CARBOHYDRATE AND LIPID METABOLISM IN MALE AND FEMALE RATS

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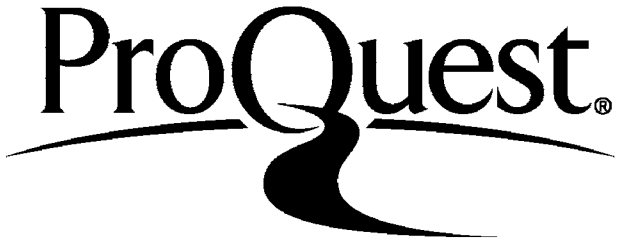

D E D I C A T I O N

To my wife, my children

and to

Our parents

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ABSTRACT

It has been suggested that there is a correlation between plasma triacylglycerol levels, carbohydrate consumption and the incidence of atherosclerosis in man. Fasting triacylglycerol levels have been reported to be related to carbohydrate intake in rats and the nature of the carbohydrate can also affect the levels of the lipid. Dietary sucrose is more hypertriglyceridemic than equicaloric glucose diets. Serum triacylglycerol levels have been also reported to be higher in males than in females, a difference which is maintained on various carbohydrate enriched diets.

In this study male and female rats have been used as experimental models to examine the effects of prolonged ingestion of sucrose and glucose, in addition to the normal CDD [R] diet, on some key lipogenic enzymes. Hepatic acetyl CoA carboxylase, fatty acid synthetase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and fructose 1, 6-bisphosphate aldolase activities were higher in adult females than in males of a comparable age. No sex related difference in the levels of microsomal glycerolphosphate acyltransferase was observed.

A comparison of the animals fed on sucrose and glucose supplemented diets shows that the disaccharide increases the activity of the enzymes examined to a greater extent than the monosaccharide with the exception of 6-phosphogluconate dehydrogenase which was lower in the sucrose-fed animals. These dietary effects appear to be common to both sexes and thus the sex differences in enzyme activities are maintained despite the changes in the diet.

No sex-related differences in enzyme activities were observed in young rats fed on Dixon .86 diet for 7 days after weaning but adult females had higher enzyme levels than male animals fed on the same diet.

Possible differences in hormone levels in male and female rats may account for the sex-related differences observed in this study.

INTRODUCTION

During the past few decades the incidence of sudden death has greatly increased. The major contributory factor is myocardial infarction, which is, in turn, attributed to coronary heart disease and in particular to atherosclerosis.

Atherosclerosis commences by thickening of the arterial wall, deformation of cells where this has occurred, inflammation of the lesion and this can lead to the occlusion of the coronary arteries, by the spontaneous formation of a blood clot (1, 2, 3). These atherosclerotic lesions called atheromatous plaques are lipid in nature and the composition of plaques is similar to blood lipid compounds, particularly with respect to triglyceride, phospholipid and cholesterol contents. At a late stage in the development of the plaque calcium is laid down in the lesion (4).

There is a close relationship between blood lipid levels and development of atherosclerosis (5, 6, 7, 8, 9). In the United States atherosclerosis and coronary heart disease have greatly increased in importance as cause of sudden death, since 1900 (10). It is also known that the incidence of the disease is much greater in men than in pre-menopausal women (10, 11, 12, 13). It has also been postulated that in man and in experimental animals, hyperlipidaemia occurs to a greater extent following fructose or sucrose feeding when compared to the ingestion of equicaloric amounts of glucose or starch. Sucrose induced hyperlipidaemia is apparently much greater in men than in pre-menopausal women (15, 16, 17, 19, 20, 21).

In 1971 Coltart and McDonald (22), reported that the increase in fasting serum triacylglycerol (TG) level in men, which occurs after sucrose ingestion, can be prevented

by oestradiol administration. McDonald (15) has also observed that the incidence of atherosclerosis is increased in young oopheroctomized and in post-menopausal women and that there may be a correlation between sex hormones, dietary carbohydrate, serum TG level and the incidence of atherosclerosis. It is of interest, therefore, to compare the effects on the components of sucrose, that is glucose and fructose, on glycerolipid metabolism.

I. LIPID METABOLISM

Storage lipids and, more directly, fatty acid are a major source of energy in mammals. Most lipid is stored as triacylglycerol (TG) in adipose tissue. As the capacity of the tissues to store carbohydrate in the form of glycogen is limited, the importance of fat reserves is clear. The source of the storage lipid may be exogenous, in the case of fat taken in in the diet, or endogenous lipid synthesized from non-fat substrates in the body tissues.

Most dietary fat is in the form of triacylglycerol which is hydrolysed by various lipases in the small intestine. The bile and pancreatic juices combine to hydrolyse triacylglycerol to yield a mixture of monoacylglycerol, and fatty acids known as chyle which is readily absorbed and then reconverted to triacylglycerol in the mucosal cells. Most of this triacylglycerol is incorporated into chylomicrons (23) which are secreted into the lymphatic system and enter the blood plasma via the thoracic duct . These chylomicrons are low density particles consisting mainly of lipid, the major component is triacylglycerol. Their major function is the transport of dietary fat (24).

The other major lipoprotein responsible for the transport of triacylglycerol is very low density lipoprotein (VLDL). VLDL is formed in the rough and smooth endoplasmic reticulium of liver and small intestine and is important in the transport of newly synthesized endogenous triglyceride (25, 26)(Fig 1) Thus VLDL accounts for much of the circulating TG produced from endogenous sources. There is a close positive correlation between VLDL synthesis and fatty acid synthesis in the perfused liver suggesting that the rate of fatty acid synthesis is an important determinant in the regulation of lipid secretion by the liver (28, 29).

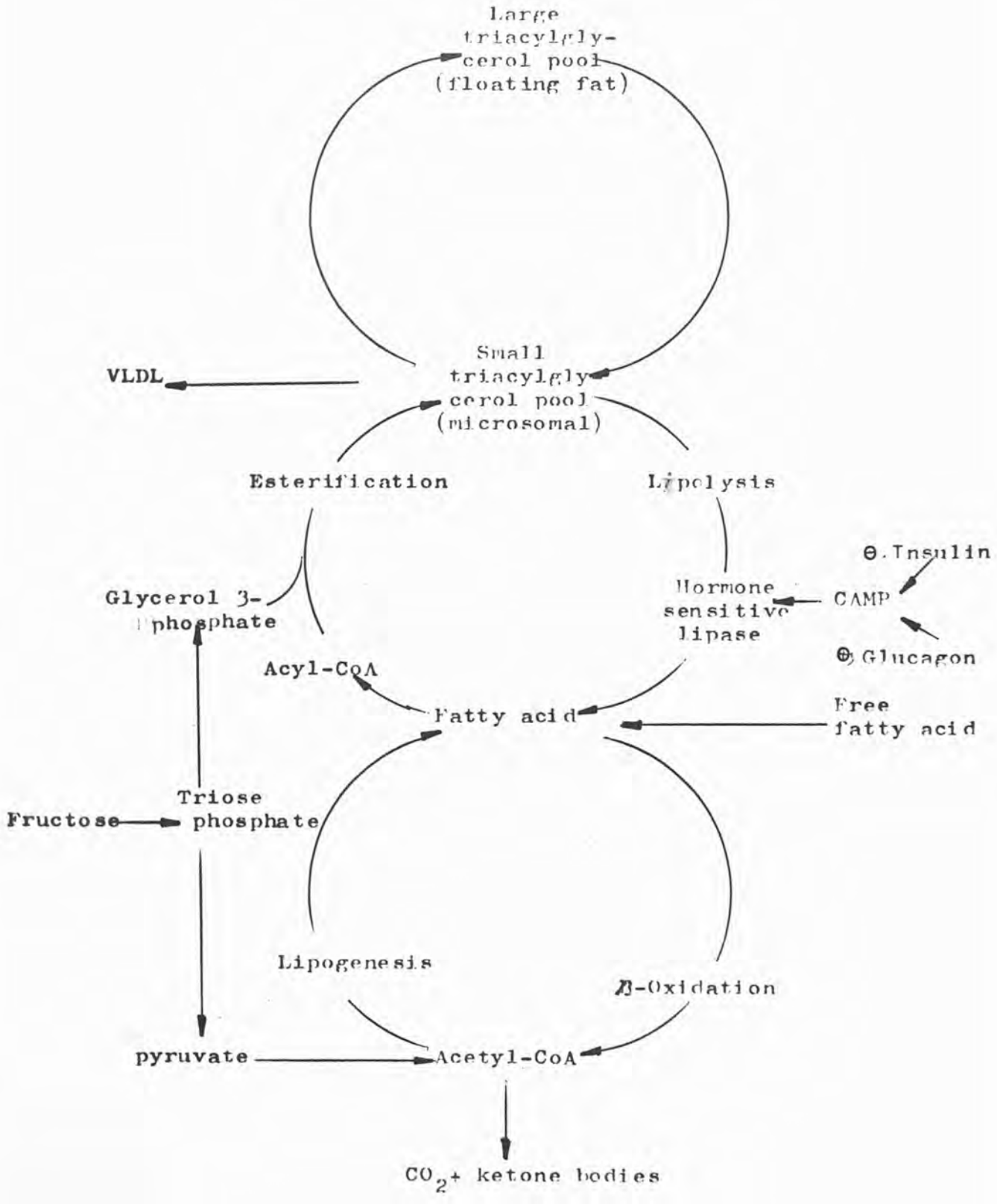


Fig. 1. Regulation of VLDL - triacylglycerol synthesis and β-oxidation of fatty acids in the liver(56)

(see pages 12,20 ,29)

Two other plasma lipoproteins occur which are involved in lipid transport, high density lipoprotein (HDL) and low density lipoprotein (LDL). These have significantly higher cholesterol and phospholipid levels than either VLDL or chylomicrons. There is evidence that LDL is formed following the breakdown of VLDL or chylomicrons by lipoprotein lipase (27).

