoted are S.D. values.

* These values differ significantly ($P < 0.05$, t-test) when ♂ rats are compared with ♀ rats in each group

TABLE IVd. Metabolite Levels in Liver of Rats on a Diet of Dixons CDD[R] chow supplement with 5% Sucrose in drinking water

Diet	DHAP	F16P	G3P	Glycerol	αGP	F1P	Lactate	Pyruvate
	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver
♂ CDD[R] + sucrose (n=10)	52 (±12)	57 (±51)	10 (± 4)	24 (±34)	1703 (±198)	273 (±104)	4081 (±457)	69 (±33)
	***	*			***		***	
♀ CDD[R] + sucrose (n=10)	36 (± 5)	25 (± 4)	12 (± 4)	5 (±10)	1115 (±122)	286 (± 81)	3343 (±542)	71 (±21)

Rats were fed for 110 days on diets of Dixon .86 chow, or Dixons CDD[R] and 5% sucrose in drinking water, or on Dixons CDD[R] and 5% glucose in drinking water. The liver was freeze-clamped 2 min after death. The metabolites in a perchloric acid extract were determined as described in Section IV.B.1.a.

Deviations quoted are S.D. values

*** These values differ significantly (P< 0.01, t-test) when ♂ rats are compared with ♀ rats in each group

* These values differ significantly (P<0.05 , t-test) when ♂ rats are compared with ♀ rats in each group.

thus this source of error can be minimized. According to Eggleston (508) this unknown interfering substance only occurs with livers of rats on normal diet but not in animals fed on high levels of sucrose but this effect was not observed in this study.

The results obtained when the liver was removed and freeze-*

*The metabolite levels were assayed when the liver was freeze-clamped 2 min after death in order to examine the possible effects of ischaemia on the general pattern of differences between various groups of animals which were observed after 45 sec.

both the αGP and lactate levels. This was expected as the levels of these metabolites are known to increase in anaerobic glycolysis (133). Glycolysis involves an NAD-dependent oxidative step and therefore it is essential to reoxidize the glycolytically generated NADH for the maintenance of glycolysis. Because pyridine nucleotides cannot cross the mitochondrial membrane, cytoplasmically generated NADH cannot be directly oxidized by the mitochondria. The "α-glycerophosphate shuttle" involving the cytoplasmic and the mitochondrial glycerol 3-phosphate dehydrogenase is one of the pathways involved in the disposal of glycolytically generated NADH in animal tissues (see section II.B.2.f.ii). The conversion of pyruvate to lactate by lactate dehydrogenase will result in the oxidation of glycolytically generated NADH to NAD, and in anaerobic glycolysis this 'blind alley' pathway is an important factor in the disposal of glycolytic NADH. Thus, in the absence of oxygen, αGP and lactate will accumulate (133).

The concentrations of F1P in livers freeze-clamped 2 min after death (Table IVd) were apparently decreased possibly due to the lack of ATP which occurs in ischaemia. Nevertheless, the pattern of sex differences remained essentially the same as in tissues where the level of anoxia was kept to a minimum. The levels of αGP, DHAP, F16P and lactate were again significantly higher in the livers of male rats compared to female rats in animals on the sucrose supplemented diet.

The sex differences in the hepatic carbohydrate metabolites of rats fed a diet supplemented with sucrose may be a function of the high specific activities of some of the hepatic fructose metabolizing enzymes in the male liver (see II.E.1). The levels of the following enzymes have been reported to be higher in males than in females, glycerol kinase (453),

fructokinase and fructose 1-phosphate aldolase (454). Pridham and Mahmoud (506) found that females had a higher level of hepatic glycerol 3-phosphate dehydrogenase than males and these results are compatible with the observation that high levels of αGP are accompanied by low levels of the latter enzyme (432). The differences in αGP levels in male and female rats may be important as this triose phosphate is an immediate precursor of triglycerides (see II.C.3.a). It is well documented that fructose is converted rapidly to glyceride — glycerol by the liver (272). Hay (509) has shown that when liver slices from both male and female rats incorporate label from [^{14}C]fructose into triglyceride, there is 13-17 fold greater incorporation into the glyceride-glycerol moiety than into the fatty acid. Zakim (272) has postulated that there is more radioactivity in glyceride-glycerol because of the dilution of label by endogenous intermediates.

Mukherjee et al. (432) found that there is no correlation between the levels of αGP and the incorporation of palmitate into hepatic lipids. These authors reported a greatly reduced hepatic level of αGP in rats fed on starch diets supplemented with either 12% fructose or 12% sucrose diet, but these rats exhibit higher rates of hepatic triglyceride synthesis. According to Mukherjee et al. (432) the decrease in αGP level occurs because it is rapidly transformed to complex lipids. In contrast, they found a direct correlation between the level of glycerol 3-phosphate dehydrogenase and the rate of hepatic glyceride synthesis. Zakim (275) has found that sucrose feeding results in the highest rate of fatty acid synthesis from acetate and this is accompanied by the lowest hepatic αGP level. Zakim et al. (273) also found that feeding diets high in either glucose or fructose (70%) did not affect the DHAP level but decreased the αGP level in the liver. They found that F16P was unchanged in animals on glucose but was significantly decreased by the high fructose diet. Pyruvate was increased by fructose but not glucose. They also observed that a diet rich in fructose increased the levels of some metabolic intermediates leading to the synthesis of fatty acids; these were malate, citrate and acetyl-CoA. However, this group of workers found that hepatic fructokinase was decreased by a diet rich in fructose whereas glucose feeding increased the level of glucokinase. Although Zakim's (273) results appear to contradict the findings reported in this thesis, a closer analysis of the data reveals many differences in the experimental technique. The major differences were that in Zakim's work the animals were fed on a high carbohydrate diet for relatively

short time periods whereas the results in Table IV were obtained in long term feeding experiments on animals fed on a relatively low carbohydrate diet.

From his work Zakim has concluded in a review (272) that fructose feeding increases the synthesis of triglyceride mainly through an increase in fatty acid synthesis and that the in vivo hepatic concentration of αGP is inversely related to the rate of fatty acid synthesis However, several different authors have shown that αGP levels are increased following the administration of fructose by various routes (263,264,257,221,265,266,253,197). Several contradictory reports have also been published (273,274,116,275,276,277,278,279) and the reasons for these discrepencies have been discussed in section II.B.3.c.

In male rats on CDD[R] diet, carbohydrate supplementation did not have a significant effect on αGP level (see Tables IVa and IVb). However, male rats fed on CDD[R] diets had higher levels of αGP than male rats fed on Dixon .86 although the levels were comparable in the females. This difference may be due to the composition of the diet or to strain differences between the groups of animals tested (cf Table IIa). The two diets differ in fat and molasses content. CDD[R] has more fat and a minimal amount of sucrose whereas Dixon .86 is low in fat and has a higher sucrose content (see Materials and Methods IV.A.3). From Table II it can be seen that rats fed on Dixon .86 had the smallest peritoneal fat content.

In rats fed on Dixon .86 no sex difference in any of the metabolite levels were found. However, rats fed on CDD[R] showed sex differences in the metabolite levels which were potentiated by the feeding of sucrose. From these results it appears that fat in the diet can influence the effects of carbohydrate supplementation of the diets. This observation has also been made by Takemoto (476).

In male rats fed a sucrose diet, a sex difference in F16P levels becomes apparent (see TablesIVa and b). F16P can regulate the rate of glycolysis by affecting the activities of fructose 1,6-phosphatase and pyruvate kinase (L form) (133). The activation of the latter and the inhibition of the former results in the production of more glycolytic intermediates for fatty acid synthesis. Galton et al. (351) found that in ob/ob mice there is an increase in plasma triglyceride because of the overproduction of glycolytic intermediates. In these animals this is due to a loss of feedback inhibition of phosphofructokinase by citrate and ATP.

Thus a liver containing high levels of DHAP, αGP and F16P may favour the endogenous synthesis of triglyceride since DHAP and αGP are precursors of glyceride-glycerol and F16P can accelerate the synthesis of glycolytic intermediates involved in the synthesis of fatty acids. If this is the case triglyceride synthesis should be greater in male rats fed on sucrose than in females on sucrose. However, there is overwhelming evidence to show that there is more hepatic triglyceride synthesized and secreted in the female rats than in males although the effect of diet has not been studied (483, 484,485,486,487,488). Some authors believe that the accumulation of αGP is due to the lower rate of esterification (275,273,432). It is also possible that αGP is higher in the male because there is a high level of glycerolkinase in the male liver compared to the female (453, 506). This enzyme can phosphorylate the glycerol released from the breakdown of triglyceride in the diet.

c. Metabolite levels in other tissues

Metabolite levels in heart, adipose tissue, kidneys and small intestine were also compared. These tissues were removed from the animal and immersed in liquid nitrogen, 4,5,6 and 7 min respectively after killing the rat. During this time the tissues became ischaemic and there were changes in the levels of some of the metabolites, therefore care has to be taken in the interpretation of the results.

In the heart (Table V) the results obtained suggested that in general there were no sex differences and that the nature of the diet did not alter the levels of the metabolites. Ischaemia had a pronounced effect on most of the metabolites and Table V shows that αGP levels were more than 15 times higher and F16P was increased by fourfold compared to values for freeze-clamped heart reported in the literature (242). The levels of F16P were higher in the heart than in all the other tissues examined. This is not surprising as the heart has a 100 times higher level of phosphofructokinase than the liver (146).

In the kidney (Table VI), the overall results do not show either any sex differences or any dietary effects on metabolite levels. The levels DHAP, F16P and αGP in non-ischaemic kidneys are 25, 17 and 131 nmoles/g wet weight respectively (242). Therefore it is evident that all these metabolites are increased due to the effects of ischaemia in the present study. The levels of αGP and F16P are lower in the kidneys than in the heart in all groups of animals studied. This presumably reflects the different functions of the organs.

TABLE V. Cardiac Metabolite levels of rats fed on diets of Dixon .86 or Dixon CDD[R] supplemented with carbohydrates

Diet	DHAP	F16P	G3P	Glycerol	αGP
	nmol/g heart	nmol/g heart	nmol/g heart	nmol/g heart	nmol/g heart
♂ Dixon .86 (n=8)	46 (±52)	171 (±46)	4 (± 2)	193 (± 47)	968 (±135)
♂ Dixon .86 (n=8)	52 (±10)	219 (±109)	5 (± 4)	239 (±120)	921 (±492)
♂ Dixon CDD[R] + sucrose (n=5)	58 (±16)	104 (± 14)	10 (± 6)	252 (± 61)	935 (±117)
♀ Dixon CDD[R] + sucrose (n=5)	62 (±10)	116 (± 19)	8 (± 7)	193 (± 38)	1026 (± 87)
♂ Dixon CDD[R] + glucose (n=5)	58 (±10)	107 (± 10)	13 (± 9)	323 (± 62)	897 (±110)
♀ Dixon CDD[R] + glucose (n=5)	61 (±19)	118 (± 26)	6 (± 4)	249 (± 88)	830 (± 99)

Rats were fed for 110 days on diets of Dixon .86 chow, or Dixons CDD[R] and 5% sucrose in drinking water, or on Dixons CDD[R] and 5% glucose in drinking water. The heart was removed and immersed in liquid N_2 4 min after death. The metabolites in a perchloric acid extract were determined as described in Section IV.B.1.a.

TABLE VI. Renal Metabolite Levels of Rats fed on diets of Dixon .86 or Dixon CDD[R] supplemented with carbohydrate

Diet	DHAP	F16P	G3P	Glycerol	αGP
	nmol/g kidney	nmol/g kidney	nmol/g kidney	nmol/g kidney	nmol/g kidney
♂ Dixon .86 (n=8)	54 (± 9)	83 (± 7)	9 (± 5)	168 (± 27)	708 (± 83)
	***	***			
♀ Dixon .86 (n=8	40 (± 5)	65 (±11)	7 (± 6)	151 (± 35)	783 (± 63)
♂ Dixon CDD[R] + sucrose (n=5)	39 (± 7)	78 (±16)	24 (± 14)	170 (± 29)	663 (± 23)
			*		
♀ Dixon CDD[R] + sucrose (n=5)	42 (± 6)	75 (± 9)	11 (± 3)	194 (± 24)	726 (± 65)
♂ Dixon CDD[R] + glucose (n=5)	51 (±13)	83 (±15)	3 (± 3)	185 (± 20)	745 (± 95)
♀ Dixon CDD[R] + glucose (n=5)	57 (± 26)	85 (±11)	11 (± 4)	175 (± 53)	823 (± 87)

Rats were fed for 110 days on diets of Dixon .86 chow, or Dixons CDD[R] and 5% sucrose in drinking water, or on Dixons CDD[R] and 5% glucose in drinking water. The kidneys were removed and immersed in liquid N_2 6 min after death. The metabolites in a perchloric acid extract were determined as described in Section IV.B.1.a.

Deviations quoted are S.D. values

* These values differ significantly ($P < 0.05$, t-test) when ♂ rats are compared with ♀ rats in each group

*** These values differ significantly ($P < 0.01$, t-test) when ♂ rats are compared with ♀ rats in each group

The kidneys, heart and liver of male rats fed on sucrose supplemented diets were much heavier than the same organs in the females (Table IIa). The levels of αGP, DHAP and F16P were also higher in the male livers. However, these metabolites were not significantly different in either the hearts or kidneys of male and female rats. This indicates that the levels of these metabolites are not related to the size of the organs. A sex difference exists in the liver presumably because this organ is important in the metabolism of carbohydrate and lipids.

Metabolite levels in the adipose tissue and small intestine were also examined and sex differences in the levels of free glycerol were observed in both cases (Table VII). Male rats fed on Dixon .86 had a higher level of free glycerol in the adipose tissue than the females (Table VIIa). The reverse situation occurred in the small intestine; a higher level of free glycerol was observed in the females (Table VIIb). The levels of αGP and G3P were higher in the small intestine from the male rats than in the female. The result suggests that the female rats can break down glycerides to glycerol and fatty acids more readily in the small intestine than males. However, there is less lipolysis in the adipose tissue in the females. Both the adipose tissue and the small intestine have important roles in the mobilization and absorption of triglycerides, therefore these findings may be relevant in the consideration of the sex differences in plasma triglyceride levels (see section II.E & Table IVc).

Lamer and Hulsmann (116) have reported levels of metabolites to be 20, 21 and 107 nmoles/g wet weight for the triose phosphate, F16P and αGP respectively in the freeze-clamped small intestine. The results shown in Table VIIb indicate that the levels of all these metabolites are increased in ischaemia. The levels of G3P were observed to be much higher in this tissue than in any of the other tissues examined (Tables IV-VII).

3. The Effect of Diet on Hepatic Protein and DNA Levels

The levels of total hepatic protein in animals fed on different diets are shown in Table VIII. In animals fed on diets containing sucrose (either Dixon .86 or CDD[R] supplemented with sucrose) there was less protein in the liver than in diets not containing the disaccharide. Total protein synthesis has been reported to be inhibited by the intravenous administration of fructose (see II.B.2.b); this may explain the

TABLE VIIa. Metabolite levels in the adipose tissue of rats fed on Dixon .86 diet

Diet	Metabolite levels nmol/g adipose				
	DHAP	F16P	G3P	Glycerol	αGP
♂ Dixon .86 (n=8)	16 (± 14)	16 (± 10)	5 (± 4)	265 (± 68)*	37 (± 25)
♀ Dixon .86 (n=8)	13 (± 7)	22 (± 17)	3 (± 2)	158 (± 79)*	31 (± 93)

TABLE VIIb. Intestinal metabolite levels of rats fed on Dixon .86 diet

Diet	Metabolite levels nmol/g intestine				
	DHAP	F16P	G3P	Glycerol	αGP
♂ Dixon .86 (n=8)	119(± 27)	94 (± 19)	53 (± 19)*	379 (± 75)*	806(± 46)*
♀ Dixon .86 (n=8)	110(± 25)	76 (± 13)	22 (± 12)*	501 (±104)*	741 (± 70)*

Rats were fed for 110 days on Dixon .86. The adipose tissue was removed and immersed in liquid N_2 5 min after death. The small intestine was removed and immersed in liquid N_2 7 min after death. The metabolites were extracted and assayed as described in IV.B.1.a.