Lipoprotein lipase is responsible for the clearance of triacylglycerol from the blood stream either for storage in the adipose tissue or for utilization of the lipid for energy requirements in other tissues.

Adipose tissue lipoprotein lipase is specific for lipoprotein triacylglycerol. Addition of heparin to adipose tissue causes the release of lipoprotein lipase into the medium, an effect which will also occur in vivo. The lipase thus released is known as post-heparin lipoprotein lipase. The activity of the adipose clearing factor lipase is postively correlated to the uptake of triacylglycerol by fat cells (30). A triglyceride lipase is also released from the liver following heparin treatment but this can be distinguished from adipose tissue lipoprotein lipase by protamine inactivation of the liver enzyme (31).

Triacylglycerol consists of a glycerol molecule esterified with three long-chain fatty acids. These fatty acids may be generated from non-fat compounds of the diet which can supply acetyl-CoA, if the necessary co-factors NADPH and ATP are present. This process is known as lipogenesis. Alternatively the fatty acid components of triacylglycerol may be derived from circulating free fatty acids (FFA) in the plasma.

The plasma free fatty acid level has been shown to be important in hepatic triacylglycerol synthesis and there is a

significant correlation between the plasma free fatty acid level and secretion of triacylglycerol from the liver (32). The synthesis and secretion of VLDL triglyceride has been shown to be stimulated by free fatty acid in a perfused liver preparation (33). FFA is the major form in which energy is transported from adipose tissues to other tissues and is subject to rapid turnover. Free fatty acids occur in saturated, mono-unsaturated and the poly-unsaturated forms. The type of free fatty acids either in number of double bounds or in chain-length, can influence the metabolism of the lipid in the liver, e.g. (1),there is a reciprocal relationship between the number of double bounds and rate of TG secretion by the liver (34, 35). (2),perfusion of palmitate and oleate do not produce identical VLDL, both the size of particles and the composition varies according to the substrate (33). Triacylglycerol synthesis by the liver also requires a glycolytic intermediate,sn-glycerol 3-phosphate. Thiscan be produced either from DHAP and NADH by the action of glycerol 3-phosphate dehydrogenase or from glycerol by glycerol kinase in the presence of ATP. The acylation of glycerol 3-phosphate is discussed in section E. The pathway for triglyceride and phospholipid synthesis is shown in Fig. 2. Reactions a, b, c, d and h are discussed in section E. Reactions (e) and (g) which utilize diacylglycerol for the formation of phospholipids are catalysed by CDP choline: 1, 2 diacylglycerol choline phosphotransferase (EC 2. 7. 8. 2) and CDP ethanolamine:1, 2 diacylglycerol ethanolamine phosphotransferase (E.C. 2. 7. 8. 1). Phosphatidyl ethanolamine and phosphatidyl serine are interconvertible (reaction (i) and (j) (36) and the former can also be converted to phosphatidyl choline (37).

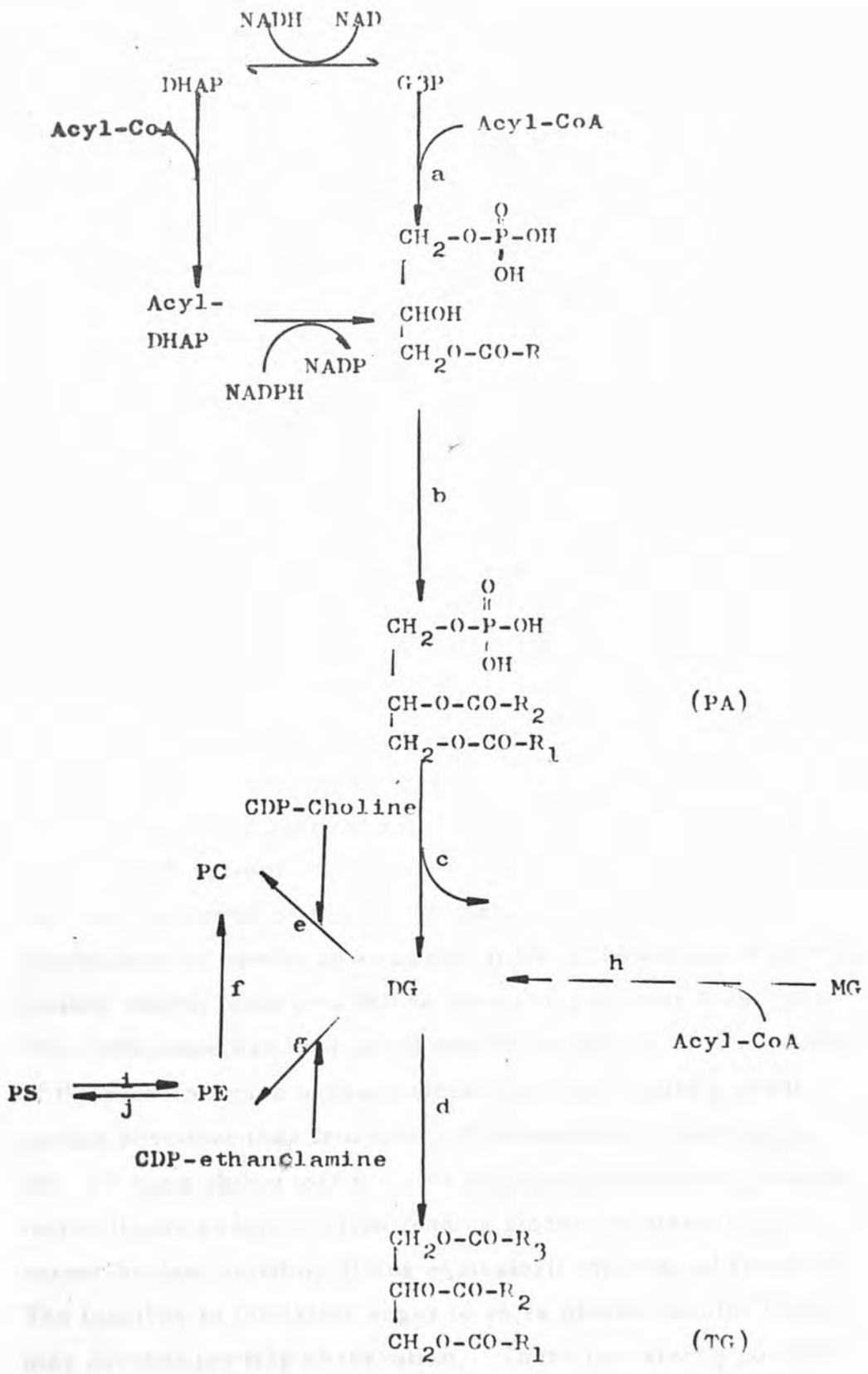


Fig.2. Pathways of triglyceride and phopholipid synthesis in the liver (38,39,40). LPA = lysophosphatidic acid. PA = phosphatidic acid, DG = diglyceride, TG = triglyceride, MG = monoglyceride, PC = phosphotidylcholine, PE = phosphotidyl ethanalamine, PS = phosphotidylserine.

A. Effect of carbohydrate on glycerolipid metabolism

There are a number of observations which suggest that hypertriglyceridaemia is related to the ingestion of dietary carbohydrate. There is a specific type of hyperlipaemia (Type IV) in humans characterized by an increased plasma triacylglycerol levels especially in the VLDL fraction. This condition is known as carbohydrate-induced lipaemia (41, 42) and usually occurs in individuals consuming large amounts of carbohydrate.