*These results differ significantly ($P < 0.05$, t-test)

TABLE VIII. Effect of dietary carbohydrate on hepatic protein levels

Diet	mg Protein/g Liver
♂ Dixon .86 (n=8)	225 (± 46)
♀ Dixon .86 (n=8)	272* (± 28)
♂ Dixon CDD[R] + sucrose (n=5)	257 (± 30) ⨍
♀ Dixon CDD[R] + sucrose (n=5)	308 (± 59)
♂ Dixon CDD[R] + glucose (n=5)	334 (± 73) ⨍
♀ Dixon CDD[R] + glucose (n=5)	325 (± 61)

Rats were fed for 110 days on Dixons .86 chow or on Dixons CDD[R] and 5% sucrose in drinking water or on Dixons CDD[R] and 5% glucose in drinking water. Protein was extracted by homogenizing the frozen powdered liver in a Potter-Elvejheim homogenizer and determined as described in IV.B.1.b.

* ♂ rats on Dixon .86 vs ♀ rats on Dixon .86 ($P < 0.05$, t-test)

⨍ ♂ rats on glucose diet vs ♂ rats on sucrose diet ($P < 0.05$, t-test)

low level of protein in livers of rats fed a sucrose diet. Another possible explanation is that there was a higher level of liver glycogen in animals fed on sucrose diets. Female rats fed on a sucrose diet have a higher liver protein content compared to males on the same diet; this is not due to differences in the hepatic lipid content (Table IXa). From Table IVa it can be seen that there was more DNA/g of fresh liver tissue in the female rats compared to male rats. It is possible that there may be more cells/g of liver in the female rats compared to the male rats. In most cases when sex differences in metabolite levels were observed, when the results were expressed in terms of nmoles/mg DNA rather than nmoles/g liver the differences were exaggerated (see Table IVa) but the general pattern of the results was unchanged.

4. Lipid Levels in Rats

The levels of lipids were assayed in the liver, serum and heart (see Materials and Methods, section IV.B.1.c). The results obtained initially were checked by at least two independent methods; the level obtained by chemical determinations of lipids compared well with values obtained by the gravimetric technique. These assay procedures were also checked against quality control sera supplied by Boehringer. Levels of triglyceride in these tissues and serum were also determined by a thin layer chromatographic method. In this method the triglyceride was charred with H_2SO_4 and subsequently assayed using a densitometer. Although the results of both the gravimetric method and the thin layer chromatographic method agreed with the results obtained by chemical analysis, the former methods lacked both precision and accuracy.

Total cholesterol, phospholipids and triglycerides were assayed in the liver, heart and serum from animals fed on various diets (Tables IXa, b and c). These experiments were done on at least four different occasions. As there is some evidence to show that lipid levels are subject to seasonal variation (160, 301), the results of these separate experiments have been represented invividually since this may be more meaningful statistically than results expressed as a combined total (see IV.B.1.d). A table summarizing these results is also shown (Table IXd).

The results in Table IXa show that in most cases the hepatic lipid levels were higher in the female than in male rats. Statistically significant sex differences in triglyceride, phospholipid and cholesterol levels were only found in animals fed on sucrose supplemented diet except for one case in which the cholesterol levels of females fed on CDD[R]

TABLE IXa. Effect of diet on the levels of lipids in the rat liver

Type of lipid	Triglycerides (mg/g)				Phospholipids (mg/g)				Cholesterol (mg/g)			
Experiment/	1///	2	3	4	1//	2	3	4	1//	2	3	4
Diet ♂ CDD[R]				5.4 ± 0.8 n=8				27.8 ±1.4 n=8				2.32 ± .25 n=8
♀ CDD[R]				5.1 ± 0.8 n=9				27.1 ±1.2 n=9				2.52* ± .16 n=9
♂ CDD[R] + sucrose	6.7 ± 1.3 n=5	2.9± 0.4 n=10	3.3± 0.7 n=5		23.1 (22.0, 24.0) n=2	27.8 ±4.4 n=10	18.3 ±4.4 n=5		2.33 (2.64, 2.02) n=2	2.47 ± .31 n=10	1.36 ± .31 n=5	
		*	*			**	*			*	**	
♀CDD[R] + sucrose	8.7± 2.5 n=5	3.5± 0.8 n=10	4.2± 0.5 n=5		24.0 (21.2, 26.7) n=2	33.3 ±3.0 n=10	25.0 ±3.2 n=5		2.46 (2.67, 2.24) n=2	2.91 ± .47 n=10	2.13 ± .29 n=5	
♂ CDD[R] + glucose	8.3± 4.8 n=5		5.3± 1.9 n=5		22.2 (22.8, 21.6) n=2		22.9 ±2.5 n=5		2.31 (2.15, 2.47) n=2		1.97 ± .26 n=5	
♀CDD[R] + glucose	9.4± 5.7 n=5		4.5± 1.3 n=5		25.1 (24.8, 25.3) n=2		24.8 ±2.5 n=5		2.77 (2.87, 2.66) n=2		2.15 ± .30 n=5	

Rats (initial weight 50g) were fed ad lib for 110 days on Dixon CDD[R],CDD[R]supplemented with 5% glucose in drinking water or CDD[R]supplemented with 5% sucrose in the drinking water. After this time the rats were killed by cervical fracture and the various lipids determined as in Materials and Methods (IV.B.1.c)

/ Experiments done during different seasons of year // Individual values and the means are given

/// Triglyceride determined by an enzymatic method (IV.B.1.c)

* $P < 0.05$, t-test; when ♂ rats are compared with ♀ rats in each group

** $P < 0.01$, t-test; when ♂ rats are compared with ♀ rats in each group

TABLE IXb. Effect of diet on the levels of lipids in the rat heart

Type of lipid	Triglycerides (mg/g)				Phospholipids (mg/g)				Cholesterol (mg/g)			
Experiment	1	2	3	4	1	2	3	4	1	2	3	4
Diet												
♂ CDD[R]				3.3 ± 0.5 n=8				19.5 ± 1.4 n=8				1.53 ± .21 n=8
♀ CDD[R]				** 4.5 ± 0.7 n=9				20.4 ± 1.2 n=9				1.48 ± .23 n=9
♂ CDD[R] + sucrose	3.8 ± 0.8 n=10	2.4 ± 0.4 n=5	3.3 ± 0.5 n=5		15.9 ± 2.6 n=10	17.2 ± 0.9 n=5	17.4 ±0.5 n=5		1.21 ± .10 n=10	1.27 ± .07 n=5	1.43 ± .07 n=5	
♀ CDD[R] + sucrose	** 5.1 ± 0.9 n=10	2.6 ± 0.3 n=5	3.7 ± 0.5 n=5		* 18.4 ± 2.4 n=10	** 19.4 ± 0.6 n=5	20.7 ± 1.8 n=5		1.25 ± .08 n=10	1.33 ± .02 n=5	1.51 ± .17 n=5	
♂ CDD[R] + glucose		2.0 ±0.6 n=5	2.7 ±0.5 n=5			17.8 ±1.2 n=5	18.7 ±1.0 n=4			1.30 ±.08 n=5	1.40 ±.07 n=5	
♀ CDD[R] + glucose		2.7 ±0.7 n=5	3.8 ±1.3 n=5			20.0 ±2.3 n=5	19.9 ±0.9 n=5			* 1.42 ±.05 n=5	1.48 ±.10 n=5	

Rats (initial weight 50g) were fed ad lib for 110 days on Dixon CDD[R], CDD[R] supplemented with 5% glucose in drinking water or CDD[R] supplemented with 5% sucrose in the drinking water. After this time the rats were killed by cervical fracture and the various lipids determined as in Materials and Methods (IV.B.1.c)
Experiments done during different seasons of year
* $P < 0.05$, t-test, when ♂ rats are compared with ♀ rats in each group
** $P < 0.01$, t-test, when ♂ rats are compared with ♀ rats in each group

TABLE IXc. Effect of diet on the levels of lipids in blood (serum)

Type of lipid	Triglycerides (mg/100ml)					Phospholipids (mg/100ml)		Cholesterol (mg/100ml)				
Experiment	1	2	3	4	5	1	2	1 ///	2	3	4	5
Diet												
♂ Dixon .86 //	69±20 n=6					123 ± 26 n=6		86±15 n=6				
♀ Dixon .86 //	** 36±15 n=6					122 ± 31 n=6		* 128±46 n=6				
♂ CDD[R]					82± 22 n=7							69 ± 7 n=8
♀ CDD[R]					64± 17 n=5							70 ± 14 n=9
♂ CDD[R] + sucrose		106 ± 24 n=10 *	120 ± 30 n=5	113 ± 38 n=5			136 ± 19 n = 10		46 ± 10 n=10 **	73±8 n=5	66 ± 12 n=5	
♀ CDD[R] + sucrose		78 ± 30 n=10	109 ± 38 n=5	111 ± 38 n=5			132 ± 19 n = 10		109± 39 n=10	65±7 n=5	74 ± 9 n=5	
♂ CDD[R] + glucose			97 ± 38 n=5	97 ± 35 n=5						59 ± 12 n=5	62 ± 7 n=5	
♀ CDD[R] + glucose			* 58 ± 10 n=5	* 50 ± 11 n=5						70 ± 12 n=5	64 ± 9 n=5	

/// Results cannot be compared with the others because the method used in the determination of cholesterol did not include a isopropanol extraction step

// Rats fed on Dixon.86 for 110 days were also used in this study.

* $P < 0.05$, t-test; when ♂ rats are compared with ♀ rats in each group

** $P < 0.01$, t-test; when ♂ rats are compared with ♀ rats in each group

Rats (initial weight 50g) were fed ad lib for 110 days on Dixon CDD[R], CDD[R] supplemented with 5% glucose in drinking water or CDD[R] supplemented with 5% sucrose in the drinking water. After this time the rats were killed by cervical fracture and the various lipids determined as in Materials and Methods (IV.B.1.c)

TABLE IXd Summary of the effect of diets on the lipid levels in the liver, heart and serum

Lipid / Diet	Triglyceride			Phospholipids			Cholesterol		
	Liver	Heart	Serum	Liver	Heart	Serum	Liver	Heart	Serum
♂Dixon .86			69 ± 20 n=6 **			123 ± 26 n=6			86 ± 15 n=6 *
♀Dixon .86			36 ± 15 n=6			122 ± 31 n=6			128 ± 46 n=6
♂CDD[R]	5.4 ± .75 n=8	3.3 ± .52 n=8 **	82 ± 22 n=7	27.8 ± 1.4 n=8	19.5 ± 1.4 n=8		2.32 ± .25 n=8 *	1.53 ± .25 n=8 *	69 ± 7 n=8
♀CDD[R]	5.1 ± .76 n=9	4.5 ± .69 n=9	64 ± 17 n=5	27.1 ± 1.2 n=9	20.4 ± 1.2 n=9		2.52 ± .16 n=9	1.48 ± .23 n=9	70 ± 14 n=9
♂CDD[R]+ sucrose	3.7 ± 1.4 n=20 ∤	3.3 ± .86 n=20 *	111 ± 28 n=20 ∤	24.5 ± 5.9 n=17 **	16.6 ± 2.0 n=20 **	136 ± 19 n=10	2.13 ± .59 n=17 *	1.28 ± .13 n=20	58 ± 16 n=20 **
♀CDD[R]+ sucrose	5.0 ± 2.6 n=20	4.1 ±1.20 n=20	94 ± 36 n=20	29.8 ± 5.3 n=17	19.2 ± 2.1 n=20	132 ± 19 n=10	2.63 ± .53 n=17	1.34 ± .15 n=20	89 ± 34 n=20
♂CDD[R]+ glucose	6.8 ± 3.8 n=10	2.3 ± .62 n=10 *	97 ± 34 n=10 **	22.7 ± 2.1 n=7	18.2 ± 1.1 n=9 *		2.06 ± .29 n=7	1.34 ± .09 n=9 **	61 ± 9 n=10
♀CDD[R]+ glucose	6.9 ± 4.4 n=10	3.2 ±1.10 n=10	54 ± 11 n=10	24.9 ± 2.1 n=7	20.0 ± 1.7 n=10		2.33 ± .39 n=7	1.45 ± .08 n=10	67 ± 10 n=10

Rats (initial weight 50 g) were fed ad lib for 110 days on Dixon .86, Dixon CDD[R] CDD[R] supplemented with 5% glucose in drinking water, or CDD[R] supplemented with 5% sucrose in the drinking water. After this time the rats were killed by cervical fracture and the various lipids determined as in Materials and Methods (IV.B.1.c).

∤ 0.10 > P < 0.05, t-test; wehn ♂ rats are compared with ♀ rats fed on CDD[R] supplemented with sucrose.

* P < 0.05, t-test; when ♂ rats are compared with ♀ rats in each group.

** P < 0.01, t-test; when ♂ rats are compared with ♀ rats in each group.

only were significantly higher than in the males. However, in general the differences between the sexes were only marginal but if the sex difference in liver weight is taken into account then the total lipid in a male liver is generally higher than in the female liver. These results are not in conflict with the finding that female rat liver synthesizes and secretes more triglyceride than male liver on a weight basis (see II.E.). Morton and Homer (479) have also found that female rats on a high sucrose, fat free diet, had livers which had higher levels of hepatic triglyceride than male rats.

In general, supplementation of the diet of both males and females with sucrose did not increase the lipid (including triglyceride) levels in the liver. The hepatic lipid levels have been shown to increase when rats are fed a high sucrose diet (see II.D.1) but in the present study a balanced, high protein diet with a relatively low level of sucrose supplement did not affect lipid levels.

The levels of triglyceride, phospholipid and cholesterol in the heart were examined after long term feeding experiments in which the animals were fed on different diets. In general, the lipid levels in females were higher than that of males, and statistically significant differences were only observed in some cases (TablesIXb and IXd). No conclusive evidence for an effect of diet on the level of cardiac lipids was obtained. The higher lipid levels in females could be related to the observation (61) that the aortas of female rats have a higher capacity for lipid synthesis from glucose than male aortas.

Serum triglyceride levels were consistently higher in male rats than in females (Table IXc). These results also show that diet can influence the serum triglyceride level. Animals fed on low fat diet (Dixon .86) had the lowest serum triglyceride levels. Animals fed on the high fat, CDD[R] diet had increased levels of serum triglyceride whereas supplementation of this diet with sucrose increases the levels of the lipid in both males and females, the sex difference in the level of triglyceride remains. No increase was observed in female rats fed on a glucose supplemented CDD [R] diet. It has been found that extrahepatic lipoprotein lipase activity is higher in females than in males (496). Glucose stimulates the release of insulin and as a consequence increases lipoprotein lipase activity and lowers serum triglyceride levels (27). These observations taken together could explain the effect of glucose supplementation of the diet on serum triglyceride in the female rats

found in this study.

There were no sex differences in the serum phospholipid levels in animals fed on either Dixon .86 or on CDD [R] supplemented with sucrose (Table IXc). However, in general, the cholesterol levels were higher in females than in males and in some experiments the sex difference was statistically significant. This finding has been reported previously (see II.E.4). There was no apparent effect of diet on the serum cholesterol levels in this study.

The long term feeding experiments have yielded information which is compatible with the results obtained by the majority of workers in this field. The results obtained indicate that the serum triglyceride level is higher in the male rat than in the female rat and that sucrose feeding raises this level in both sexes. In contrast, the levels of triglyceride in the hearts and livers of rats were higher in females and prolonged supplementation of the diet with sucrose did not cause any noticeable alteration in the lipid levels in these two organs.

The results of the metabolite studies have shown that males have a higher hepatic αGP, DHAP and F16P level than females and that sucrose feeding exaggerates this sex difference. Clearly, the long term feeding experiments have not produced enough evidence to explain the sex difference in serum triglyceride levels. It is possible that there is more hepatic synthesis of triglyceride in the male or that there is a faster clearance of triglyceride from the serum in females. However, the data presented does indicate that there are sex differences in the response to dietary sucrose.

B. SHORT TERM FEEDING EXPERIMENTS

One major problem in the study of the long term effects of dietary carbohydrates is that it is difficult to control the food intake of animals. In short term experiments this problem can be overcome by intragastric feeding experiments.

1. Preliminary Experiments

In this series of experiments the short term effect of the ingestion of fructose on male and female rats was studied. In a preliminary study a mature rat was anaesthetized by nembutal injection and a fine plastic tube inserted via the nostril into the stomach. The peritoneal cavity was then exposed. After 15 min a lobe of liver was freeze-clamped and removed to serve as a control. Fructose was introduced into the stomach

via the fine plastic tube. After 45 min the remaining liver was freeze-clamped and removed from the body cavity. The metabolites were extracted and assayed as described in Materials and Methods (IV.B.1.a) and the results obtained are shown in Table X.

There was a 5 fold increase in the level of F1P which demonstrates that some fructose was absorbed by the intestine and transported to the liver. However, the effects of anaesthesia and surgery were to increase the levels of αGP and lactate compared to the values obtained when the liver is freeze-clamped 45 sec after death (see Table IV). The high control levels of these metabolites indicated that this experiment was unsuitable for the study of the effect of fructose on the metabolite levels in the liver. The increase in αGP and lactate may be due to physiological stress, anoxia or anaesthesia and not wholly due to the effects of fructose ingestion.

TABLE X. Gastric intubation of fructose and hepatic metabolite levels - the effects of surgery and anaesthesia

Liver	nmol/g liver							
Metabolites	αGP	glycerol	DHAP	G3P	F16P	F1P	Lactate	Pyruvate
Control (15 min)	1180	ND	46	12	23	210	3090	99
Fructose Fed (2ml, 70%; 45 min)	2530	340	47	12	36	1060	4960	77

A male rat weighing 355g was anaesthesized with nembutal (see Materials and Methods, IV.B.2.a). A lobe of liver was removed and freeze-clamped 15 min after the operation. This served as the control. Fructose (70%, 2ml) was then introduced into the stomach via a fine plastic tube and the rat was left for a further 45 min. Then the remaining liver was freeze-clamped. The metabolites were extracted and assayed as described in IV.B.1.a.

In an effort to study the fate of the intragastric fructose preliminary experiments using [^{14}C]fructose were carried out. A young male rat was fasted overnight and the [^{14}C]fructose was introduced into the stomach (see Materials and Methods, IV.B.2.a). Biopsies from the exposed liver of the anaesthesized rat were taken at 2 min intervals

and extracted with perchloric acid and the extracts were assayed for radioactivity by scintillation counting. The results in Fig. 10a show that fructose was taken up from the small intestine into the liver 18 min after the administration of the fructose.

In a similar experiment the radioactivity in the portal blood serum was measured. The results shows that the uptake of [^{14}C]fructose into the portal system is time dependent (Fig. 10b).

From the results in Fig 10a and Fig. 10b it was assumed that fructose was taken up by the small intestine and within 20 min the [^{14}C]fructose appeared in the liver. In the latter experiment the amount of unabsorbed fructose remaining in the gastrointestinal tract after 45 min was greater than 81% of the total intubated fructose. A perchloric acid extract of the small intestine and stomach contained 3% of the total radioactivity administered and 14% of the total radioactivity was found in the perchloric acid insoluble precipitate. From this experiment, it was deduced that only a small proportion of the administered fructose was absorbed by the liver (approx. 2%) after 45 min. This is equivalent to approximately 2mg fructose. From Table X, the increase of F1P level was found to be 850nmole/g liver after 45 min. For a 10g liver the total accumulation of F1P level could be assumed to be 8.5μmole/whole liver. Thus 1.53mg of fructose would have to be converted to F1P to account for the increase in F1P level. These two sets of data from Fig. 10b and Table X are in broad agreement. Both experiments indicated that the quantity of fructose absorbed under these conditions was very low. These findings are in agreement with other work on fructose absorption by rat small intestine (83, 88). The results also suggest that the rate limiting factor in hepatic fructose metabolism is the rate of absorption of the hexose by the small intestine.

Finally, in this series of experiments the incorporations of [^{14}C]-fructose into the various organs of a male rat were studied. The fructose was administered to a nembutal anaesthesized rat. After 3 hr the rat was killed and various tissues sampled and assayed for radioactivity as described (see Table XI). The results are shown in Table XI. It is apparent that although 3 hr had elapsed since fructose administration there was considerable amount of label left in the gastro-intestinal tract. However, this amount is less than that found in rats killed after 45 min. Apart from the organs involved in digestion, the liver, kidneys and lungs have highest total radioactivity. The liver is the

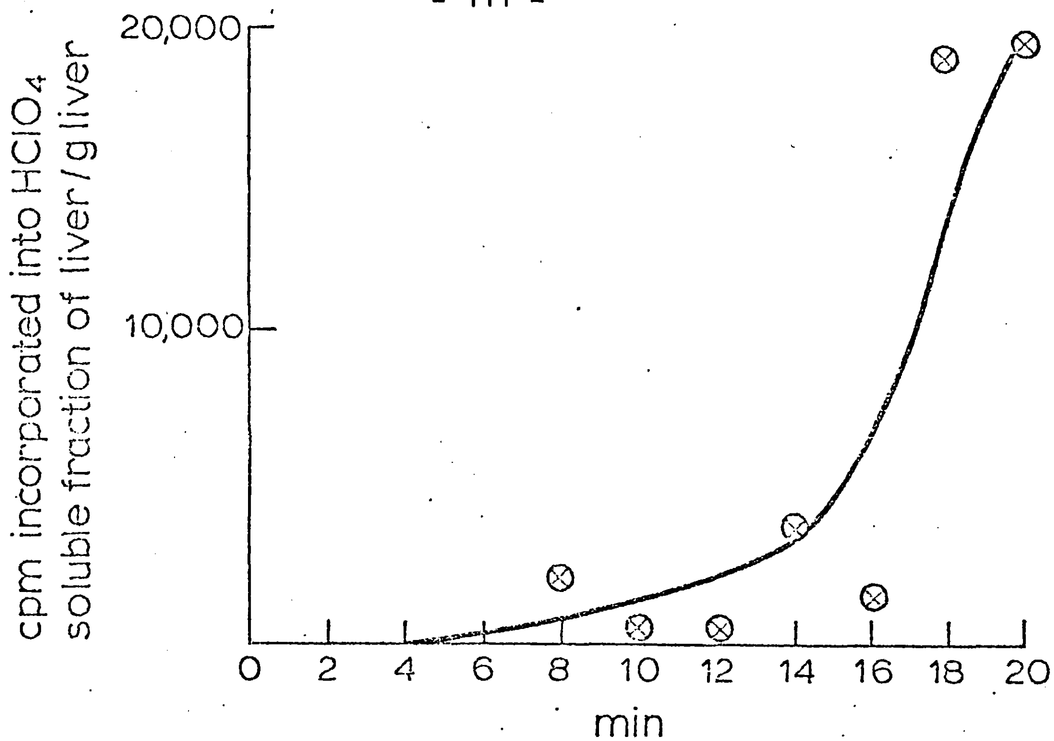


Fig. 10a. Uptake of fructose by the small intestine into the $HC10_4$ soluble fraction of liver.

A male rat weighing 90g was fasted overnight and 2ml of 5% [^{14}C]fructose containing 8μCi was introduced intragastrically via stomach tube. Biopsies from the exposed liver of the anaesthetized rat were taken at 2 min intervals and extracted in 0.5ml $HC10_4$ (6% w/w). The perchloric soluble extract obtained after ultrasonication and centrifugation was neutralized with a saturated solution of K_2CO_3 and an aliquot of 0.1ml removed and assayed for radioactivity by liquid scintillation counting (Efficiency 88%) (see IV.B.2.a).

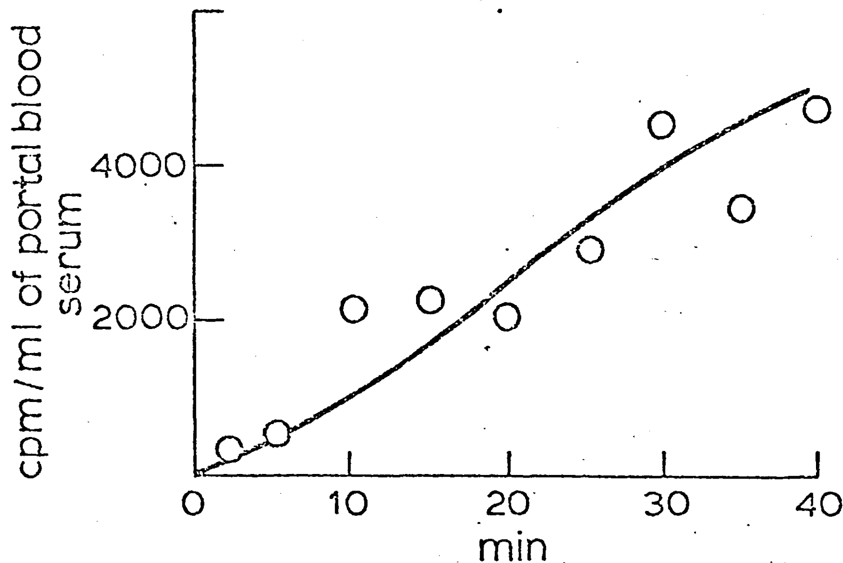


Fig. 10b. Uptake of fructose from small intestine into the portal blood stream.

A male rat weighing 260g was used. Fructose was administered as described in the legend of Fig. 10a. Blood samples were collected from the hepatic portal vein by a small syringe. Bleeding from the vein was prevented by clamping the puncture with artery forceps. Serum (0.05ml) was assayed for radio activity by liquid scintillation counting (Efficiency 80%). After 45 min the gasterointestinal tract contained the following proportions of radioactivity: $>$81% in unabsorbed 'fructose', 3% in $HC10_4$ soluble extract of this organ and 14% in the $HC10_4$ insoluble precipitate.

TABLE XI. Incorporation of $[U-^{14}C]$fructose into the various organs of a ♂ rat

Tissue	dpm/100mg tissue	dpm/total tissue
Liver	2,733	337,314
Heart	1,587	15,870
Thoracic fat (near heart)	2,489	7,686
Testis	523	15,998
Vas deferen	1,544	20,541
Pancreas	621	20,324
Diaphragm	1,170	4,588
Back muscles	1,660	-
Perirenal fat	294	29,000
Kidney	3,162	71,864
Epididymus fat	336	11,525
Blood vessels	1,254	-
Lungs	1,939	54,292
Spleen	2,202	18,350
Stomach	5,508	113,400
Small Intestine	17,216	1,893,760
Large Intestine	1,054	60,416
Serum ≠	2,269	-
Red blood cells ≠	320	-

≠ values of these samples expressed as dpm/0.1ml

The organs of a ♂ rat weighing 330g were removed and weighed 3 hours after an intragastric load of 1ml 5% $[^{14}C]$fructose containing 4μCi. (see IV.B.2.b. - this rat was anaesthesized with 0.33ml nembutal). 0.1g of tissue sample was digested overnight with NCS (see IV.B.2.c) and the radioactivity measured by scintillation counting.

main organ responsible for fructose metabolism and therefore it is not surprising that there is more label in this organ than in other non-digestive tract tissues. Surprisingly, the kidneys show a very high content of radioactivity and this suggests that a certain amount of metabolism or excretion of fructose may occur. Reinecke (182) found evidence to indicate that fructose will prolong the life of eviscerated rats so long as these animals retain their kidneys (also see II.B.2.a). The specific radioactivities of the perirenal and epididymal fat tissues were low but the fat in the thoracic cavity (near the heart) had a high level of radioactivity associated with it. The significance of this finding is not clear.

These preliminary findings obtained when fructose was administered intragastrically to rats indicate that experiments conducted on animals subjected to anaesthesia and to surgical treatment are not suitable for the study of the fate of fructose in the animal. Although fructose is taken up into the hepatic portal blood Fig. 10a and into the liver Fig. 10b, the amount absorbed is low. After 3 hr there was more fructose absorbed but a substantial amountof the hexose remains in the gastro-intestinal tract.

2. Sex Differences in Metabolite Levels Following Fructose Administration

Young rats (50g) were fed on Dixon CDD [R] diet for 110 days. After this period of feeding the rats were starved for 4-5 hr, anaesthesized with diethyl ether and 70% fructose (2ml) was administered by intragastric intubation. The animals were then allowed to recover and were killed by cervical fracture 3 hr after the administration of the fructose. The livers were freeze-clamped and removed 45 sec after death. The small intestines were removed and freeze-clamped 1.5 min after death. The tissues were extracted with perchloric acid and the metabolite determined as described in section IV.B.1.a. The metabolite levels found in the liver are shown in Table XIIa. Fructose administration increased the αGP levels slightly in males. A mean value of 764 ($\pm$ 163) was obtained in this experiment compared to a mean value of 651 ($\pm$ 187) obtained for animals which had not been administered fructose. This difference is, however, not statistically significant ($P > 0.05$). However, the short term administration of fructose to female rats significantly increased the hepatic αGP levels, in comparison to those found in female rats on CDD [R] alone ($P < 0.01$). The sex difference in hepatic αGP observed in long term feeding experiments (Table IVa and b) was not apparent after

TABLE XIIa. Metabolite levels in livers[//] of rats after gastric incubation of 2ml of 70% fructose

Diet /	DHAP	F16P	G3P	Glycerol	αGP	F1P	Lactate	Pyruvate
	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver	nmol/g liver
♂ Dixon CDD[R] (n=4)	81 (± 37)	62 (± 30)	9 (± 4)	ND	764 (±163)	1565 (± 66)	3570 (±773)	162 (± 16)
♀ Dixon CDD[R] (n=5)	57 (± 36)	39 (± 11)	7 (± 5)	ND	763 (± 96)	1610 (±134)	4081 (±423)	147 (± 45)

/ Rats were fed on CDD[R] diet for 110 days after which 2ml of 70% fructose was introduced via a gastric tube into the stomach (see Materials and Methods, IV.B.2.b. and IV.B.1.a).

// Removed 45 sec after death and freeze-clamped.

For details - refer to text.

TABLE XIIb. Metabolite levels in small intestines of Rats after gastric incubation of 2ml of 70% fructose

Diet ⸸	DHAP	F16P	G3P	Glycerol	αGP	F1P	Lactate	Pyruvate
	nmol/g tissue	nmol/g tissue	nmol/g tissue	nmol/g tissue	nmol/g tissue	nmol/g tissue	nmol/g tissue	nmol/g tissue
♂ Dixon CDD[R] (n=4)	97 (± 15)	69 (± 15)	25 (± 15)	134 (± 66)	489 (± 118)	6156 (± 711)	4851 (± 570)	166 (± 67)
♀ Dixon CDD[R] (n=5)	89 (± 29)	74 (± 32)	22 (± 10)	** 270 (± 53)	512 (± 79)	6617 (± 660)	* 5705 (± 164)	163 (± 50)

⸸ Rats were fed on CDD[R] diet for 110 days after which 2ml of 70% fructose was introduced via a gastric tube into the stomach. The small intestine was immersed in liquid nitrogen 1.5 min after death. (see Materials and Methods IV.B.2.b. and IV.B.1.a).

* $P < 0.05$

** $P < 0.02$

For details - refer to text

Deviations quoted are S.D. values

intragastric fructose loading. DHAP and F16P levels were higher in the male than in the female after fructose intubation but these differences were not statistically significant ($P>0.05$) (see Table XIIa).

The short term feeding of fructose increased the levels of all the liver metabolites studied, except for G3P and glycerol, in both male and females (cf Tables XIIa and IVb). The levels of F1P, lactate and pyruvate were markedly raised in both males and females. As expected the levels of F1P were increased 5 fold in both males and females compared to the control. The lactate and pyruvate levels were increased approximately 2 fold.

From the results shown in Table XIIa there is no doubt that fructose administration increased the levels of most of the metabolites studied. The surprising finding was the lack of sex difference in αGP levels after short term fructose feeding. The reason for this finding is not clear but it is apparent that the αGP level is raised to greater extent in females than in males. The results suggest that the males do not synthesize αGP from fructose at a faster rate than the females. The level of hepatic αGP in the male liver is already high and is not increased to any significant extent by fructose administration. However, in the female rat the lower hepatic αGP level is raised to a higher level by the administration of fructose. The sex difference in αGP levels in animals on a normal CDD [R] diet (Table IVa) may be due either to a greater rate of lipid synthesis in the female liver or to a higher glycerol kinase level in the male liver. A differential effect of anaesthesia on males and females cannot be ruled out but is unlikely since these animals were only mildly sedated and were then allowed to recover.

The small intestine was removed from the rat 1.5 min after death and therefore the metabolite levels found in this tissue were generally lower than in experiments where the intestine had been subjected to a longer period of ischaemia (Tables XIIb, cf VIIb), Following intragastric fructose administration a significant sex difference in the level of glycerol was observed but this had also been found to occur in normal animals. Male rats had less free glycerol than female rats in both experiments. The significance of this finding has been discussed previously (IIIA.2.c). No sex differences in αGP and DHAP levels were observed in this experiment (Table XII.b). The concentration of F1P

in the small intestine following fructose administration was four times that found in the liver. This was expected as the small intestine absorbs and transports fructose into the hepatic portal vein at a very slow rate and there is sufficient fructokinase in the small intestine to phosphorylate the fructose absorbed into the sucosal cells (II.A.2). The high level of F1P suggests that the intestine is capable of fructose metabolism and this tissue may be important in the synthesis of lipid from this hexose in the rat. A high level of fat in the diet, may provide favourable conditions for the formation of glyceride via the phosphatidic acid pathway (see II.A.3). Preliminary studies (151) have shown that fructose can be converted to triglyceride in the intestinal mucosa but further work is required to demonstrate the importance of this synthesis in relation to triglyceride synthesis by the liver.