Carbohydrate ingestion by experimental animals can also lead to hypertriglyceridaemia and there appears to be a relationship between the type of carbohydrate consumed and the severity of the condition. The increase in plasma triglycerides level in rats given sucrose is greater than in animals fed on glucose or starch (43, 44, 45).

Dietary sucrose also causes a rise in the fasting TG level in both normoglyceridaemic and hyperglyceridaemic humans. Sucrose feeding of patients with carbohydrate-induced hyperglyceridaemia results in an aggravation of the condition whereas dietary starch leads to a fall in serum triglyceride level (46). This difference has been attributed to the degree of stimulation of lipoprotein lipase because dietary glucose elicits a greater insulin response than fructose. For example, Cryer et al. (30, 47) have shown that the rise in rat adipose tissue clearing factor lipase resulting from feeding glucose to starved rats cannot be duplicated by giving equicaloric amounts of fructose. The inability of the latter sugar to raise plasma insulin levels may account for this observation. There is a strong positive correlation between clearing factor lipase activity and triglyceride uptake in rat adipose tissue.

In addition, it has been suggested that the hepatic synthesis and secretion of triacylglycerol may be higher in fructose fed than in glucose fed animals. This has been attributed to the relative ease of conversion of fructose to both precursors, i.e. glycerol 3-phosphate and fatty acids. It is well known that fructose is metabolized at a much faster rate than glucose in the liver probably because of the high ketohexokinase and the relatively low glucokinase and phosphofructokinase levels (48) (see section II).

Maruhama and Macdonald (49) have shown that rats fed ad lib on a high fructose diet for 5 weeks have a greatly increased hepatic TG level. A smaller, but significant, increase was observed in animals fed a high glucose diet. This increase in hepatic TG was accompanied by an increase in plasma TG level, assayed 2 h. after the administration of intragastric fructose but no significant alteration was observed when glucose was given. They also examined the distribution of label in TG from liver, plasma and adipose tissue following the intragastric administration of either [^{14}C] glucose or [^{14}C] fructose. As expected the specific activity of the glycerol moiety was greater than that of the fatty acid in both cases.

In addition there was a correlation between the specific activity of the fatty acid in both liver and plasma triglyceride, suggesting that the fatty acid moiety of plasma triglyceride, which arises from both dietary fructose and dietary glucose, is synthesized in the liver. The increased liver and plasma triglyceride levels in the case of animals fed fructose can be correlated to an increase in the specific activity of both fatty acid and glycerol moieties. However, this data does not resolve the question as to whether the increased TG

levels are a result of increased synthesis or decreased clearance of the lipid.

Macdonald (50) has suggested that the greater contribution of fructose toward hepatic glyceride formation, compared to glucose, is the result of the ease of the conversion of the former to glycerol 3-phosphate. To support this view he has found that acute and chronic injection of glycerol will also cause a marked hyperglyceridaemia. An interesting observation in this context is that,glycerol,unlike glyceraldehyde and DHA, when given at the same time as [^{14}C] fructose will increase the incorporation of label into both the glycerol and fatty acid moieties of liver TG. This effect of glycerol was not apparent when [^{14}C] glucose was the precursor. The explanation for this phenomenon is not clear, but it is suggested that glycerol 3-phosphate may regulate triglyceride synthesis.

Dietary carbohydrate has been shown to affect hepatic lipogenesis. Zakim (51) has demonstrated that the incorporation of [^{14}C] fructose into fatty acids by liver slices is always greater than the incorporation of [^{14}C] glucose and short-term fructose feeding increases incorporation in both cases. However, Chevalier *et al.*(52) found that glucose was a better precursor of fatty acids than fructose. This discrepancy may be related to the observation of Romsos and Leveille (45)who found that at 10 mM hexose the incorporation was greater than fructose but at 100 mM hexose glucose was a betterprecursor. This is presumably related to the lowering of ATP levels by high concentrations of the ketose (47). However Romsos and Leveille (45) have found that long term feeding of fructose results in an elevation of hepatic ATP levels.

When *in vivo* fatty acid synthesis was measured by the incorporation of either [^{14}C] acetate or $^{3}H_2O$ into fatty

acids a higher rate of hepatic lipogenesis from both substrates was observed in the fructose fed animals. Their observations are consistent with the fact that fructose metabolism is confined to the liver (54) and that fructose depresses serum insulin levels in comparison with diets containing only glucose (55).

It has been demonstrated that in perfused liver of fed mice circulating glucose at a concentration less than 17 mM is not a major source of newly synthesized fatty acid, whereas lactate markedly stimulates lipogenesis from $^{3}H_{2}O$ and also contributed C_2 units for fatty acid synthesis. Glycogen also contributed significantly to fatty acid synthesis (53). It has been suggested that fructose could contribute to lipogenesis to a greater extent than glucose since fructose metabolism does not involve glucokinase and phosphofructokinase. (280)

Topping and Mayes (56) have examined the effect of insulin and fructose on lipid metabolism in the perfused liver. When livers from fed rats were perfused with blood containing either high insulin or fructose, there was an increased esterification and a decreased oxidation of free fatty acids (see Fig I). In addition increased secretion of VLDL triglyceride was observed in both cases. In all cases the effects were additive i.e. there were no significant interactions between the effects of fructose and insulin. They found that lipogenesis from [^{14}C] acetate was not stimulated by fructose but was stimulated by fructose in the presence of bovine insulin.

McGary and Foster (57) found that perfusion of the liver from fasted rat with fructose increased the recovery of [^{14}C] oleate in the lipid fraction, and decreased the rate of ketogenesis. Glucose produced identical but less marked effects. Ontko (58) found similar effects with isolated hepatocytes.

Lamb and Fallon (59) have examined the effects of

dietary sugars on the esterification of DHAP by rat liver microsomes. High fructose or glucose diets fed to rats over a 6 day period resulted in increased esterification by an *in vitro* system containing ATP, palmitate, CoA, NADH and microsomes. The increase observed was correlated to the increase in serum TG levels. Since DHAP acyltransferase and GPAT are thought to be functions of an identical protein (see I.E. 1) these changes probably represent changes in the total rate of esterification under different dietary conditions.

The nature of the fat in the diet also seems to influence the rate of glycerolipid synthesis. Macdonald (60) observed a sucrose-induced hyperglyceridaemia in humans fed a saturated fat diet but not in those fed on unsaturated fat. The effects of glucose ingestion were not significant. There is also a report that feeding a mixture of cholesterol and oleic acid in addition to fructose increases the hypertriglyceridemic effect of the hexose. It is of interest that glucose by itself did not produce hypertriglyceridaemia but when cholesterol was given in addition, the serum triglyceride levels were raised threefold (61).

Bruckdorfer *et al.*(62) have also reported that plasma triglyceride levels are raised by feeding rats on 50% sucrose for 150 days. The presence of saturated fat in the diet further elevated the plasma TG levels. However, the activity of various key lipogenesis enzymes were not affected by the nature of the fat in the diet although they were raised by sucrose feeding. They suggested that sucrose elevates plasma TG by stimulating hepatic lipogenesis whereas saturated fat may produce the same effect by reducing the rate of plasma clearance.

It seems clear that fructose can increase the rate of hepatic esterification of fatty acids. The exact mechanism of

the effect is not clear, it may possibly be the result of an inhibition of fatty acid oxidation or an effect on the enzymes involved in the esterification process. There also seems to be some evidence that fructose stimulates lipogenesis to a greater extent than glucose. It has been suggested that the effect of fructose on lipid metabolism is a secondary effect via insulin (56) or glucocorticoids (271).

B. Sex hormones and lipid metabolism

There is a considerable body of evidence which suggests that there are significant differences in lipid metabolism in male and female animals and that the sex steroids may play a role in these differences.

The output of triglyceride by isolated perfused livers from female rats is 2-fold greater than that by livers from male rats. Ovariectomy reduces the secretion of triglyceride and oestrogen administration to ovariectomized animals tends to return the output to normal levels (64, 65, 66, 67). Orchidectomy has no effect on the release of hepatic triglyceride from male animals (65). The exogenous fatty acid levels were kept constant in each case. The rates of uptake of FFA per liver by livers from female rats exceeds that from males. In each case the incorporation of exogenous oleate into triglyceride, phospholipid and oxidation products is proportional to FFA uptake. However livers from female rats incorporate more oleate into triglyceride and less into phospholipids and oxidation products than livers from males (66).

VLDL secreted by livers from female rats is a larger particle containing a smaller ratio of phospholipid and cholesterol to triglyceride than VLDL from male rats (67). The administration of ethynyl oestradiol raises serum TG levels in both males and females and also increases the

secretion of VLDL lipids. The rate of perfused liver secretion of VLDL in females exceeds that of males in both control and oestrogen treated animals (68).

The levels of serum TG in fed rats are greater in females than in males (64). However VLDL-triacylglycerol after a 16 h. fast period is higher in males (0.43 μmoles/ml) than in females (0.16 μmoles/ml) (67). Taken together this evidence points to the conclusion that although the output of VLDL-triacylglycerol by livers from female animals is greater than in the males the rate of utilization of VLDL-triacylglycerol must be greater in the female. Thus the turnover of plasma triglyceride in the female is slower than that in the male. Heimberg and his group (67) suggest that this is the result of hormonally mediated control mechanisms and that the sex steroid hormones and/or pituitary gonadotrophins may play a regulatory role.

The rate of glycerolipid synthesis by hepatic microsomes from female rats is greater than that from male animals. In contrast, the active accumulation of calcium ion and the subsequent inhibition of phosphatidate synthesis from glycerol 3-phosphate is lower in hepatic microsomes from female rats than from males (69). This may account for sex differences in hepatic triacylglycerol synthesis.

The work of Mandour et al.(70) suggests a possible mechanism for these sex differences. They found that oestrogens administered to ovariectomized female rats increased fasting plasma triglyceride levels and this change was correlated to an increase in both ACC and FAS activity. Progesterone administration was ineffective in this respect. An interesting observation was that the insulin/glucagon ratio increased by oestrogen administration. These workers suggest that the effect of oestrogen on hepatic TG metabolism is an indirect

effect mediated via changes of glucagon and to some extent insulin levels. There is one report (71) however, of a direct in vitro effect of oestrogen and testosterone on isolated rat liver cells. Lipogenesis from acetate was increased by both steroids. Most studies on the effect of oestrogen on lipid metabolism have involved the administration of the hormone in vivo.

In this context it is also interesting that the oral administration of ethynyl oestradiol to normal female rats leads to an increased serum TG level and to an increased lipoprotein lipase activity in the adipose tissues (30).

Hypertriglyceridaemia also occurs in pregnancy. The levels of plasma TG are markedly increased in the pregnant rat especially in the latter stages of gestation. The increased TG is mainly associated with an increase in VLDL-triacylglycerol and to a smaller extent in chylomicron triglyceride levels. These observations also may be associated with increased oestrogen levels which are known to occur during pregnancy (72). Knoop et al., (73) have reported that the hypertriglyceridaemia which occurs in pregnancy is the result of increased production of the lipid.

Kekki and Nikkila (74) have reported the elevation of plasma triglyceride in premenopausal women using oestrogen/progestogen oral contraceptives is associated with an increased turnover of the lipid including effects on both synthesis and clearance. The hypertriglyceridaemia is associated with increased VLDL levels (69).

Oestrogen administration in chicks has been reported to induce the synthesis of VLDL as judged by immunochemical methods (76).

Macdonald et al., (177, 78, 79) have found that men and

postmenopausal women who consumed a high fructose-low fat diet had a higher serum TG level than when on a normal diet whereas premenopausal women showed a decreased serum glyceride level on the same diet. Their work suggested a possible sex difference in response to dietary sucrose which may be related to the level of ovarian sex steroids.

Coltart and Macdonald (22) report that the increase in fasting serum TG levels in male baboons following administration of dietary sucrose can be prevented by oestradiol administration. No increase in fasting serum TG was observed in females. They suggested that oestrogen may enhance TG clearance.

Bruckdorfer et al. (80) demonstrated an elevation of plasma TG, in both male and female rats fed on sucrose, in comparison with those fed on starch. In this study male plasma TG levels were higher than females. A similar result was observed by Tay (81). He found that dietary sucrose elevated the TG level in both males and females but no increase was observed in females fed on a glucose supplemented diet. Again this evidence points to a possible sex difference in response to dietary carbohydrate.

Takemoto (82) found that when rats were fed for 30 days on fat free diet containing 77% carbohydrate, it caused changes in both serum and hepatic lipid levels. Fructose fed males had a low hepatic lipid and a high serum lipid level compared to males on other types of carbohydrates. In female rats the liver lipid levels were similar on all diets. The lowest serum lipid levels were found in glucose fed rats.

Jeffery and White (83) have suggested that oestradiol and progesterone have a synergistic effect on lipid metabolism. They found that administration of either steroid to male rats lowers the hepatic TG level but has no effect on serum TG.

However when the hormones are given together to rats fed on sucrose the liver TG levels are raised.

C. Hormonal regulation of glycerolipid metabolism

1. Lipogenesis

Insulin has been reported to stimulate lipogenesis in vivo and in vitro by several investigators. Jeannet et al. (84) have measured hepatic lipogenesis from ^3H_2O in perfused liver from fed mice in the presence of glucose. The rate of lipogenesis in insulin pre-treated animals was higher than that of animals which were treated with anti-insulin serum prior to the perfusion. Short-term stimulation of fatty acid synthesis by insulin in isolated preparations has not been consistently demonstrated (85) but recently Geelen et al. (87) have shown that fatty acid synthesis from ^3H_2O by isolated hepatocytes is stimulated by insulin and inhibited by glucagon. The rate of lipogenesis was found to be directly related to ACC activity measured in the absence of citrate (87).

Topping andMayes (86) have reported that when rat liver is perfused with a medium containing low levels (0.3 mM) of free fatty acid then insulin has no effect on the rate of lipogenesis. Increasing the FFA levels up to 1.9mM resulted in an inhibition of lipogenesis which could be partially overcome by addition of insulin into the perfusate.

Glucagon inhibits fatty acid synthesis in perfused mouse liver at glucagon concentrations greater than 10^{-9} M (88). This concentration is higher than that required for the stimulation of glycogen breakdown. These authors concluded that the action of the hormone is secondary to the depletion of favoured substrates of lipogenesis such as glycogen or lactate. (88, 85). In vitro and in vivo hepatic fatty acid synthesis has also been reported by Cook et al. (89) to be inhibited 15 min. after glucagon administration. This inhibition can correlate

to a lowering of hepatic malonyl-CoA levels in the *in vivo* experiment. However, the activity of ACC was not affected by the glucagon treatment and cholesterol synthesis from ^3H_2O was not inhibited. They concluded that the only explanation for these observations was that ACC was inhibited *in vivo* by an unknown mechanism. However, it should be pointed out that the *in vitro* effect on lipogenesis in isolated hepatocytes was obtained with a level of 1 μM glucagon.

Ochs and Harris (90) have found that both glucagon and dibutyryl c AMP inhibit lipogenesis in isolated rat hepatocytes at concentrations of hormone similar to those which produce change in glucose utilization, gluconeogenesis and pyruvate kinase activity. Thus they suggest that the diverse effect of the hormone is integrated in some way.

McGarry *et al.* (91) have examined the effect of glucagon on rat hepatocytes from fed animals and have found that the hormone stimulated the direction of fatty acid metabolism from synthesis to oxidation. Lactate and pyruvate had an opposite effect. Changes in the citrate and malonyl-CoA levels indicated that glucagon exerts its inhibitory effect on lipogenesis both by blocking glycolysis and by partial inhibition of ACC. These authors suggest a regulatory role for malonyl-CoA in altering the balance between fatty acid oxidation and in synthesis. They propose that in the fed animal insulin/glucagon ratio and malonyl-CoA level are high and fatty acid synthesis is rapid whereas fatty acid oxidation is reduced to a minimum. In the fasted or diabetic animal insulin/glucagon ratio and malonyl-CoA levels are low and fatty acid oxidation predominates.

A number of other hormones have been shown to exert a short-term effect on lipogenesis. Angiotensin II, adrenaline (92) and vasopressin (93, 94) inhibit lipogenesis in perfused mouse livers. These hormones do not apparently exert their effects via changes in hepatic c AMP levels (94).

Adrenalectomy has been reported to impair synthesis and secretion of triglyceride fatty acid as assayed in a perfused liver system (95, 96, 97). Normal release of triglyceride is restored by steroid treatment (95). This suggests that glucocorticoids can exert long-term effects on both lipogenesis and triacylglycerol secretion. Kirk et al. (97) have suggested that the action of adrenal glucocorticoids is mediated by insulin since a mild insulin deficiency is found in adrenalectomized rats.

2. Glycerolipid synthesis and secretion

Triacylglycerol release by perfused livers from alloxan diabetic rats has been reported to be reduced (98). Topping and Mayes (56) using perfused rat liver have demonstrated that insulin has a direct role in the regulation of hepatic lipid metabolism. Insulin enhances the secretion of triglyceride in VLDL and also causes more of the FFA taken up to be converted to VLDL. This is presumably because of the inhibitory effect of the hormone on β-oxidation. In addition, Topping and Mayes (86) have reported that insulin also enhances the export of newly synthesized FA in VLDL.