A high lactate level was observed in the small intestine of both male and female rats. A similar effect of fructose on hepatic lactate levels has been documented previously (see II.B.2.b).

C. IN VITRO EXPERIMENTS WITH ISOLATED HEPATOCYTES

The method used for the preparation of isolated hepatocytes is described in section IV.B.3.a. Hepatocyte preparations were used instead of liver slices because of the difficulties encountered previously (see section III.A.2.a and Fig. 9) when liver slices were used in the study of metabolite levels. Hepatocytes, however, are comparable to perfused liver in a variety of biochemical parameters including gluconeogenesis, cholesterol synthesis, and protein synthesis (510). These isolated cells also have several advantages as an *in vitro* system for the study of carbohydrate and lipid metabolism. The major advantage is that it is possible to do various experiments on a single batch of cells and this is very useful in the detection of significant differences between control and treated cells. However, there are limitations to the use of hepatocytes : i, there is no contact between the plasma membranes of cells; ii, only one hepatic cell type (parenchyma) is present; and iii, when comparing the biochemical functions of hepatocytes from different animals another variable is introduced because of differences arising during the preparation of cells.

1. Preparation and Evaluation of Isolated Hepatocytes

Although hepatocytes were routinely prepared from perfused liver, four attempts were made to prepare these isolated parenchyma cells from

liver slices (see IV.B.3.a). This method recently published by Fry et al. (511) involves the collagenase/hyaluronidase digestion of liver slices. This technique proved to be unreliable since the hepatocytes produced were not viable as judged by trypan blue exclusion and also the yield of cells was very low. The best preparation yielded 50mg of hepatocytes from 3g of tissue and 24% of the isolated cells excluded trypan blue. The problem associated with this method is that the plasma membranes of the hepatocytes can be damaged when incubated with collagenase and hyaluronidase when liver slices are incubated under the conditions described by Fry et al. (511). Perhaps cell yields and the viability of these cells could have been improved by decreasing the incubation time.

During the preliminary studies on the isolation of hepatocytes using the perfused liver system an observation indicated that the 'viability' of the cells as judged by the ability to exclude trypan blue, can be changed by the presence of fatty acid free bovine serum albumin (cf Fig. 11a and Fig. 11b). The presence of albumin decreases the number of cells stained blue by the dye. This effect was probably due to the colloid osmotic effect of albumin. A high concentration (70%) of fructose added to an equal volume of cells has a similar effect. Trypan blue staining is widely used as an index of cell viability. Some workers claim viabilities of 90-100% for hepatocyte preparations in the presence of albumin (158). It is therefore evident that great care must be taken in the interpretation of data obtained with hepatocytes treated in this way.

Some biochemical parameters were studied using hepatocytes prepared by liver perfusion. The leakage of K^+ from hepatocytes into the Waymouth incubation medium was studied. Table XIII shows that very little K^+ leaks into the medium from isolated cells incubated at 37°C for up to 3 hr but after 4 hr a large amount of K^+ could be detected. This increase in K^+ corresponded to a decrease in the cell viability as judged by trypan blue exclusion in the absence of albumin. This experiment shows that hepatocytes prepared by the perfusion technique had intact cell membranes and very little loss of K^+ occurred within 3 hr.

The rate of gluconeogenesis was also studied in isolated hepatocytes by measuring the rate of glucose synthesis from [^{14}C]lactate. Hepatocytes were preincubated for 1 hr to restore the ATP/ADP ratio to normal (174) and then [^{14}C]lactate was added. The [^{14}C]glucose produced was assayed

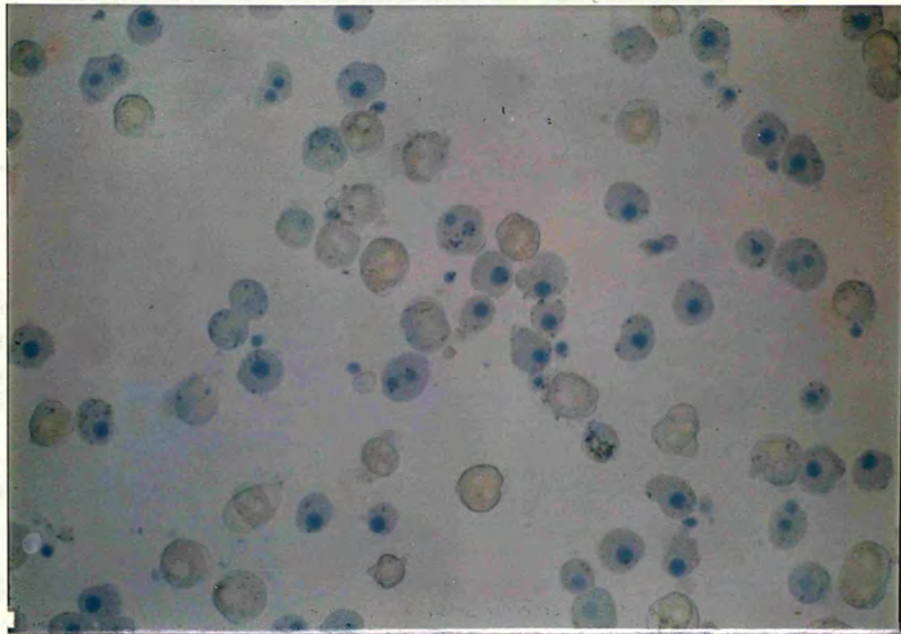


Fig. 11a. Microscopic appearance of hepatocytes in KRB.

Hepatocytes were isolated from perfused liver (see Materials and Methods, IV,B.3.a). Non viable cells take up dye and the nuclei are stained. These cells were incubated in KRB without albumin, X200.

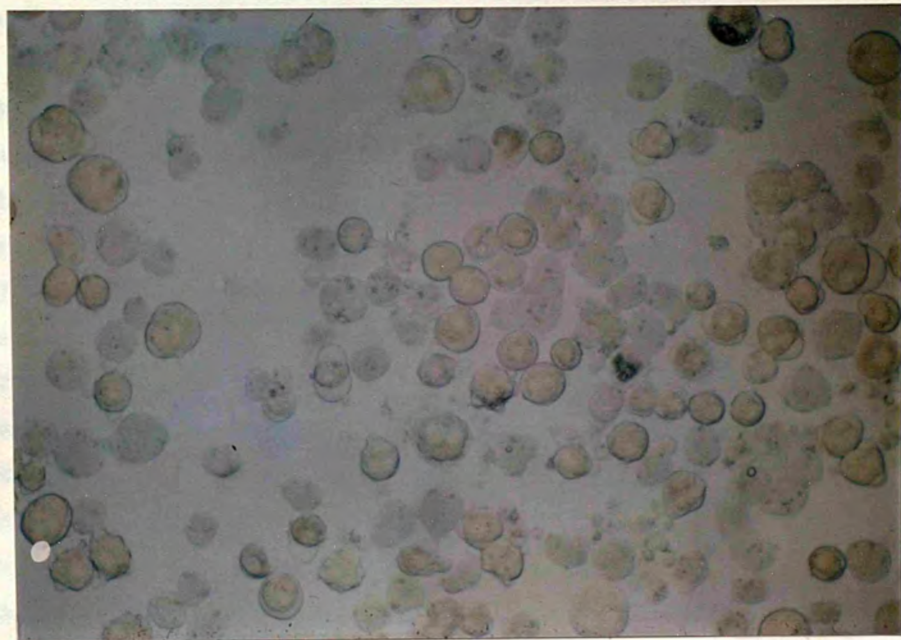


Fig. 11b. Microscopic appearance of hepatocytes in KRB with 1.5g albumin/100ml buffer.

The same preparation of hepatocytes was incubated in KRB in the presence of albumin (1.5g/100ml), X200.

TABLE XIII. Exclusion of K^+ from hepatocytes suspended in Weymouth's medium

Time	0	1hr	2hr	3hr	4hr	24hr
K^+ conc in Waymouth's Media meq/l	4.0	6.0	6.7	5.6	>10	>10
% cells stained blue with trypan blue (without albumin)	34%	39%	50%	42%	62%	100%

Hepatocytes were prepared from perfused liver (see IV.B.3.a) and suspended in Waymouth's medium (5.6×10^6 cells/ml). The cells were continuously gassed with 95% O_2/5% CO_2 and incubated at 37° in a shaking water bath. At the appropriate times the cells were separated from the medium by centrifugation. The K^+ levels in the cell free media were determined by flame photometry (EEL flame photometer). The concentration of K^+ in Waymouth's media was 3.2 meq/l.

as described in section IV.B.3.b. Gluconeogenesis from L-[U-^{14}C]lactate by isolated hepatocytes was linear for up to 30 min (Fig. 12a). Incubation of the cells with glucagon stimulated gluconeogenesis but insulin did not have a significant effect on the process. The result in Fig.12b shows that insulin had very little effect on the rate of gluconeogenesis at concentrations between 10^{-9} to 10^{-6}M. This finding has previously been reported by Pilkis et al. (171). The effects of insulin on isolated hepatocytes are known to be more variable than the effects of glucagon (see section II.B.1.d). Glucagon always stimulated gluconeogenesis from lactate in seven different hepatocyte preparations.

The effect of concentration of L-[U-^{14}C]lactate on gluconeogenesis was also studied (Fig. 12c). The rate of gluconeogenesis increases with increasing lactate concentration and the rate of gluconeogenesis is similar to values reported by Wagle (152). This author reported a gluconeogenic rate of 62.3 ± 7.0 μ moles/g hepatocytes/hr at a concentration of 10mM lactate. The rate obtained in the study was 60μmoles/g hepatocytes/hr (Fig. 12c).

Thus hepatocyte preparations were produced which had similar

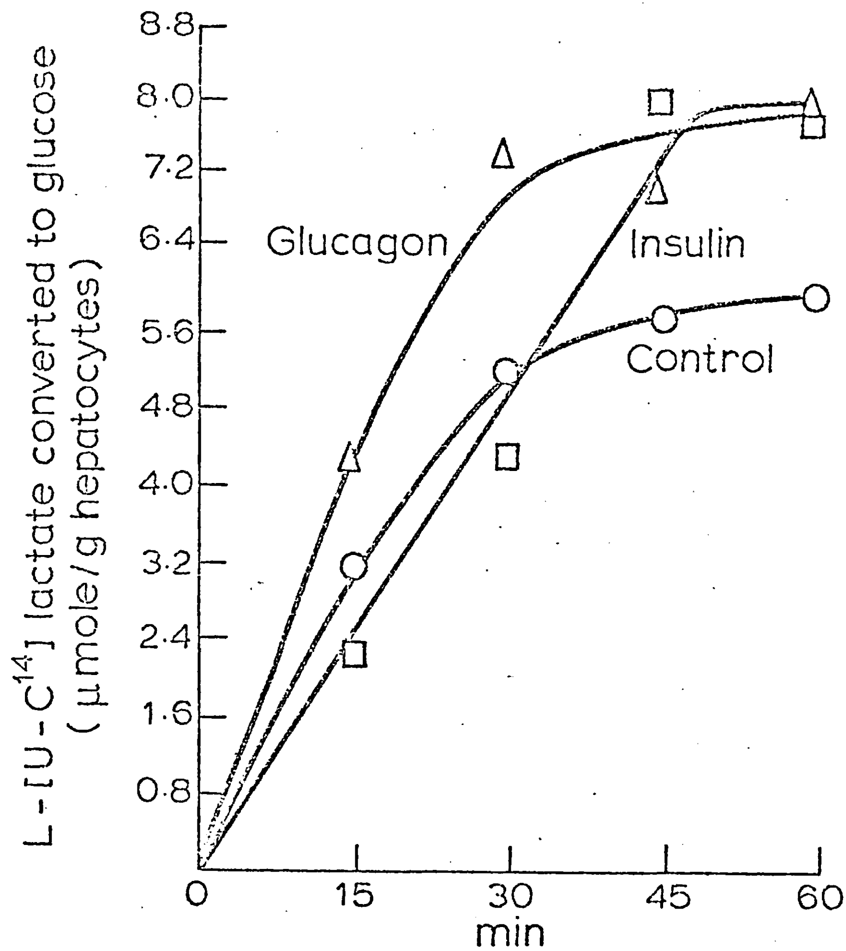


Fig. 12a. Rate of gluconeogenesis from L-[U-^{14}C]lactate

Isolated hepatocytes (3.2 x 10^{6} cells/ml) from fed rats were preincubated for 1 hr without substrate and then with lactate (2mM) for various times. Glucagon (10µM) and insulin (10µM) were added at zero time. Experimental details described in IV.B.3.b.

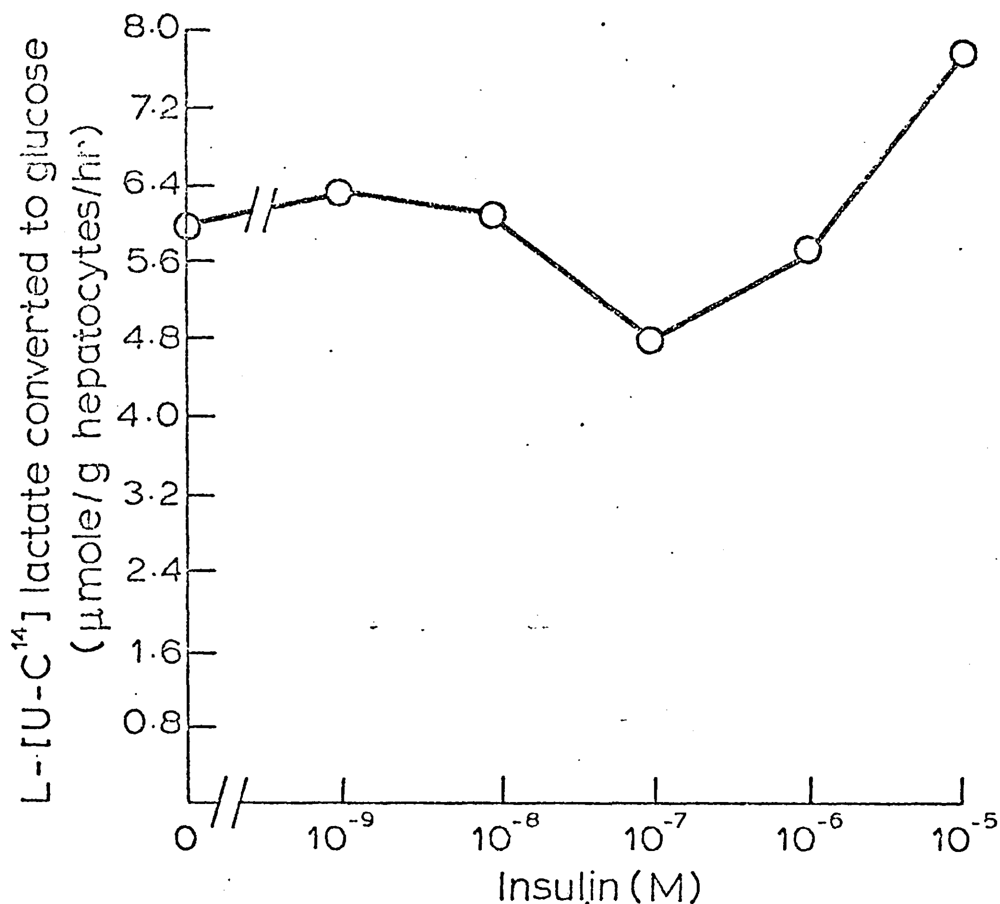


Fig. 12b. Effect of insulin concentration on the rate of gluconeogenesis.

Hepatocytes (3.2×10^6 cells) were preincubated for 1 hr and then with [U-^{14}C]lactate (2mM) for 1 hr. The glucose synthesized was assayed by the method detailed in section IV.B.3.b.