Woodside and Heimberg (99) found that when anti-insulin serum (AIS) is given to rats the concentration of blood glucose, ketone bodies and plasma FFA increase rapidly whereas plasma TG levels are increased more slowly. These factors are rapidly restored to normal by insulin administration.

The rate of ketogenesis from oleate by isolated perfused liver is accelerated by AIS administration and secretion of TG is suppressed. Maximal changes were observed 10 h after treatment of the animals with AIS. However, treatment of the animal with insulin resulted in a restoration of the normal secretion but only after 20 h , suggesting that the insulin

deficiency induces secondary metabolic changes, for example decreased availability of carbohydrate, which take some time to correct.

Insulin has been reported to depress TG degradation in perfused mouse liver (100) possibly via an effect on an insulin sensitive hepatic triacylglycerol lipase

Glucagon, on the other hand, has been reported to increase the rate of depletion of hepatic TG in a perfused rat liver (101), possibly by a stimulation of lipolysis (102). In addition TG export in VLDL is inhibited by glucagon (103).

When the liver is perfused with either glucagon or dibutyryl cAMP a decreased conversion of FFA into VLDL triacylglycerol and an increased rate of β-oxidation is observed (104, 105).

Topping and Mayes (56) suggest that glucagon raises the CAMP levels thus activating a hormone sensitive lipase which acts on a small precursor pool of VLDL-triacylglycerol (see Fig. 1). This accounts for the decrease in triglyceride export and synthesis from both exogenous and endogenously synthesized fatty acid.

Klausner and Heimberg (95) have examined the effect of adrenalectomy on TG release by perfused rat liver. The export of the lipid is reduced in the adrenalectomized animal and is restored by the treatment with cortisone. Addition of cortisol to perfusion media was also reported to enhance TG output in both normal and adrenalectomized rats.

Kirk et al. (97) have described an impaired synthesis of TG fatty acid in adrenalectomized rats whereas an effect on the synthesis of phospholipid fatty acid was not as apparent. They suggest that adrenalectomy could result in a preferential inhibition of triacylglycerol synthesis. This may be related

to the observation that the level of phosphatide phosphohydrolase (PPH) is lower in adrenalectomized animals, (see section I.E.2.). Glenny and Brindley (106) have examined the effect of cortisol on rates of hepatic glycerolipid synthesis in vivo. Treatment with cortisol increases the relative rate of glycerolipid synthesis from [^{3}H] glycerol and also raises the activity of PPH. The change in enzyme activity may be a direct or indirect effect of the hormone but is not mediated by an increase in insulin level since the level of the enzyme is increased both in starvation and in diabetes (see I.E.2.).

Reaven et al. (107) have found that corticosteroid treatment of rats and mice results in increases of plasma TG levels, an increased rate of accumulation of TG following inhibition of VLDL removal by Triton WR 1339, and an increase in the size and number of Golgi associated VLDL particles in hepatocytes. The results suggest that corticosteroid induces hyperlipoproteinemia through increased hepatic production of VLDL.

3. Triglyceride clearance

It is evident that hypertriglyceridaemia can result from a wide variety of causes. Indeed, there is a multiplicity of forms of hypertriglyceridaemia which occurs as a result of alterations in insulin secretion or action (108). At one extreme severe insulin deficiency leads to an impaired tissue lipoprotein lipase (LPL) activity, and consequently impaired clearing of both VLDL and chylomicrons from the plasma. On the other hand, hyperinsulinaemia, often associated with obesity, can induce hypertriglyceridaemia by a mechanism involving enhanced lipogenesis and enhanced TG synthesis and secretion (108, 109).

There is considerable evidence that plasma triglycerides are hydrolysed by LPL in extrahepatic tissues mainly adipose tissue and muscle (30). The LPL is associated with the endothelial cell surface of the blood capillaries but it is thought that, in the adipose tissue, it is synthesized in the adipocytes, secreted and transported to the capillary cells (111, 112). The enzyme can associate with a specific protein present in the plasma lipoprotein and this leads to a breakdown of the triacylglycerol component of the lipoproteins (113).

In rats the adipose tissue LPL is positively correlated with triglyceride uptake and also with plasma insulin levels in different nutritional states. For example, the enzyme level is high in the fed animal and falls to low levels on starvation (30).

Cryer et al. (30) have shown that the increase in adipose tissue LPL that results from feeding glucose to starved animals cannot be duplicated by giving either sucrose or fructose and they suggest that this is due to the inability of the latter sugars to raise plasma insulin levels (47).

Insulin has been shown to stimulate epididymal tissue LPL when administered in vivo. This effect is abolished by cycloheximide treatment (114). One report of an in vitro stimulation of LPL in this tissue by insulin has appeared (115). Adrenaline has been shown to inactivate the enzyme in vitro by a mechanism which is independent of protein synthesis and appears to involve enzyme processing following the translation process (116). Glucagon, ACTH and TSH have been reported to inhibit the increase in enzyme activity which occurs when the fat pads are incubated in a defined medium (112). The first two hormones are thought to exert this effect via changes in cyclic AMP levels. Thus the LPL is clearly distinguished from the activity of the triacylglycerol mobilizing lipase which is

responsible for the breakdown of the lipid to yield the plasma free fatty acids.

D. Lipogenic Enzymes

The de novo synthesis of fatty acid from carbohydrate involves the generation of pyruvate by glycolysis. Pyruvate is then converted to acetyl CoA which is used in the synthesis of the fatty acid. The process involves the cooperation of some key mitochondrial and cytoplasmic enzymes. (See Figs. 3 and 4).

1. Pyruvate dehydrogenase (E.C.1.2.4.1.), or, Pyruvate dehydrogenase complex

The pyruvate dehyrogenase complex (PDH) catalyses the oxidative decarboxylation of pyruvate, yielding acetyl-CoA. This irreversible reaction is an obligatory step for the entry of all carbohydrates into tricarboxylic acid cycle. The enzyme consists of three subunits, E_1, E_2, and E_3. The overall reaction:

$$\text{pyruvate} + \text{NAD} + \text{CoA}\xrightarrow{-SH} \text{acetyl-CoA} + \text{NADH} + H^+ + CO_2.$$

The individual reactions catalysed by each subunit occur as follows:

(1) $\text{Pyruvate} + [\text{thiamine-PP}]\text{-}E_1 \longrightarrow [\text{acetaldehyde-thiamine -PP}]\text{-}E_1 + CO_2.$

(2) $[\text{Acetaldehyde-thiamine-PP}]\text{-}E_1 + [\text{Lip } S_2]\text{-}E_2 \longrightarrow [\text{acetyl-S-Lip-SH}]\text{-}E_2 + [\text{thiamine-PP}]\text{-}E_1.$

(3) $[\text{Acetyl-S-Lip-SH}]\text{-}E_2 + \text{CoA - SH} \longrightarrow \text{acetyl-S-CoA} + [\text{Lip}(SH)_2]\text{-}E_2$

(4) $[\text{Lip}(SH)_2]\text{-}E_2 + (\text{FAD})\text{-}E_3 \longrightarrow [\text{Lip } S_2]\text{-}E_2 + [\text{reduced -FAD}]\text{-}E_3$

(5) $[\text{Reduced -FAD}]\text{-}E_3 + NAD^+ \longrightarrow [\text{FAD}]\text{-}E_3 + \text{NADH} + H^+$

Sum:-

$$\text{Pyruvate} + \text{CoA - SH} + NAD^+ \longrightarrow \text{acetyl-CoA} + CO_2 + \text{NADH} + H^+$$

E_1 = pyruvate dehydrogenase, E_2 = dihydrolipoyl transacetylase, E_3 = dihydrolipoyl dehydrogenase (117, 118).

Pyruvate dehydrogenase is located in mitochondria (119) in many different animal tissues, such as liver, kidney, heart and adipose tissue. It has been postulated that PDH appears in two forms, a phosphorylated, inactive form and a dephosphorylated, active form (120). Their interconversion is catalyzed by a PDH kinase and a PDH phosphatase (118). The two forms have been extracted from rat liver mitochondria by Roethenhouse and Wieland (121). The ratio of active/inactive forms varies between different tissues, e.g. in liver from normal fed animal 1/6 of the total PDH is in an active form, whereas in heart, kidney and muscle the ratio of 2/3 (122).