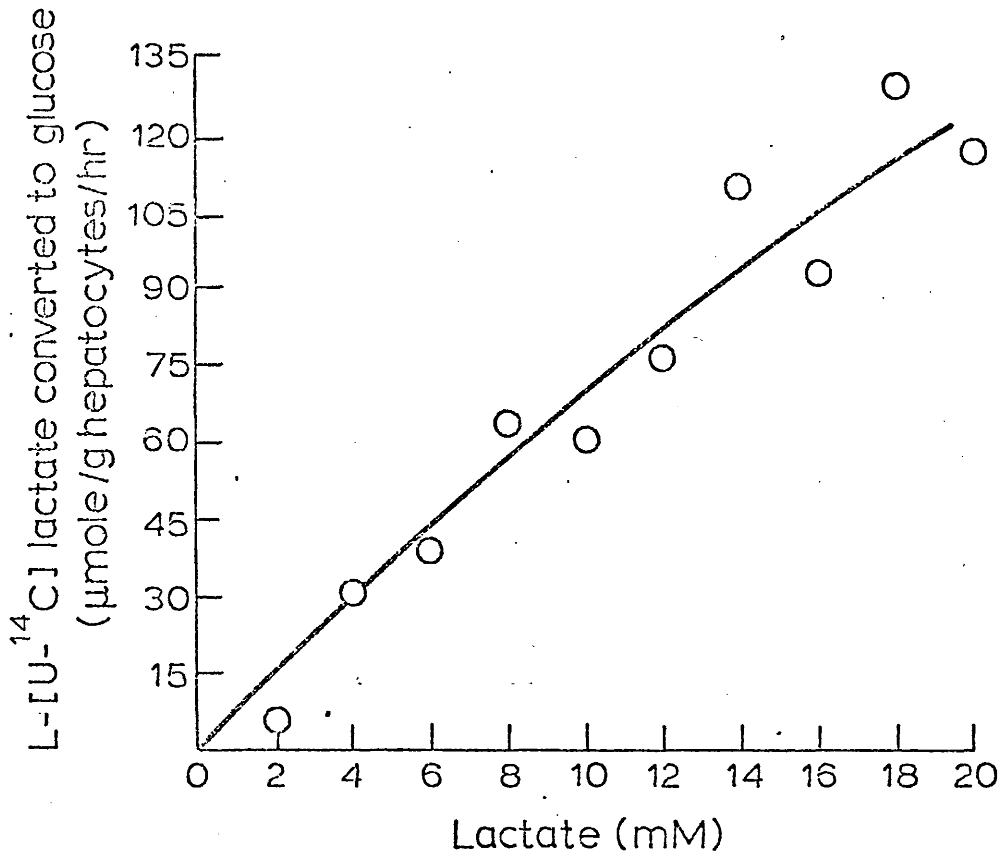


Fig. 12c. The effect of lactate concentration on the rate of gluconeogenesis

Hepatocytes (4.6 x 10^6 cells/ml) from fed rats were preincubated for 1hr without substrate and then with various concentrations of L-[U-^{14}C]lactate for 1 hr.
Experimental details in Materials and Methods, IV.B.3.b.

properties to isolated cells prepared by other workers with respect to trypan blue exclusion, K^+ leakage and glucagon stimulation of gluconeogenesis.

2. Metabolite Levels in Isolated Hepatocytes

The effects of incubating isolated hepatocytes with either glucose (9.33mM), fructose (9.33mM) or glycerol (9.33mM) on the αGP and glycerol levels were examined. Initial experiments using cell concentrations similar to those used for the determination of gluconeogenesis (3.4×10^6 cells/ml) were unsuccessful because the metabolites could not be detected. When the hepatocyte concentration was increased to 20×10^6 cells/ml the αGP and glycerol levels were detectable (Table XIVa). The metabolites were extracted with perchloric acid, the supernatant neutralized and the αGP and glycerol were assayed as described in section IV.B.1.a. The results in Table XIVa clearly show that the levels of glycerol and αGP are initially raised above the normal levels found in freeze-clamped liver (cf Tables IVa and b). This was expected since Claus et al. (174) have reported abnormal ATP/ADP ratio in isolated hepatocytes which can be returned to normal by incubation of the hepatocytes in a suitable medium for between 20 and 40 min.

In the present study the levels of glycerol and αGP were raised in liver slices presumably due to the effects of anoxia (see Fig. 9). It can be seen that the levels of glycerol and αGP are lowered by the incubation of the hepatocytes in KRB gassed with 95% O_2 and 5% CO_2 (Tables XIVa and b). Indeed, the level of αGP can be lowered to a level which is much lower than that found in a 45 sec freeze-clamped liver tissue. However, this low level of αGP may possibly reflect the physiological situation (cf Fig. 8) and see section II.B.3.a. From the results in Table XIVa it can also be seen that the incubation of hepatocytes with either glucose or fructose did not raise the αGP levels. However, when glycerol was added to the cells the level of αGP increased compared to the control and after 120 min incubation the level of αGP was 2.8 fold higher than the control level. In another experiment (Table XIVb) the effect of a prolonged preincubation (4 hr) on the metabolite levels was examined. In this case glucose was added at the start of the pre-incubation period to maintain the cell in a viable state. Two concentrations of glucose, which are widely used for hepatocyte work, were tested. KRB containing either 100mg glucose/100ml (as in Hanks solution) or 500mg glucose/100ml (as in Waymouth incubation medium), were used in

TABLE XIVa. Metabolite levels in hepatocyte following incubation with various substrates

Hepatocyte αGP levels (nmol/g hepatocytes)

Time	Control	Fructose (9.33mM)	Glucose (9.33mM)	Glycerol (9.33mM)
30 min	989	524	815	1280
60 min	844	524	815	1222
90 min	582	524	466	931
120 min	291	234	582	815

Hepatocyte Glycerol levels (nmol/g hepatocytes)

Time	Control	Fructose (9.33mM)	Glucose (9.33mM)	*Glycerol (9.33mM)
30 min	233	204	233	-
60 min	233	204	291	-
90 min	116	58	116	-
120 min	ND	58	175	-

Hepatocytes (20 x 10^6 cells/ml) were preincubated in KRB for 1 hr and then incubated with 9.33mM fructose, glucose or glycerol. Untreated cells served as controls. $HClO_4$ was then added at intervals of 30, 60, 90, 120 min and the metabolites αGP and glycerol analysed as described in section IV.B.1.a.

* Levels of glycerol not determined because of the extremely high content of glycerol in the extract.

A ♀ rat (360g) was used for the preparation of the hepatocytes (IV.B.3.a)

TABLE XIVb. Metabolite levels in hepatocytes following incubation with glucose (100mg/100ml or 500mg/100ml) and added substrates

Substrate added	CONTROL		GLUCOSE		FRUCTOSE		GLYCEROL	
	(a) Cells in KRB with 100mg % glu	(b) Cells in KRB with 500mg % glu	(a) Cells in KRB with 100mg % glu	(b) Cells in KRB with 500mg % glu	(a) Cells in KRB with 100mg % glu	(b) Cells in KRB with 500mg % glu	(a) Cells in KRB with 100mg % glu	(b) Cells in KRB with 500mg % glu
Metabolite ≠≠ / Time	αGP		αGP		αGP		αGP	
1 hour	256	291	326	326	326	419	2241	2718
2 hours	122	198	192	262	204	291	3026	3492
3 hours	58	116	116	146	192	239	2619	2805
Metabolite ≠≠ / Time	Glycerol		Glycerol		Glycerol		≠ Glycerol	
1 hour	29	64	12	58	116	134		
2 hours	29	29	12	41	58	58		
3 hours	6	12	6	52	23	41		

Hepatocytes (20 x 10^6 cells/ml) were preincubated for 4hr in KRB with either 100mg% or 500mg% glucose and then treated with fructose, glucose or glycerol (9.33mM final concentration of all substrates). Untreated cells served as controls. The cells were extracted with $HClO_4$ at intervals of 1,2 and 3 hr and αGP and glycerol were assayed in the extract.

≠ Level of glycerol was not determined because of the extremely high content of glycerol in the extract

≠≠ Values expressed as nmol/g hepatocytes.

A female rat weighing 370g was used in the preparation of hepatocytes (IV.B.3.1)

this study. These cells were preincubated for 4 hr to minimize the effects of ischaemia on the cells. This treatment decreased the control levels of both glycerol and αGP in the cells (see Table XIVb and cf Table XIVa). Further incubation led to a fall in the level of both metabolites. Hepatocytes incubated in media containing 500mg glucose/100ml and cells similarly treated in media of 100mg glucose/100ml have comparable metabolite levels.*

*The viabilities of the hepatocyte preparations used in this study were found to be unchanged during the 6 hr incubation period as judged by three different criteria. The percentage of the hepatocytes which excluded trypan blue in the presence of albumin remained constant at approximately 90%. The rates of lipid synthesis from [^{14}C]glucose were found to be linear for at least 6 hr after the preparation of the isolated cells. The rates of total protein, albumin and α2u globulin synthesis by the same preparations of hepatocytes were found to be linear for at least 6 hr. A rate of α2u globulin synthesis of 0.15μg/hr/10^6 cells was observed in hepatocytes from male animals (512). This rate is comparable to the rates found by other workers using isolated hepatocytes, perfused liver systems and intact animals (513, 514).

synthesis (for discussion see II.C.3.c).

The rates of lipid synthesis by hepatocytes from fructose, glucose or glycerol (all 9.33mM) are shown in Fig. 14 (see Materials and Methods, IV.B.3.c). These cells were preincubated in KRB for 2 hr before the addition of the radioactive precursors. Glycerol was found to be the most effective precursor, while glucose was not incorporated as efficiently as fructose. The results also show that the synthesis of lipid from all three precursors was linear for at least four hr. If the preincubation period is taken into account it seems that isolated hepatocytes are viable, in terms of lipid synthesis at least, for up to 6 hr after preparation.

4. Sex Differences in Lipid Synthesis and αGP Levels in Hepatocytes

In this study, hepatocytes were prepared from male and female rats. These rats were bred in our laboratory and fed on a diet of CDD [R] for ca 110 days prior to the preparation of isolated hepatocytes. The rates

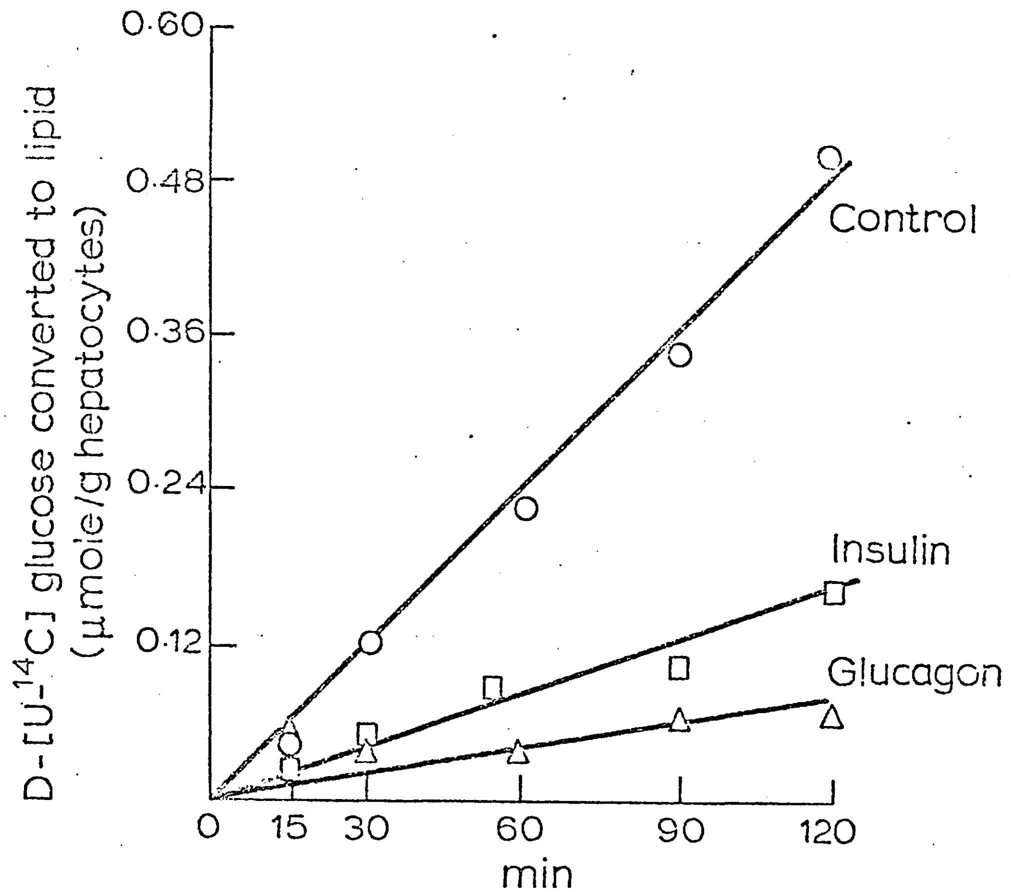


Fig. 13. Effects of insulin and glucagon on lipogenesis from D-[U-^{14}C]glucose

The concentration of glucose was 9.33mM

Insulin = 40µM and glucagon = 10µM

Hepatocytes (1ml, 7.3 x 10^6 cells) were incubated with glucose (9.33mM) in the absence and presence of hormones (insulin, 40µM and glucagon, 10µM). The reaction was stopped by the addition of 0.5ml of 5% $ZnSO_4$ and 0.5ml of 0.15M $Ba(OH)_2$. Total lipid obtained by Folch extraction was assayed for radioactivity (see Materials and Methods, IV.B.2.c.)

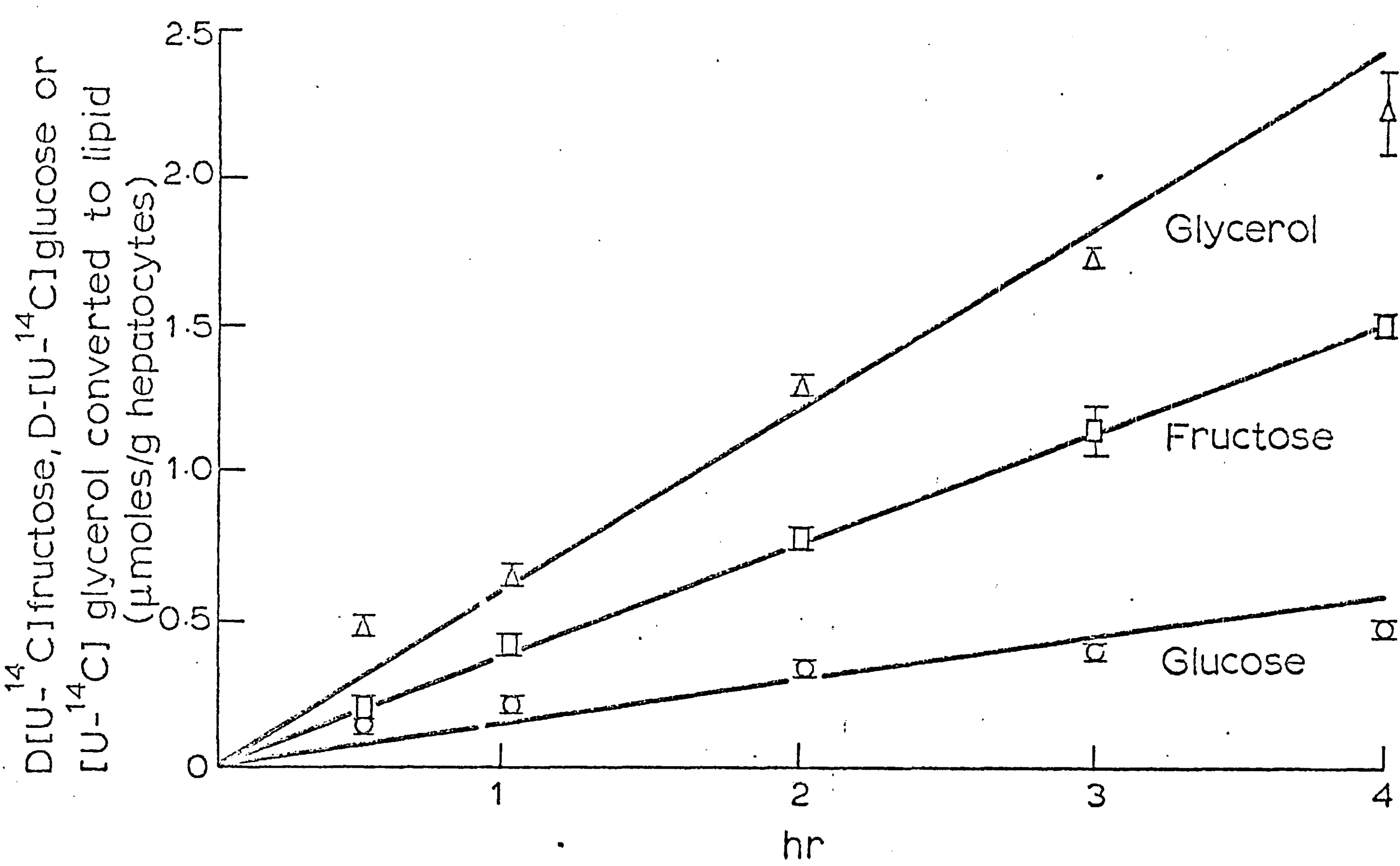


Fig 14, Rates of lipogenesis from fructose, glucose or glycerol by isolated hepatocytes

Hepatocytes were prepared from female rats. Fructose, glucose or glycerol (9.33mM each substrate) was added to 1ml of cells (4.0×10^6 cells/ml) which were preincubated at 37^oC for 2 hr in KRB. The reaction was terminated by the addition of 6% $HClO_4$ and the lipid extracted by Folch solvents as described in Materials and Methods, IV.B.3.c.

of lipogenesis from glucose, fructose or glycerol (9.33mM each substrate) was studied after a preincubation period of 2 hr in KRB. αGP levels were determined using the same batch of cells after a preincubation period of 4 hr in glucose in KRB (100 mg/100ml) followed by the addition of substrates and a further incubation for 2 hr. The longer preincubation period was necessary because a higher concentration of cells was required in this study in order to be able to detect the metabolite. As a result the cells required a longer preincubation period in order to reach a stable condition and thus any effect observed on the level of αGP was due to the addition of substrate. The results in Table XVa show that fructose did not have any effect on the level of αGP in the hepatocytes from either male or female rats whereas the addition of glycerol to the cells greatly increased the level of αGP in hepatocytes from male rats. However, the increase in αGP observed after the addition of glycerol to hepatocytes from female rats was only minimal in comparison. The αGP has been previously shown (Table XIV) to be independent of the glucose concentration in the medium.