The hepatic enzyme activity has been estimated to be 123 mu/min /g wet wt. tissue (122). PDH can be converted to active form by incubating mitochondria in the presence of pyruvate. Maximal activation occurs at 2 mM pyruvate and half maximal activation occurs between 0.3 mM and 0.4 mM (123, 121). ADP also stimulates the formation of the active PDH. Palmitoyl-L(-)-carnitine counteracts both effects of pyruvate and that of ADP (121). The degree of phosphorylation of the enzyme is also regulated by a number of different factors, including ATP/ADP (121, 124, 122, 125), NADH/NAD (126, 127), and acetyl-CoA/CoA (126, 128, [illegible]). The concentration of Ca^{++} and Mg^{++} are also important in determining enzyme activity (118, 123, 130, 124). PDH kinase is also inhibited by pyruvate, ADP, NAD and CoA-SH, whereas NADH and acetyl-CoA as activators (120).

The oxidation of pyruvate is known to be inhibited by fatty acids. This may be the result of enzyme inhibition by products of β-oxidation, NADH and acetyl-CoA. Alternatively, β-oxidation may result in an increased ATP/ADP ratio in the mitochondria (125). Another possible explanation is that, fatty acids cause an acceleration of pyruvate transport into the

mitochondria (131). PDH is also subject to competitive inhibition by citrate (132). This is found to occur both in intact mitochondria and in a soluble enzyme preparation. The liver PDH activity seems to be regulated by various diets. Soling and Bernhard (133) found that in fructose fed rats, liver PDH activity was increased, and subsequently, acetyl-CoA production was elevated. They suggested that this sugar may change the state of enzyme from inactive form to active form by a phosphorylation/dephosphorylation mechanism. Hepatic PDH activity was lowered in starvation and increased after refeeding (122). The enzyme activity in normal fed rats is 123mU/g , whereas after 24 hr fasting it is decreased to 80 mU/g , after 90 min. refeeding with glucose the activity increases to 149 mU and, if refeeding is continued for 4 hr, the activity reaches 162 mU/g of liver. They suggested that interconversion of active and inactive forms of PDH may be regulated by the plasma concentration of fatty acid. For example, in starvation or in alloxan-diabetic rats, the level of fatty acid increases and a simultaneous inactivation of PDH occurs which results from conversion of active form to inactive form. In refed animals, however, the ratio of active form to inactive form increases. The effect of fatty acids on perfused rat liver is similar i.e., the enzyme activity is lowered. Glucose perfusion however leads to an increase in enzyme activity (122) which must be independent of the effect of the fatty acid.

The effect of some hormones on PDH levels have been investigated. Insulin injection by intraperitoneal route, causes an increase in rat liver PDH after 10 minutes which is maximal after 20 minutes (122). It was suggested that the mechanism of insulin action in this case may be via a lowering

plasma free fatty acid level. Oleate infusion has been shown to abolish the increase in PDH activity caused by insulin. Stanbie et al. (338) have studied the acute, in vivo effect of anti-insulin serum on the liver PDH activity. They found that although changes in the rate of fatty acid synthesis estimated by the incorporation of label from ^3H_2O were found, no change in PDH was observed. In contrast to the liver enzyme, adipose PDH activity was found to parallel changes in fatty acid synthesis in the above experiments. Claus and Pilkis (134) have found that glucagon has no effect on rat liver PDH activity, either in isolated hepatocytes or liver homogenate from fed and starved animals. Therefore, they believe that PDH is not regulated by glucagon.

2. Citrate cleavage enzyme (E.C.4.1.3.8), ATP:citrate oxaloacetate-lyase (CoA - acetylating and ATP dephosphorylating) (CCE)

$$\text{Citrate} + \text{ATP} + \text{CoA} \xrightarrow{\text{CCE}} \text{acetyl-CoA} + \text{oxaloacetate} + \text{ADP} + P_i$$

Translocation of acetyl-CoA from mitochondria into cytoplasm is an obligatory process in de novo fatty acid synthesis. There are two hypotheses for the mechanism of this transfer, firstly, that free acetate leaves the mitochondria and is then converted to acetyl-CoA by acetyl-CoA synthase Secondly, that citrate generated by the action of mitochondrial citrate synthase leaves the nitochondria and is used to generate acetyl-CoA in the cytoplasm by the action of citrate cleavage enzyme (135) (Fig. 3). Acetyl-CoA synthase and CCE are both present in rat liver (136, 137, 138), but the level of the latter is much higher, suggesting that the citrate shuttle is the most important mechanism for acetyl-CoA transfer from mitochondria to cytoplasm. CCE is a cytoplasmic enzyme (139)

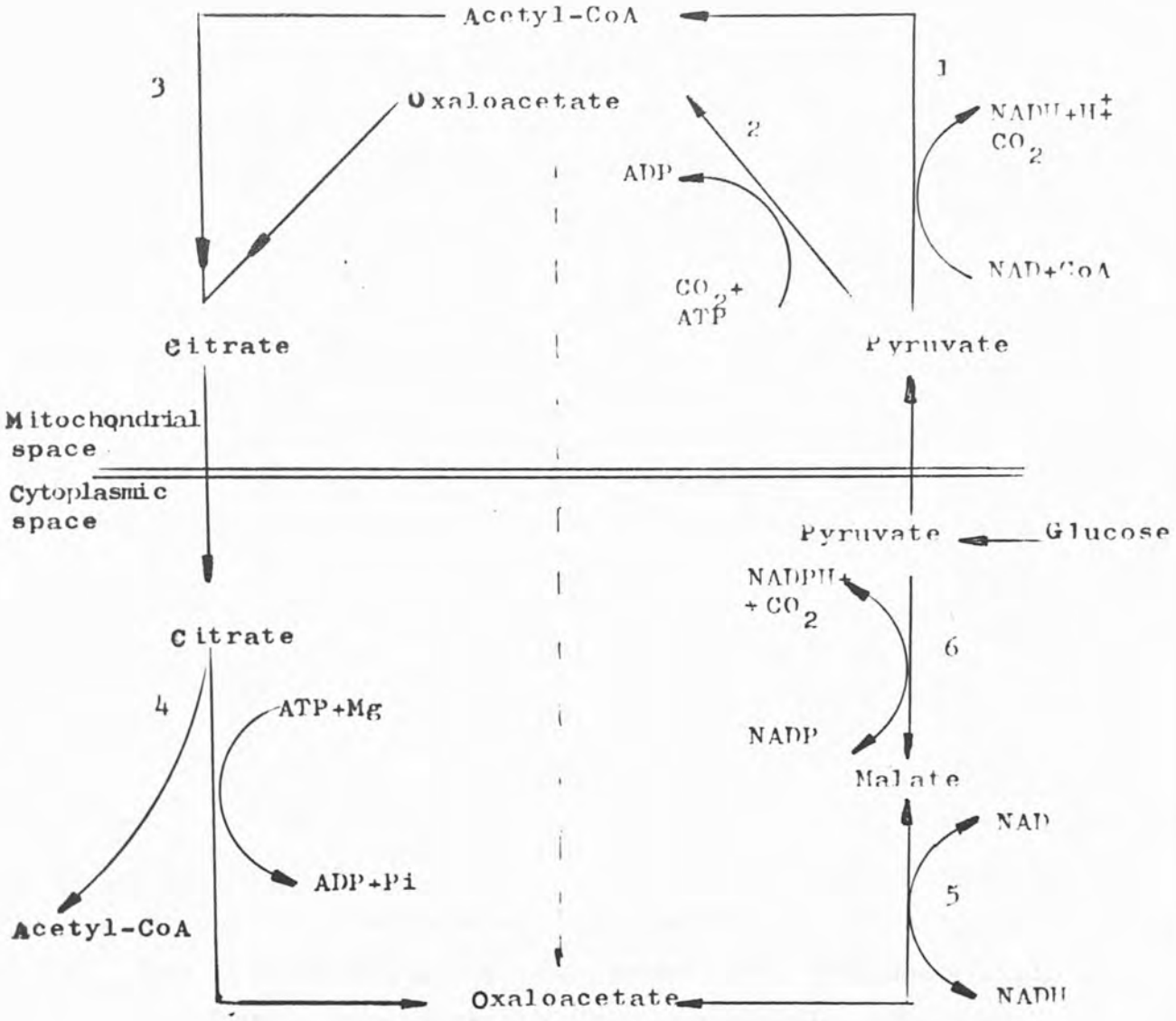


Fig. 3. Scheme for the generation of cytoplasmic acetyl-CoA from pyruvate showing the involvement of the mitochondria.

the km for citrate is 5.8×10^{-4} M (140). The specific enzyme activity is reported to be 0.012 μmol/min/mg protein (141) It has been shown that Mg^{++} stimulates the enzyme activity (142, 143) and ADP is a competitive inhibitor, ki = 1.71×10^{-4} M. The enzyme is susceptible to oxidation of the sulfhydryl group at the active site and EDTA is also needed to prevent enzyme inactivation. (143).

Citrate cleavage enzyme is influenced by different nutritional states. Rat liver enzyme activity decreases in fasting and in alloxan-diabetic animals and in rats fed high-fat diets (138). Refeeding by diets enriched in carbohydrate, causes a marked increase in the rat liver and adipose enzyme activity (145, 139). Gibson et al. (139) showed a six-fold increase in hepatic CCE following re-feeding (139) of fasted rats. They also observed that the rate of enzyme synthesis increases eleven-fold following this treatment. Acute insulin administration to the rat or to isolated perfused normal liver (146) causes an increase in enzyme activity. The effects of chronic hyperinsulinaemia in young rats is an increase in rat liver CCE activity after a 6 day period (146). Greenberg et al. (147) found a higher enzyme activity in obese mice with hyperinsulinism in comparison to that in lean mice with lower plasma insulin level. They suggested that there is a positive correlation between the plasma insulin level and citrate cleavage enzyme activity.

3. Acetyl-CoA carboxylase (E.C., 6.4.1.2) or: Acetyl-CoA-CO_2 ligase (ADP-forming)

Malonyl-CoA formation from acetyl-CoA and HCO_3^- is catalysed by acetyl-CoA carboxylase (ACC) in the presence of ATP and citrate. This cytoplasmic enzyme is found in several different animal tissues (148, 149, 150, 151, 152).

Gregolin et al.(153) believe that the enzyme exists in two forms, a less active or inactive monomeric form and an active polymeric form. Citrate or iso citrate, and Mg^{++} or Mn^{++} ions are required for the in vitro conversion of the monomeric form into the polymeric form (154, 148, 151). Acetyl-CoA carboxylase is an allosteric enzyme, with a biotin prosthetic group and consists of three subunits; biotin carboxylase (BC), biotin carboxy-carrier protein (BCCP), and transcarboxylase (TC). The carboxylation process involves two partial reactions:

$$ATP + H\overset{-}{C}O_3 + BCCP \xrightarrow[Citrate+Mg^{++}]{BC} \overset{-}{C}O_3 - BCCP + ADP + P_i$$

$$\overset{-}{C}O_3 - BCCP + CH_3CO\text{-}S\,CoA \xrightarrow{TC} -O_2CCH_2CO\text{-}SCoA + BCCP$$

Acetyl-CoA carboxylase catalyzes the initial committed step in de novo fatty acid synthesis and is thought to serve a regulatory function (155). Liver ACC activity is closely related to the rate of fatty acid synthesis (156). The Km for acetyl-CoA is 3×10^{-5} M, the Km for bicarbonate is 6×10^{-3} M and for ATP; 2×10^{-4} M under optimum assay conditions (157, 156, 148).

Liver and adipose tissue ACC are inhibited by palmitoyl-CoA and also by long-chain fatty acids (129, 158, 151, 156). Dorsey et al. (159) and Taketa and Pogell (144) believe that this inhibition is non-specific and it may be attributed to the detergent properties of acyl-CoA. Ogiunara et al. (160) found that liver ACC is competitively inhibited by palmitoyl CoA, even in the presence of phosphatidyl choline which is known to bind palmitoyl-CoA and thus overcome the detergent effect of the latter. They suggest that long-chain acyl-CoA is a specific, physiological inhibitor of liver ACC.

Nikawa et al. (161) have examined the structural requirements for the inhibitory effect of long-chain acyl CoA and concluded that the inhibitor binds to a specific site on the ACC molecule.

The discovery of a fatty acid and acyl-CoA binding protein (FABP) from rat liver cytosol which enhances the activity of ACC and overcomes the inhibition of the enzyme by palmitoyl-CoA has led to the theory that FABP may participate in the short-term regulation of lipogenesis (162).

The hypotriglyceridaemic agent, clofibrate, when given to rats increases the concentration of FABP in the liver cytosol two-fold. This can be correlated to an increase in the uptake of FFA by perfused livers from clofibrate-treated rats. However, there has been no evidence for a change in the rate of esterification of FFA following drug treatment (168). The β-oxidation capacity of mitochondria has been reported to be enhanced by clofibrate (169). Thus, Renaud et al. (168) suggest that FABP plays an important part in the uptake and metabolism of FFA in the liver. Malonyl-CoA has also been reported to be a competitive inhibitor of liver ACC with respect to isocitrate ($K_i = 1\text{-}4 \times 10^{-5}$ M) (163).

Craig et al. (164) showed parallel changes in ACC and fatty acid synthetase in response to different nutritional states. It has been shown that rat liver ACC activity increases at the time of weaning (165). This change has been related to a change from a high-fat to a high-carbohydrate diet.

A decrease in enzyme activity was found to occur in fasted rats (166, 156), and it was suggested that this may be due to the accumulation of fatty acids which may, in turn, inhibit the ACC activity (166). An increase in ACC activity was observed on re-feeding the fasted animals with a high-carbohydrate diet (156, 164, 149, 139). Nakanishi and Numa (167)

have examined the rate of synthesis and degradation of ACC in different nutritional and hormonal states, and have shown that changes in enzyme activity were accompanied by proportional changes in the amount of immunochemically reactive protein. The relative rate of synthesis of acetyl-CoA carboxylase as measured by the incorporation of [^{3}H] leucine were decreased 1.9 and 1.7 fold by fasting and diabetes, respectively. However, the rate of enzyme degradation is the same in normal, re-fed and diabetic rats (half-life, 59 hr), but is accelerated in fasted rats (half-life 31 hr). They suggest that the former animals are in a steady state whereas the latter is adjusting to a new environment and this adjustment results in accelerated enzyme degradation. Tanabe et al. (170, 171) have identified a specific polysome fraction involved in the synthesis of liver ACC by binding of ^{125}I-labelled antibody against ACC to these polysomes. They showed that the binding of [^{125}I]-anti acetyl-CoA carboxylase to the isolated polysomes from fasted rats is two-fold lower than from normal animals, whereas this binding is four-fold higher when the polysomes are isolated from liver of a re-fed rat. Thus the hepatic content of ACC synthesising polysomes is closely correlated to changes in ACC synthesis due to different nutritional states, the translational process is not greatly affected.

The effect of different hormones on the ACC activity has been studied by several workers. Halestrap et al. (129) found that exposure of adipocytes to insulin (in the presence of glucose) causes an increase in initial enzyme activity (assayed in the absence of citrate) whereas a decrease in the initial ACC activity occurs with adrenalin (in the presence of glucose and insulin). Total activity (after incubation with citrate) does not

change following the above treatments. They found no evidence that phosphorylation-dephosphorylation mechanism is involved in these changes of activities. They suggested that the concentration of citrate (as activator) and fatty acyl-CoA thioesters (as inhibitor) may play an important role in the regulation of ACC activity in the adipocytes. Yen et al. (147) however found no correlation between liver ACC activity and plasma insulin level in obese and normal mice, suggesting that insulin has no direct effect on the enzyme activity.

Fatty acid synthesis from $^{3}H_2O$ can be inhibited by glucagon in perfused mouse liver but only at high concentrations ($>10^{-9}$M). No effect on ACC activity was observed, and it was suggested that the effect of the hormone on fatty acid synthesis was secondary to the stimulation of glycogenolysis and that liver preparations treated with glucagon would gradually convert the favoured precursors for lipogenesis (glycogen and lactate) with a less favoured precursor glucose (88).

Glucagon administration can prevent the increased hepatic ACC activity produced by re-feeding fasted rats. The hormone has no effect on the adipose tissue enzyme (173).

Evidence from in vitro studies has been put forward to suggest the view that rat liver ACC is regulated by a phosphorylation-dephosphorylation mechanism. It has been postulated that one of the subunits of the enzyme is phosphorylated and that this leads to a decrease in enzyme activity (174, 175). However, when chicken liver cells are grown in monolayer in the presence of insulin the ACC becomes extremely phosphorylated without any apparent change in enzyme activity. Dibutyryl cAMP did

not affect the phosphorylation (176) of the enzyme suggesting that a cAMP independent protein kinase is involved.

Adrenalectomy does not affect rat liver ACC activity , but the adipose enzyme increases following the surgical treatment, and decreases after glucocorticoid administration to the animals (173).