Statistical analysis of the results indicated that although the levels of αGP in male hepatocytes incubated with either glucose or glucose plus fructose were less than that of cells incubated with glucose plus glycerol this was not significant at the 5% level ($0.10 > P > 0.05$, t-test). Although hepatocytes prepared from female rats incubated with glycerol had a higher mean αGP level compared to cells incubated with either glucose or fructose, the differences were not statistically significant. Sex difference in αGP levels following incubation of hepatocytes with glycerol can be related to a sex difference in glycerol kinase activity observed by Fathipour and Pridham (454) and Pridham and Mahmoud (506). The levels of the enzyme in the male liver are 50% higher than in the female. Alternatively the difference in αGP levels may be related to the rate of lipid synthesis and thus the rates of lipogenesis from various substrates by the hepatocytes were determined.

In all cases studied lipid synthesis by the hepatocytes was linear for at least 3 hr for all substrates. The rates obtained in Table XVa were calculated from four individual determinations at two different time intervals (2 and 3 hr). In all the hepatocyte preparations examined the rate of lipid synthesis from glycerol was greater than from fructose which, in turn, was greater than that from glucose. The possibility that there was radioactive dilution of [^{14}C]glucose by the endogenous level of glucose and glycogen cannot be discounted.

TABLE XVa. Rates of lipogenesis from glucose, glycerol and fructose and the concentrations of αGP in isolated hepatocytes

	Lipogensis from D-[U-^{14}C]glucose µmole/g hepatocytes/hr.	Level of αGP in hepatocytes incubated with glucose nmol/g hepatocytes/hr.	Lipogenesis from D-[U-^{14}C]fructose µmole/g hepatocytes/hr.	Level of αGP in hepatocytes incubated with fructose nmole/g hepatocytes/hr.	Lipogenesis from [U-^{14}C]glycerol µmole/g hepatocytes/hr.	Level of αGP in hepatocytes incubated with glycerol nmole/g hepatocytes/hr.
Males						
1.	0.16	966	0.42	811	0.51	5699
2.	0.20	580	0.38	773	0.53	2724
3.	0.07	773	0.13	676	0.18	966
4.	0.08	705	0.17	802	0.21	966
5.	0.11	551	0.29	502	0.36	2106
Mean	0.12 ± 0.06	715 ± 167	0.28 ± 0.13	713 ± 129	0.36 ±0.16	2490±1945
Females						
1.	0.04	560	0.07	580	0.13	541
2.	0.08	725	0.27	599	0.41	1662
3.	0.12	869	0.22	1333	0.35	860
4.	0.10	869	0.24	647	0.37	811
5.	0.16	-	0.37	-	0.63	-
Mean	0.10 ± 0.04	756 ± 147	0.23 ± 0.11	790 ± 363	0.38 ± .18	969 ± 483

Hepatocytes were prepared from rats fed on CDD[R] diet for 110 days. The weights of these rats were ♂ = 392±31, & ♀ = 255±31. Hepatocytes (4 x 10^{-6} cells/ml) were preincubated in KRB for 2 hr and the rates of lipogenesis from ^{14}C substrates (9.33mm) were determined for 2 and 3 hr as described in section IV.B.3.c. For the study of αGP the cells (20 x 10^{6}ml) were preincubated for 4 hr in KRB plus 100mg% glucose before the addition of the substrate (9.33mm) and the cells were further incubated for a period of 2 hr. αGP level in the hepatocytes was determined as described in section IV.B.1.a.

TABLE XVb. Distribution of label in various lipids following lipogenesis by hepatocytes from various substrates

	% of radioactivity as triglycerides			% of radioactivity as phospholipids †		
	glucose	fructose	glycerol	glucose	fructose	glycerol
Cells from ♂ rat	48.0	60.3	63.6	34.9	24.5	23.3
Cells from ♂ rat	57.0	63.4	75.3	19.1	24.0	16.8
Cells from ♀ rat	47.3	59.0	66.1	25.2	29.1	25.0
Cells from ♀ rat	-	63.5	66.4	-	23.4	18.9

Lipogenesis by isolated hepatocytes was carried out as described in Table XVa. The total lipid components were separated on Silica gel TLC(petroleum ether : ethyl ether : acetic acid, 90:10:1 v/v) and the various fractions were assayed for radioactivity by liquid scintillation counting (IV.B.2.c).

† Phospholipid fraction may also contain monoglycerides

Williamson and Bronson (242) have obtained data concerning the glucose and glycogen levels in the liver tissue from fed rats which suggest in this experiment that the [^{14}C]glucose may be diluted up to 2 fold. This could account for the difference in the rate of lipogenesis from glucose compared to that from fructose. However, the hepatocytes have been preincubated for 2 hr in KRB and during this time the level of glucose and glycogen in the hepatocyte should have decreased. A better method of resolving this problem would be to starve the animals for 48 hr before the preparation of hepatocytes.

No sex differences in the rates of lipogenesis from glucose, fructose or glycerol were observed. Cole and Margolis (504) found that there is no sex difference in the basal rates of lipid synthesis from [^{14}C]acetate by isolated hepatocytes. Maruhama and Macdonald (153) have also shown that there is no sex difference in the synthesis of hepatic triglycerides from [^{14}C]fructose or [^{14}C]glucose, given intragastrically. However, Heinberg et al. (485) have shown a sex difference in the hepatic output of triglyceride in the perfused liver but they suggest that this is a result of a sex difference in the uptake of free fatty acids by the liver.

In the present study there was, however, some correlation found between the level of αGP in isolated hepatocytes and the rate of lipid synthesis (correlation coefficient $r = 0.62$). This positive correlation is significant at the 1% level (Table XVa). In general, high levels of αGP were accompanied by high rates of lipid synthesis in isolated hepatocytes. From the results of feeding experiments Zakim (273) and Mukherjee (432) have postulated that there is a negative correlation between the level of this metabolite and the rate of lipogenesis.

In some experiments, the total lipid was fractionated by TLC into triglyceride, cholesterol fractions and also a fraction containing phospholipids and monoglycerides, (Table XVb). Very little labelled cholesterol or cholesterol esters was observed in any of the experiments. The majority of the radioactivity was associated with the triglyceride and phospholipid-monoglyceride fractions.

A greater proportion of the total lipid was [^{14}C]triglyceride following incubation of the cells with [^{14}C]glycerol than was labelled after incubation of the cells with either [^{14}C]glucose or [^{14}C]fructose. In turn, a large percentage of the total lipid was labelled triglyceride following incubation of the hepatocytes with fructose than after the

treatment of the cells with glucose. This result in addition to the data in Table XVa suggests that glycerol is a better precursor of αGP and triglyceride than fructose which, in turn, is a better precursor than glucose.

CONCLUSIONS

Sex differences in carbohydrate and lipid metabolism, in particular in the metabolism of fructose, were examined in this study in an attempt to explain the hypertriglyceridaemic nature of fructose.

The effects of feeding rats with dilute solutions of glucose and sucrose, in addition to the normal chow diet, over a relatively long period were examined. The results obtained showed that both male and female rats on the sucrose diet showed greater increases in both the body and organ weights than either the glucose fed or the control animals. Sex differences were always observed in animals on the CDD [R] diet. An interesting feature was the large increase in the weights of both the liver and the small intestine on sucrose feeding. This was more in the male rats than in the females. Another relevant observation was that there was a considerably larger ratio of peritoneal adipose tissue to body weight in the females than in the males.

The levels of αGP, DHAP and F16P were higher in male liver than in female when the rats were fed on CDD [R]. Sucrose feeding appeared to exaggerate these sex differences. However, no statistically significant sex differences were found in hepatic F1P levels but sucrose feeding increased the level of this metabolite in the male.

These sex differences in the metabolite levels taken together with the differences in liver weights may explain the hypertriglyceridaemic effect of dietary sucrose in the male rat. The differences in the metabolite levels may favour the synthesis of triglyceride in male rats since DHAP and αGP are precursors of triglycerides and F16P can accelerate the synthesis of glycolytic intermediates involved in the synthesis of fatty acids. However, the possibilities that the sex difference in αGP is the result of either a higher hepatic glycerol kinase activity in the male or the higher rate of esterification in the female, cannot be excluded.

The long term feeding of sucrose resulted in an increase in serum triglyceride in both male and female rats but the levels were always higher in the male rats than the females. An increase in the levels of hepatic lipid has been reported to occur when a high concentration of sucrose is fed to rats (see II.D.1), however, in this study this was not observed. This was thought to be due to the comparatively low level of sucrose in the diet. However, it appeared that there was more lipid in the livers of female rats than of male rats on all the diets administered but this difference was only statistically significant in the sucrose fed

animals. In general the cardiac lipid levels were higher in females than in males. It is difficult to explain the reasons for these differences. The high level of hepatic lipid in the female may be correlated to a greater rate of lipid synthesis in female liver. It has been widely reported that females secrete more triglyceride than males (II.E.2). However, it is also possible that there is a greater uptake of plasma lipids into female rat heart, liver and adipose tissue. The higher serum triglyceride in the male could be related to sex differences in the activity of post-heparin lipoprotein lipase. In this context, Nikkila and his co-workers (496) have recently reported that men have a higher level of this enzyme than women. A higher rate of removal of triglyceride from the plasma in the female is compatible with the finding that there is more peritoneal adipose tissue deposited in the female rats than in the males.

The results of the short term intragastric intubation experiments, indicated that fructose was absorbed slowly but F1P accumulated in the liver. However, there was no apparent sex difference in the level of this metabolite. As expected the hepatic lactate and pyruvate levels were raised in response to intragastric administration of fructose in both male and female animals. The increased lactate levels observed may favour the synthesis of fatty acids and glycerides (398). A surprising observation was the lack of sex difference in the hepatic αGP levels following fructose intubation. It appears that the level of this metabolite was raised to a greater extent in the female than in the male.

A higher level of F1P was found in the small intestine than in the liver of both male and female rats following fructose intubation. There has been very little work done on the synthesis of lipids from carbohydrate in the small intestine. F1P can be metabolised to αGP in the small intestine and in the presence of fatty acids, which are readily available as a result of the hydrolysis of fats in the diet, there may be an increased esterification of this metabolite to form triglyceride (151).

The results of the *in vitro* experiments using isolated hepatocytes showed that there was no apparent sex differences in the rates of lipogenesis from glucose, fructose or glycerol. The rates of lipogenesis from glycerol were greater than from fructose which was in turn greater than from glucose in all cases examined. There was also evidence that a greater proportion of the total lipid was triglyceride when glycerol was used as a substrate. In addition incubation of hepatocytes with glycerol

increased αGP levels in the hepatocytes from either male or female rats but incubation of hepatocytes with either fructose or glucose had no effect on the metabolite level. There was a significant correlation ($P < 0.01$) between the levels of αGP and the rates of lipogenesis from carbohydrate, $r = .62$.

In conclusion, this study has demonstrated a higher level of serum triglyceride in the male rats than in females, and sucrose feeding raises the level of serum triglyceride in both sexes but the sex difference persists. However, there is no evidence to suggest that male rat synthesize more lipid than females _in vitro_ although the hepatic αGP level is higher in the male _in vivo_. Nevertheless, there is evidence to suggest a correlation between the _in vitro_ rates of lipogenesis and the _in vitro_ levels of αGP in isolated hepatocytes.

This study has demonstrated some sex differences in the metabolism of carbohydrate but these observations are not sufficient to explain the sex difference in serum triglyceride levels. A possible explanation for this observation may be that sex differences exist in the rate of clearance of triglyceride from the plasma.

MATERIALS AND METHODS

A. MATERIALS

1. Chemicals: All the chemicals used were of Analar grade and all solutions were prepared in glass distilled, deionised water. Unless otherwise stated all enzymes were obtained from Boehringer Mannheim.

2. Rats: All rats used in this study were of the Wistar strain. These rats were either bred in this laboratory or obtained from A. Tucks and Son, Rayleigh, Essex.

3. Diets: Two types of commercial diets were used, Dixon .86 and Dixon CDD[R]. The compositions of these two diets are shown in Table XVIa.

TABLE XVIa. Composition of Dixon CDD[R] and Dixon .86 diets*

Dixon .86	cwt/ton	DixonCDD[R]	cwt/ton
Wheat	10	Wheat	103
Barley	5	Maize	1.5
Concentrated meat meal	1.5	Soya meal	2.5
White fish meal	1.4	White fish meal	3
Grass	1	Milk powder	0.5
Yeast	1	Yeast	0.39
Molasses	1	Shredded suet fat	1.2
Salt	0.2	Pirkbent mineral salt	0.25
Vitamin 706	0.022	Salt	0.16
		Vitamin 17	0.223

*Approximate quantities, composition supplied by manufacturer: E. Dixon & Sons (Ware) Ltd., Crane Mead Mills, Ware, Herts.

The calculated composition of these two diets is given in Table XVIb.

TABLE XVIb. Calculated composition of Dixon CDD[R] and Dixon .86 diets*

	Dixon .86 (%)	Dixon CDD[R] (%)
Crude oil	2.03	8.34
Crude protein	19.29	24.18
Crude fibre	3.01	1.88
Digestible crude oil	1.44	7.18
Digestible crude protein	15.73	19.75
Digestible crude fibre	1.90	1.01
Digestible carbohydrate	50.83	42.84
Saturated fatty acids	0.42	2.82
Linoleic acid	0.72	1.98
Other unsaturated fatty acids	0.88	3.52
Cals/Kg		
Gross energy	3,942	3,931
Metabolizable energy	3,548	3,538

*values obtained from manufactuere: E. Dixon & Sons (Ware) Ltd, Crane Mead Mills, Ware, Herts.

4. Perfusion cabinet

A perfusion cabinet was made in this laboratory from a discarded glass-drying oven, a fan heater connected to a thermostat and a glass "lung" blown to specification (131) by the university glass-blower. A Watson-Marlow H.R. flow-inducer (Marlow, Bucks) was used to pump the perfusion system. Swinnex-13 filter units (Buc.France) was used to filter the circulating perfusate. Fig. 15a is a photograph of the finished product. Fig. 15b is a diagrammatic representation of the liver perfusion equipment.

B. METHODS

1. Long Term Feeding Experiments

Both male and female Wistar rats were used in this study. Rats (50g) were fed on diets of Dixon .86 or CDD[R] for 110 days. These diets were supplemented with either 5% sucrose or 5% glucose in the drinking water. Rats were allowed to feed *ad lib.* but were restricted from food intake 4 hr before sacrifice. The intake of sucrose or glucose depended on the age of the animal but there was no marked sex difference in the amount of sucrose or glucose consumed. Approximately 7.5g/day of each sugar was taken in by the mature animals.

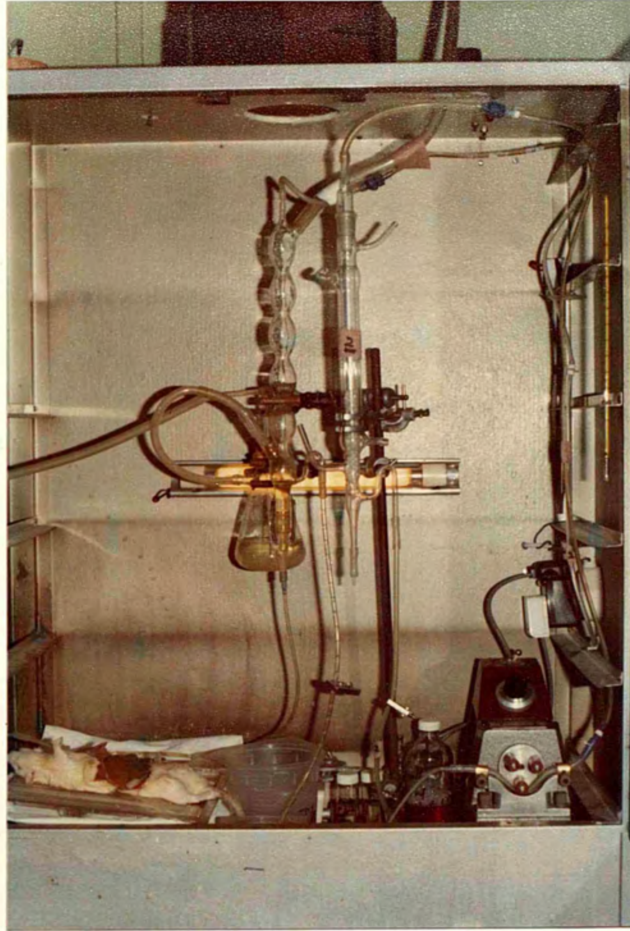


Fig. 15a. The perfusion cabinet used in this laboratory

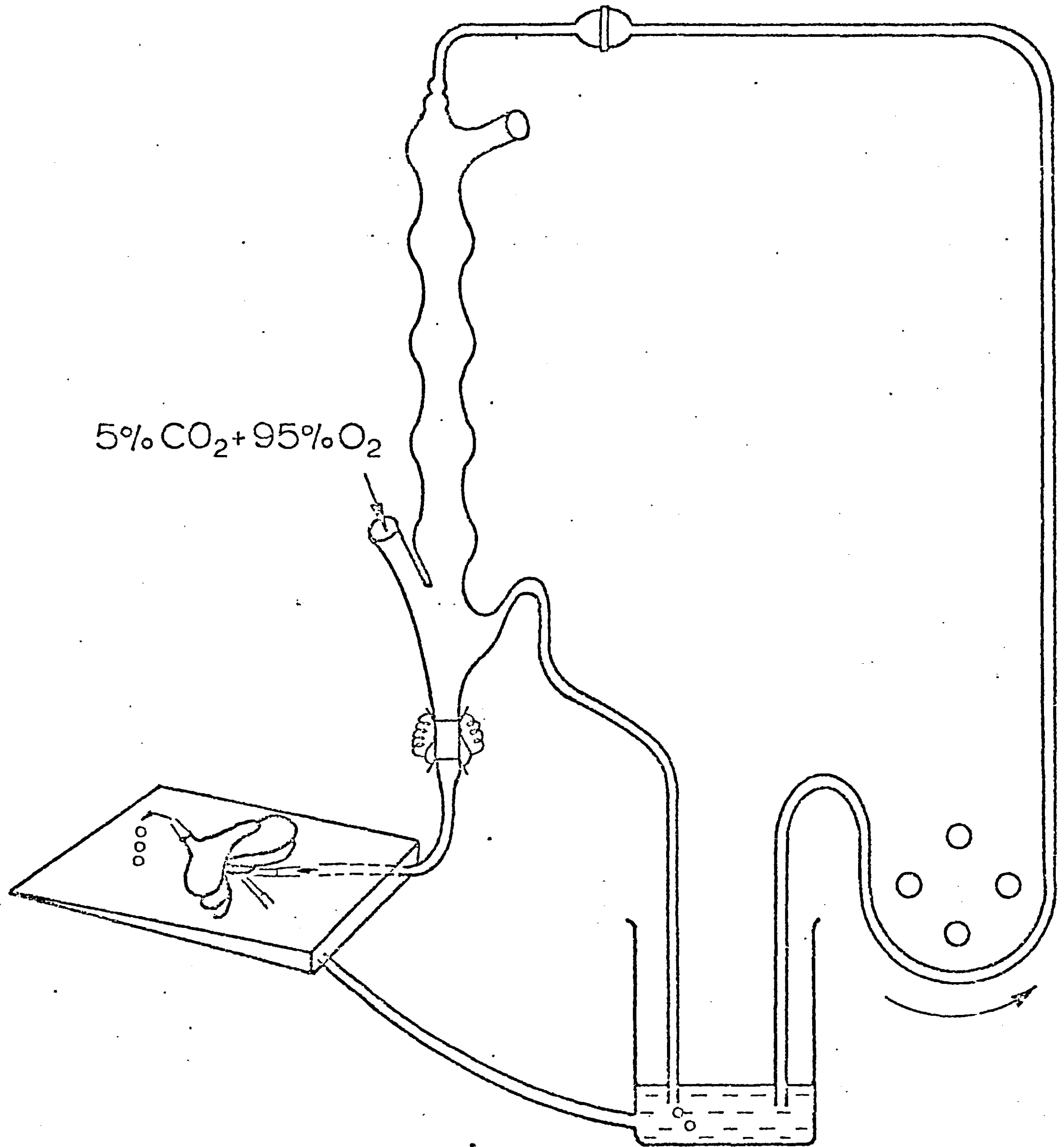


Fig. 15b. Diagram of the apparatus used in the preparation of isolated liver cells

(4) Metabolite Assays

Rats were killed by cervical fracture and a portion of the liver freeze-clamped with a pair of copper tongs (241) and then removed 45 sec after death. Another portion of the liver was also freeze-clamped and removed 2 min after death. The heart, the adipose tissue from the peritoneal cavity, the kidneys and the small intestine were then removed and immersed into liquid nitrogen, 4,5,6 and 7 min respectively after death. The tissues were weighed and care was taken to ensure that the tissues did not thaw out after freezing. These organs were stored at $-70^{\circ}C$ until used for assays.

In the preparation of protein-free extracts of animal tissues the frozen tissue was powdered in a porcelain mortar, pre-cooled with liquid nitrogen. A centrifuge tube containing 5ml of 6%(w/w) perchloric acid solution was weighed. Approximately 1g of the powdered tissue was added and quickly mixed with the contents of the centrifuge tube. The centrifuge tube was reweighed and the suspension of the sample in perchloric acid solution was homogenized using a Potter Elvejhem homogenizer. The precipitate obtained after centrifugation at 3,000g for 10 min at $4^{\circ}C$ was reextracted with 3%(w/w) $HClO_4$ (2ml). The supernatant obtained after centrifugation at 3,000g for 10 min was combined with the previous extract and the total volume made up with water to a concentration of 1g tissue per 8ml extract. This extract was neutralized with a small volume of 5M K_2CO_3 using methyl orange as an indicator. The precipitate formed was centrifuged at 3,000g for 10 min at $4^{\circ}C$ and the supernatant obtained was then used for the assay of metabolites. This method is a modification of the method of Michal and Beutler (141).

(i) Determination of F16P, DHAP, G3P and pyruvate. These metabolites were assayed by the enzymic method of Michal and Beutler (141). Triethanolamine buffer (0.4M, pH 7.6, 1.25ml), NADH (5mM, 0.02ml) and deproteinised sample (1.0ml) were placed in a quartz cuvette (1cm light path). The decrease in absorbance at 340nm was followed using a Pye Unicam SP 1800 recording spectrophotometer following the addition of various enzymes (10µl each enzyme). Glycerol 3-phosphate dehydrogenase (0.3 units) was added initially and the decrease in absorbance was related to the amount of DHAP present in the extract. Then triose phosphate isomerase (1.9 units) was added and the further decrease in absorbance related to the G3P level in the extract. This was followed by the addition of F16P aldolase (0.1 units) and the further decrease in absorbance was related to the F16P level.

Finally lactate dehydrogenase (2.8 units) was added and the decrease in absorbance was used to assay the pyruvate content of the extract. The enzyme system was checked by adding a known amount of F16P and thus any errors caused by insufficient NADH levels were prevented. The metabolite levels were calculated assuming that NADH has a molar extinction coefficient of 6.22×10^6 cm^2/mole at 340nm

(ii) Determination of αGP, glycerol and lactate. The method of Wieland (183) was used to measure these metabolites. Hydrazine-glycine buffer (0.2M glycine, 1M hydrazine, 2mM $MgCl_2$; pH 9.8; 1.40ml), ATP (50mM, 0.05ml), NAD (20mM, 0.05ml) and deproteinised sample (0.5ml) were placed in a quartz cuvette. The increase in absorbance at 340nM was followed using a Unicam SP 1800 recording spectrophotometer after the sequential addition of 20μl of each of the following enzymes: glycerol 3-phosphate dehydrogenase (8 units), glycerol kinase (17 units) and lactate dehydrogenase (10.6 units). The increase in optical density following the addition of each enzyme was related to the αGP, glycerol and lactate levels in the extract, respectively. At the end of the experiment a standard amount of αGP was added which served as a check on possible errors in the assay system.

(iii) F1P determination. The method of Eggleston (508) was used to determine F1P. Liver aldolase catalyses the breakdown of F1P to glyceraldehyde and DHAP. The DHAP formed can be reduced to αGP by glycerol 3-phosphate dehydrogenase coupled to the oxidation of NADH to NAD.

Liver aldolase was prepared from 3 rats killed by cervical fracture. Hepatic blood was washed from the liver by the slow perfusion of 150mM KCl (10ml) at 2°C. The combined livers were homogenized in 4 volumes of 150mM KCl and centrifuged at 3000g for 20 min. $(NH_4)_2SO_4$ (27.7g/100ml) was added to the supernatant with stirring and the final pH was adjusted to 7.8 with 2N NaOH. After allowing this solution to stand for 60-90 min in an ice bath, this mixture was centrifuged at 3000g for 20 min and a further portion of $(NH_4)_2SO_4$ (6.5g/100ml supernatant) was added. This solution was again centrifuged and the precipitate dissolved in a small volume of water and dialysed against 200 volumes of distilled water. All operations were carried out at 2°. After 4 hr the solution was centrifuged and 0.1ml of EDTA (0.1M, pH 7.4) was added to 4ml of supernatant and the mixture was allowed to stand at room temperature for 60 min. This last treatment destroys any polyol dehydrogenase present.

In the assay of F1P the following amounts of reagents in the assay

mixture were used: Tris buffer, (0.1M Tris; pH 7.4, 1.50ml), NADH solution, (4mM NADH, 0.05ml), 0.10ml of lactate dehydrogenase/F16P aldolase/glycerol 3-phosphate dehydrogenase and Triose phosphate isomerase enzyme mixture (15 units LDH, 0.42 units aldolase, 0.3 units GDH, 4.2 units TIM), sample (1.15ml), and liver F1P aldolase (48munits, 0.2ml). The decrease in optical density at 340nm (cuvette light path: 1cm, final volume 3.0ml) was followed using a Pye Unicam SP 1800 recording spectrophotometer.

(b) Protein and DNA determinations

The Biuret method was used for protein determinations, (187). Bovine serum albumin was used as a standard.

The method of Schneider (188) was used in the analysis of DNA. In this assay 1ml of the nucleic acid extract is mixed with 2ml of diphenylamine reagent [diphenylamine (1g) glacial acetic acid (100ml) and concentrated H_2SO_3 (1.75ml)] and heated for 10 min in boiling water. The absorbance was measured at 600nm. A standard curve was prepared using calf thymus DNA (Sigma, Na salt).

(c) Lipid determinations

(i) Lipid extraction from heart and liver. Lipids from these tissues were extracted by a simplified version of the 'classical' Folch (189) procedure. The method of Kates (190) was used. In this procedure, 1g (wet weight) of tissue was added to 0.6ml of water and homogenised by ultrasonication, (MSE ultrasonic disintegrator, maximum amplitude) for 1 min. Methanol-chloroform (2:1, v/v) (6ml) was then added and the mixture ultrasonicated for 2 min. The homogenate was centrifuged using a bench centrifuge, the supernatant decanted and the residue re-extracted with 7.6ml of methanol-chloroform-water (2:1:0.8) by homogenization for 2 min. After centrifugation, the combined supernatants were diluted with 4ml of chloroform and 4ml of water. The two phases were separated by centrifugation the aqueous phase was removed by aspiration and the lower chloroform phase was filtered through phase separating paper (Whatman IPs) to remove protein and water. The chloroform extract was then made up to 25ml. Two 2ml aliquots and one 1ml aliquot were taken and blown down to dryness under nitrogen. The 2ml aliquots were then analysed for cholesterol and triglyceride. Phospholipids in the 1ml aliquot were assayed. The remaining chloroform extract was evaporated to dryness under nitrogen in a weighed vial, left overnight in a desiccator containing anhydrous $CaCl_2$ and reweighed.

(ii) Triglyceride determination. For the routine analysis of triglycerides, a method based upon the techniques of Fletcher (191) and Kessler and Lederer (192) as adapted by Sigma (Technical Bulletin No. 405) was used. Isopropanol (5.0ml) was added either to serum (0.2ml) or to tubes containing lipid (see IV.B.1.c.i) and 0.2ml water. The mixtures were shaken for at least 5 min in the presence of activated alumina (Sigma triglyceride purifier). After centrifugation at 3,000 rpm for 5 min in a bench centrifuge (MSE Minor), 2.0ml of the clear supernatant was transferred into test tubes and saponified with N KOH (0.5ml) at 60°C for 5 min. Periodate solution [135mg sodium periodate in 50ml of 2N acetic acid (0.5ml)] was added and after 10 min 3ml of colour reagent [2M ammonium acetate (20ml); isopropanol (40ml); acetyl acetone (0.15ml)] was added and the mixture incubated at 60°C for 30 min. The absorbance of the resulting solutions were measured at 410nm in a Pye-Unicam SP 1800 spectrophotometer. A standard curve was prepared using triolein. Quality control sera (Precilip, Boehringer Mannheim) were routinely used to check the accuracy of the estimation.

An enzymatic analysis of liver triglyceride was used in one set of experiments. The method by Eggstein and Kuhlmann (208) was slightly modified. The glycerol liberated following saponification was estimated by the method of Wieland (183). As this method did not include the removal of phosphatides, it was discontinued because it resulted in an overestimation of the level of hepatic triglyceride. A standard curve was prepared using tripalmitin.

Triglyceride levels in tissues and serum was also determined by thin-layer chromatography. The results obtained were compared against values obtained by chemical determination. The method of Mantel *et al.* (385) was followed. Lipids in serum or tissue were separated on plastic TLC plates coated with silica gel G (Fertigfolien, Macherey-Nagel & Co., Germany) developed in petroleum ether 60-80°C/diethyl ether/glacial acetic acid (90:10:1, v/v) solvent system and the lipids were detected by spraying with 10% H_2SO_4 and heating at 140° for 20 min. Lipids were quantitated using a scanning densitometer (Chromoscan, Joyce Loebl).

(iii) Cholesterol determination. Cholesterol was determined by the method of Watson (386). The lipid sample (0.1ml) was added to 2.5ml of Watsons reagent (0.05M dimethylbenzene sulphonic acid: 7.0M acetic acid: 6.5M acetic anhydride), allowed to stand for 5 min and then reacted with 0.5ml concentrated H_2SO_4. The absorbance was measured at 580nM in a spectrophotometer. A standard curve was prepared using a standard cholesterol solution supplied by Sigma.

In cases where the serum was contaminated with haemoglobin a sample (0.2ml) was treated with isopropanol (5ml). The tubes were capped and shaken for 5 min then centrifuged at 3000 rpm for 5 min. The supernatant extract was evaporated to a minimum volume under nitrogen. The recovery of cholesterol by this procedure was 85%. This method of purification is adapted from the procedure described by Leffler (464).

(iv) Phospholipid determination. The method used was adapted from Kramil (210) and Zilversmit *et al.* (307). Kraml's method of digesting phospholipids at 200-225° in 1·2ml of 70% $HClO_4$ was followed. After cooling, the volume was made up to 5ml and the phosphate determined colorimetrically by the method of Zilversmit *et al.* (307), using ammonium molybdate and 1-amino,2-naphthol,4 sulphonic acid reagents. Since serum contains inorganic phosphate, TCA precipitation was necessary. The precipitate obtained, containing the phospholipid, was then digested as described above.

Since phospholipid-phosphorus is about 4% of the total phospholipid, a conversion factor of X 25 was used to determine the total phospholipid. Quality control sera (Precilip, by Boehringer Mannheim) were used to check the accuracy of the method.

(d) Statistical Analysis

The treatment of statistics were obtained from the article by Reed and Hendry (346).

Standard deviation was calculated from the formula

$$SD = \sqrt{\frac{\Sigma(x^2) - [(\Sigma x)^2/N]}{N-1}}$$

where Σ = "sum of"

$\bar{x}$ = mean of arithmetic average $\Sigma x / N$

x = observed values

N = number of observations

t-test for comparison of results used the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(SD_1)^2}{N_1} + \frac{(SD_2)^2}{N_2}}}$$

("degrees of freedom" = n - 2)

The t-test is a parametric method of statistical analysis and it assumes Gaussian distribution. In its proper use, the t-test is applicable only when data are Gaussian distributed. However, the t-test has been shown to be "robust" (ie not invalid) if the data exhibit moderate departure from the Gaussian distribution. However, it is not valid in cases of extremely non-Gaussian data.

One of the common misconceptions concerning statistical analysis has to do with the 5% level of significance. There is nothing magical or fundamental about a 5% level. It is only by convention that differences that occur with $P < 0.05$ are called "statistically significant". The other misconception has to do with the interpretation of a test of significance when the number of samples are too few for the statistical analysis to have any value. When the number of samples used are small, the probability of type II error is high. Type II error occurs when true differences are not indicated by a statistical test.

Recently, in an article by Zivin and Bartko (296), the inappropriate use of the t-test has been criticised. One criticism is in the use of t-test to test differences of means among more than 2 groups. In applying this test to each of the possible pairs, the number of such comparisons rapidly increases as the number of groups increases (if there are G groups, the number of comparisons is G(G-1)2). Because so many comparisons are made some pairs may randomly appear to be significant.

Another possible error may arise from errors in variances ($SD = \sqrt{variance}$). When variances are measured in several identical groups, some variance will randomly be smaller than others. If two groups have abnormally small variances and randomly different means, a "significant difference" would appear when the t-test is applied. When this experiment is repeated the results are not reproducible. This error is known as type I error and it occurs when a statistical test indicates that a significant difference exists where, in fact, there is no difference.

Conversely, and more likely, among groups whose means do differ significantly, one will randomly measure some abnormally large variances. When t-test is applied, a significance that should be present will not be found (Type II error).

Zivin and Bartko (296) proposed the use of analysis of variance for testing differences among more than two groups of result. An attempt to use an user programme, for the analysis of variance (Royal Holloway College, Department of Statistics) was made. However, the number of

observations in each group must be the same before the analysis of variance can be applied. Therefore some results have to be randomly discarded before this programme can be used.

Finally, a decision was made to use t-test for the statistical analysis of results bearing in mind its limitations.

The coefficient of correlation (r) of x and y values is given by the following equation:-

$$r = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^{n} (y_i - \bar{y})^2}}$$

The correlation coefficient can be tested for statistical significance by the equations

$$F = r^2(N-2)/(1-r^2)$$

or $$t_{N-2} = r[(N-2)/(1-r^2)]^{\frac{1}{2}}$$

2. Intragastric experiments

(a) Gastric intubation of fructose in rats under anaesthesia with exposed peritoneal cavity.

Mature rats were anaesthesized by nembutal injection (60mg of pentabarbitone/ml; AbboH,U.K.; 0.1ml/100g rat). A mixture of 95% O_2-5% CO_2 gas was administered to the rats to aid respiration. A fine plastic tube was inserted via the nostril, or through the mouth, into the stomach. The peritoneal cavity was then exposed and covered with a cotton wool pad moistened with KRB (287). The pad was maintained at 37° ($\pm 3^o$) with an electrical lamp. The appropriate amount of unlabelled or $[^{14}C]$fructose was then given to these rats.

(b) Gastric intubation of fructose in intact rats under anaesthesia

Young rats (50g) were obtained from a commercial source (see Materials) and kept on a diet of Dixon CDD[R] for 110 days. Fructose (70%, 2ml) was given via intragastric tube to diethyl ether anaesthesized rats which had previously been starved for 4-5 hr.

After the administration of fructose these rats were allowed to recover. Exactly after 3 hr these rats were killed by cervical fracture and the livers removed and freeze-clamped 45 sec after death. The small

intestines were removed and freeze-clamped 1.5 min after death.

In one preliminary experiment a rat weighing 330g was anaesthesized with 0.33ml nembutal and 1ml 5%[^{14}C]fructose containing 4µCi administered. The incorporation of [^{14}C]fructose into the various organs was measured after 3 hr.

(c) Liquid scintillation counting

All radioactivity was measured using a Parkard Tri-Carb liquid scintillation spectrometer. For lipid analysis toluene scintillant (5g PPO/L toluene) was routinely used. Aqueous samples (eg gluconeogenesis from [^{14}C]lactate) were assayed in a mixture of 1:2 ratio of Triton X-100 (Sigma): Toluene scintillant (5gPPO/L toluene). NCS tissue solubilizer (0.6N solution in toluene; Amersham/Searle) was used to digest tissues (1.1ml NCS/0.1g; digested overnight at 38°C) and the digest was counted in 10ml of 5gPPO/L toluene scintillation fluid.

3. In vitro experiments

(a) Hepatocyte preparation

Hepatocytes were prepared by a method adapted from the methods of Krebs et al. (158) and Wagle (152). In the procedure, rats were anaesthesized with nembutal (60mg of pentabarbitone/ml; Abbott, U.K.; 0.1ml/100g rat), the peritoneal cavity was opened and the portal vein and the inferior vena cava located. Two ligatures were loosely placed around the portal vein and a third ligature was placed loosely around the inferior vena cava just anterior to the branch point of the right renal vein.

The hepatic portal vein was then cannulated with a sterile luer (Braunula sterile Luer; Armour pharmaceutical Co. Ltd., Sussex) and firmly attached to the portal vein by the ligatures. KRB without Ca^{++} which had been preincubated at 37°C, gassed with 95% O_2 and 5% CO_2 and which was circulating in the perfusion apparatus, was allowed to flow into the liver. The inferior vena cava was immediately severed to prevent the liver from swelling. Immediately following this the rib cage was cut, exposing the thoracic cavity. An incision was made in the right atrium of the heart and a cannula introduced into the inferior vena cava via the atrium. The inferior vena cava in the peritoneal cavity was then tied and the liver perfused with KRB ($-Ca^{++}$) at a rate of approximately 25ml/min. When the liver appeared pale and the perfusate was free of blood cells (usually after 2 to 3 min), 20mg of crude collagenase (Boehringer; the enzyme tested for suitability, some batches caused the

isolated hepatocytes to be "leaky") was added to the remaining 100ml of KRB(-Ca^{++}) and the liver perfused until it was smooth and swelling; and leakage of medium from the liver was rapid (usually after 15 to 20 min). The liver was then removed and placed in a 250ml beaker containing 50ml of KRB, finely minced with a pair of scissors and gassed gently with 95% O_2 and 5% CO_2 for 1 min. A plastic funnel with a nylon mesh (Nybolt, No. 10; 132 micron, J.Staniar & Co., Manchester) placed over the top was used to filter the cells into preweighed centrifuge tubes (MSE 50ml capacity). These samples were centrifuged at 50g for 1 min in a bench centrifuge washed with KRB and recentrifuged at 50g for 1 min. After re-weighing these centrifuge tubes, the pellet in one tube was resuspended in exactly 16 volumes of KRB containing 1.5g/100ml of essentially fatty acid-free albumin. This was used in the study of lipogenesis from carbo-hydrate and gluconeogenesis. The pellet in another tube was resuspended in exactly 3 volumes of KRB containing 100mg glucose/100ml . and 1.5g of essentially fatty acid-free albumin. This was used in the study of αGP levels in the hepatocytes. The hepatocytes used in the study of both gluconeo-genesis and lipogenesis were preincubated for 1 hr and 2 hr respectively. In the study of αGP levels the cells were preincubated for 4 hr.

(b) <u>Gluconeogenesis from [^{14}C]lactate by isolated hepatocytes.</u>

Hepatocytes were preincubated for 1 hr to restore the ATP/ADP ratio to normal (174). The cell suspension (1ml) was added to a solution of [^{14}C]lactate (10 µl) in plastic tubes. Insulin or glucagon was added where indicated. The cells were incubated for the appropriate time and the reaction was stopped by the addition of 0.5ml 5% $ZnSO_4$ and 0.5ml of 0.15M $Ba(OH)_2$. Water (2ml) was then added and the protein precipitate removed by centrifugation. The protein free supernatant was mixed with 200mg of moist Dowex 50-x8 (H^+ form, 200 to 400 mesh) and 400mg of moist Duolite ES561 (dia-prosim, France) and shaken. This extraction procedure was repeated. When tested this amount of resins could remove 85% and 98.6% of 20mM D-[U-^{14}C]lactate after the first and second treatments respectively. However, this amount of resin was subsequently found to be insufficient, and did not completely remove 2mM L-[U-^{14}C]lactate when the specific activity was increased by 10 fold to 1µCi/40µmole L-[U-^{14}C]lactate. At least 800mg of moist duolite was required. Thus the accuracy of the method of Claus <u>et al.</u> (174) can be affected if the amount of Duolite introduced is not kept constant.

Appendix 1a.

<u>Effect of dietary carbohydrates on the weights of rats and of various organs.</u>

Tissue / Diet	Body weights Final mean wts. (g)					Livers Final mean wts. (g)					Hearts Final mean wts. (g)				
Experiment ✓	1	2	3	4	total	1	2	3	4	total	1	2	3	4	total
♂ Dixon .86	331± 35 (n=8)				331± 35 (n=8)	10.7± 1.8 (n=8)				10.7± 1.8 (n=8)	1.23± .19 (n=8)				1.23± .19 (n=8)
♀ Dixon .86	305± 40 (n=8)				305± 40 (n=8)	8.4± 3.7 (n=8)				8.4± 3.7 (n=8)	1.22± 0.16 (n=8)				1.22± 0.16 (n=8)
♂ Dixon CDD[R]	418± 40 (n=8)				418± 40 (n=8)	11.9± 2.4 (n=8)				11.9± 2.4 (n=8)	1.43± .34 (n=8)				1.43± .34 (n=8)
♀ Dixon CDD[R]	287± 29 (n=9)				287± 29 (n=9)	8.8± 1.2 (n=9)				8.8± 1.2 (n=9)	0.97± 0.35 (n=9)				0.97± 0.35 (n=9)
♂ Dixon CDD[R] + sucrose	443± 55 (n=5)	488± 39 (n=10)	440± 44 (n=5)	396± 45 (n=5)	450 ± 54 (n=25)	15.4± 2.9 (n=5)	16.7± 2.1 (n=10)	15.0± 2.1 (n=5)	14.3 ±2.7 (n=5)	15.6± 2.4 (n=25)	1.59± 0.17 (n=5)	1.49± 0.26 (n=10)	1.51± 0.25 (n=5)	1.35 ±.06 (n=5)	1.48± 0.22 (n=25)
♀ Dixon CDD[R] + sucrose	325± 66 (n=5)	306± 10 (n=10)	288± 16 (n=5)	285± 30 (n=5)	301 ± 34 (n=25)	9.6± 1.2 (n=5)	10.7± 0.8 (n=10)	10.1± 0.5 (n=5)	11.3 ±1.0 (n=5)	10.4 ± 1.0 (n=25)	0.88± 0.12 (n=5)	1.17± 0.26 (n=10)	1.02± 0.03 (n=5)	0.97 ±.05 (n=5)	1.04± 0.21 (n=25)
♂ Dixon CDD[R] + glucose	421± 56 (n=5)		436± 8 (n=5)	405± 4 (n=5)	420 ± 32 (n=15)	11.6± 2.3 (n=5)		13.2± 0.9 (n=5)	12.3 ±0.5 (n=5)	12.3 ± 1.5 (n=15)	1.42± 0.19 (n=5)		1.42± .31 (n=5)	1.12 ±.04 (n=5)	1.33± 0.24 (n=15)
♀ Dixon CDD[R] + glucose	267± 22 (n=5)		250± 14 (n=5)	277± 14 (n=5)	264. ± 19 (n=15)	8.1± 0.6 (n=5)		8.9± 0.8 (n=5)	10.7 ±0.3 (n=5)	8.9 ± .9 (n=15)	1.05± 0.13 (n=5)		1.00± 0.02 (n=5)	1.01 ±.06 (n=5)	1.02± 0.08 (n=15)

Deviations quoted are S.D. values.

Rats (50g) were fed <u>ad lib</u> for 110 days on diets of:
(i) Diet - Commercial diet Dixon .86 with water to drink
(ii) Diet - Commercial diet Dixon CDD[R] with water to drink
(iii) Diet - Commercial diet Dixon CDD[R] and 5% carbohydrate in drinking water

✓ Results were obtained from separate experiments

Appendix 1b. continued

Effect of dietary carbohydrates on the weights of rats and of various organs.

Diet / Tissue	Intestines (small) Final mean wts. (g)					Adipose tissues Final mean wts. (g)					Kidneys Final mean wts. (g)				
Experiment ⁄	1	2	3	4	total	1	2	3	4	total	1	2	3	4	total
♂ Dixon .86	7.2± 1.10 (n=8)				7.2± 1.10 (n=8)	3.5± 2.55 (n=8)				3.5± 2.55 (n=8)	2.55± 0.32 (n=8)				2.55± 0.33 (n=8)
♀ Dixon .86	8.0± 1.10 (n=8)				8.0± 1.10 (n=8)	5.9± 5.08 (n=8)				5.9± 5.08 (n=8)	2.40± .23 (n=8)				2.40± .23 (n=8)
♂ Dixcn CDD[R]	6.0± .87 (n=8)				6.0± .87 (n=8)	12.8± 5.40 (n=8)				12.8± 5.40 (n=8)	2.71± .35 (n=8)				2.71± .35 (n=8)
♀ Dixon CDD[R]	5.1± 0.66 (n=9)				5.1± 0.66 (n=9)	14.9± 4.91 (n=9)				14.9± 4.91 (n=9)	1.82± .19 (n=9)				1.82± .19 (n=9)
♂ Dixon CDD[R] + sucrose	9.3± 2.03 (n=5)	9.9± 0.90 (n=10)	9.8± 1.40 (n=5)	7.7± 0.88 (n=5)	9.31± 1.48 (n=25)	7.0± 1.62 (n=5)	20.5± 5.74 (n=10)	14.8 ±4.77 (n=5)	9.6± 3.17 (n=5)	14.46± 7.06 (n=25)	3.31± .62 (n=5)	3.24± 0.40 (n=10)	2.49± .48 (n=5)	2.95± .27 (n=5)	3.07± .57 (n=25)
♀ Dicon CDD[R] + sucrose	7.7± 1.36 (n=5)	7.8± 0.60 (n=10)	8.4± 0.39 (n=5)	6.6± 1.63 (n=5)	7.65± 1.11 (n=25)	11.3± 5.44 (n=5)	13.1± 4.38 (n=10)	13.0± 2.99 (n=5)	9.6± 5.01 (n=5)	12.01± 4.45 (n=25)	2.09± .24 (n=5)	2.17± 0.19 (n=20)	1.81± .20 (n=5)	1.84± .18 (n=5)	2.01± .25 (n=25)
♂ Dixon CDD[R] + glucose	7.6± 1.56 (n=5)		9.2± 0.72 (n=5)	7.3± 1.10 (n=5)	8.04± 1.39 (n=15)	10.0± 2.65 (n=5)		11.9± 2.52 (n=5)	11.1 ±2.69 (n=5)	11.00± 2.55 (n=15)	2.89± .47 (n=5)		2.44± .15 (n=5)	2.83± .22 (n=5)	2.72± .36 (n=15)
♀ Dixon CDD[R] + glucose	6.7± 0.89 (n=5)		7.3± 0.44 (n=5)	5.7± 0.46 (n=5)	6.53± 0.90 (n=15)	9.5± 4.16 (n=5)		7.2± 1.84 (n=5)	10.0 ±1.05 (n=5)	8.90± 2.80 (n=15)	1.79± 0.30 (n=5)		1.77± .18 (n=5)	1.94± .05 (n=5)	1.33± .20 (n=15)

Deviations quoted are S.D. values

Rats (50g) were fed *ad lib* for 110 days on diets of:

(i) Diet - Commercial diet Dixon .86 with water to drink
(ii) Diet - Commercial diet Dixon CDD[R] with water to drink
(iii) Diet - Commercial diet Dixon CDD[R] and 5% carbohydrate in drinking water

⁄ Results were obtained from separate experiments

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