Ovariectomized animals have a lower hepatic ACC activity than normal animals, and oestrogen administration results in increased enzyme activity (178), but this recovery does not occur in ovariectomized/adrenalectomized animals. Afolabi et al. (178) found that corticosterone is necessary for the oestrogen-induced increase in ACC activity. On the other hand, Diamant et al. (179) have found that oestrogen administration in large doses to normal female rats over a two-day period depressed the liver enzyme activity. Mandour et al. (70) have also found an increase in liver ACC activity when ovariectomized rats are treated with 17 β-oestradiol. This increase was accompanied by a rise in the fasting serum TG level and an increase in the portal vein insulin/glucagon ratio. They suggested that the increase in ACC activity and plasma TG level may be related to the change in the hormone ratio rather than a direct effect of the oestrogen on the liver.

4. Fatty acid synthetase

In the presence of NADPH, fatty acid synthetase (FAS) catalyzes the formation of fatty acid from acetyl-CoA and malonyl-CoA. This cytoplasmic enzyme (180, 181) has been resolved into seven subunits (182, 183) each with a separate enzymic capability:

1. Acetyl-CoA ACP transacylase
2. Malonyl-CoA ACP transacylase
3. β-Ketoacyl ACP synthase
4. β-Ketoacyl ACP reductase
5. β-Hydroxybutyryl ACP dehydrase
6. Enoyl ACP reductase and
7. Fatty acyl-S ACP thioesterase

The seven reactions catalysed by the subunits are as follows:

$$\text{Acetyl-S-CoA+ACP-SH} \xrightarrow{1} \text{Acetyl-S-ACP+CoA-SH}$$

$$\text{Malonyl-S-CoA+ACP-SH} \xrightarrow{2} \text{Malonyl-S-ACP+CoA-SH}$$

$$\text{Acetyl-S-ACP+Malonyl-S-ACP} \xrightarrow{3} \text{Acetoacetyl-S-ACP+} CO_2 + \text{ACP-SH}$$

$$\text{Acetoacetyl-S-ACP+NADPH+H}^+ \xrightarrow{4} \text{D(-)-β-Hydroxybutyryl-S-ACP+NADP}^+$$

$$\text{D(-)-β-Hydroxybutyryl-S-ACP} \xrightarrow{5} \text{Crotonyl-S-ACP+}H_2O$$

$$\text{Crotonyl-S-ACP+NADPH+H}^+ \xrightarrow{6} \text{Butyryl-S-ACP+NADP}^+$$

$$\text{Fatty acyl-S-ACP+}H_2O \xrightarrow{7} \text{Fatty acid+ACP-SH}$$

(ACP = Acyl Carrier Protein)

The subunits of mammalian FAS are tightly bound together, and loss of activity is observed on dissociation (184). FAS from mammalian tissues contains one mole of 4-phosphopantotheine as a prothetic group per mole of enzyme (185, 186). Fatty acid synthetase from liver, mammary glands and adipose tissues of the same species have similar molecular weights, and are inactivated, by dissociation into subunits, at the same rate (187).

Hepatic FAS has a Km for acetyl-CoA of 4.4×10^{-6} M

for malonyl-CoA the Km is 10×10^{-6} M (190) and for NADPH the Km is 4.0×1^{-5} (180). Citrate and Mg^{++} both stimulate the enzyme activity (181) and the PH optimum is between 6.8 to 7.0 (185, 189, 190). Sarvaggas and Porter (191) found that both acetyl-CoA and malonyl-CoA competitively inhibit the enzyme activity at high concentrations.

The liver enzyme is also competitively inhibited by palmitoyl-CoA and long-chain fatty acids (192). This inhibition occurs at a palmitoyl-CoA concentration of 20×10^{-6} M (337). Knoche et al. (192) found that BSA protects the FAS activity from the inhibitor. Dorsey et al. (159) believe that the inhibition of FAS by long-chain acyl-CoA is nonspecific and is due to the detergent properties of the compound. Heavy metal ions, such as Ca^{++} can inhibit the FAS activity (181), EDTA is therefore required to protect the enzyme. The sulphydryl group of the active site is sensitive to oxidation, therefore, cysteine or 2-mercaptoethanol is required for both the in vitro assay and the storage of the enzyme (193).

Rat FAS activity is low in foetal liver and in suckling animals, but dramatically increases after weaning and remains at a high level in adult animals (180). This change seems to be related to the change in food intake, from the high-fat diet in the suckling animals to the low-fat diet after weaning.

Volpe et al. (194) using isotopic immunochemical techniques have found that the diet-induced differences in FAS activity resulted from changes in both enzyme synthesis and degradation. They found an increase in enzyme synthesis after

weaning but the rate of enzyme degradation does not change. However, the rate of enzyme degradation increases in fasted rats (194). [The half-life in normal fed animals is 6.5 days but in fasted rats (194)] The half-life in normal fed animals is 6.5 days but in fasted rats it drops to 18 h. Other workers have also found similar changes in enzyme activity on fasting and refeeding (194, 75, 196, 197, 198, 156).

A fructose-containing fat free diet when fed to the fasted animals leads to a higher increase in FAS activity than when other carbohydrates are given (55, 44, 62).

Volpe and Vagelos (199) found that liver FAS activity can be affected by various nutritional states, for example, FAS decreases in starved and diabetic rats and a sharp increase occurs after refeeding a fat-free diet to the starved animals. They also found that the turnover of the FAS protein is dramatically changed in response to dietary states, and the changes in FAS activity are related entirely to the changes in the amount of enzyme protein.

Craig and Porter (200) found that parenchymal cells isolated from animals re-fed a fat-free diet for 16 h following a 48-h period of fasting, incorporated L-(U-^{14}C) Leucine into FAS at a rate 16.2 times faster than cells isolated from normal fed animals. In fasted and alloxan-diabetic animals the rates of incorporation were 0.34 and 0.11 respectively in comparison to normal rats. However, they showed that the rate of total soluble protein synthesis in rat liver fed a fat-free diet after 48-h period fasting is much higher than in control animals and the absolute FAS synthesis is close to the rate of soluble protein synthesis.

A specific polysome fraction which participates in the synthesis of the liver FAS has been identified by Alberts et al. (201). These polysomes can bind to the ^{125}I-labelled antibody against FAS, then have been used to study the relationship between changes in FAS synthesis and dietary factors. There is a decrease in the number of polysomes synthesising FAS in starved animals and an increase in animals refed a fat-free diet.

Liver FAS can also be affected by different hormonal states. Insulin, glucagon, glucocorticoid, thyroxine and oestrogen have been shown to change the enzyme activity. Greenberg et al.(147) and Hems et al. (188) have found a correlation between liver FAS activity and plasma insulin level in obese mice. Bruckdorfer et al. (55) have compared the effect of various high carbohydrate diets on liver and adipose FAS. They found that fructose feeding resulted in high levels of hepatic FAS and plasma TG but the lowest adipose FAS and plasma insulin levels. They could find no correlation between plasma insulin levels and hepatic FAS activity but there was a correlation between the latter and plasma TG levels when the animals were fed on fructose-containing diets. They suggest that when fructose or sucrose is fed to rats then the rate of hepatic lipogenesis becomes an important factor in determining plasma TG levels. A lower FAS activity occurs in the diabetic rat which is restored to normal after insulin administration (200, 202). The increase in enzyme activity appears to be the result of an increase in the rate of enzyme synthesis (202). In contrast, Volpe and Vagelos (203) have found no evidence that insulin is necessary for the regulation of liver FAS activity. Their data shows an increase in liver enzyme activity following

fructose feeding for a two-week period of both normal and diabetic rats. They suggest that it is the carbohydrate intake which is causing the increase in FAS rather than a specific insulin effect.

The induction of liver FAS in fasted-refed rats can be inhibited by glucagon and theophylline administration (173). Lakshmanan et al. (202) also found that both glucagon and cAMP administration reduce the increase in the liver FAS activity following the feeding of a fat-free diet to previously fasted rats. They suggested that the enzyme activity is regulated by the relative levels of insulin and glucagon in the blood.

In the hyperthyroid state hepatic FAS activity increases and in hypothyroid animals enzyme activity is lower than in controls (180, 204). Neither adrenalectomy nor hydrocortisone administration have any effect on liver FAS activity, but, adipose FAS activity is decreased in animals treated with the hormone and increased in adrenalectomized animals (204). This change in adipose enzyme activity is due to change in the rate of enzyme synthesis. Volpe and Maraza (173) demonstrated that although adrenalectomy of normal rats has no effect on liver FAS activity, adrenalectomy of diabetic rats results in restoration of the decreased FAS which is associated with diabetic animals. Hypophysectomy also causes a decrease in liver FAS activity (173). Volpe and Maraza have suggested that the effect of the pituitary gland on both FAS and ACC mediated via the thyroid hormones.

Bruckdorfer and Yudkin (80) found no statistically significant difference in hepatic FAS activity in male and female rats. White and Tulloch (29) showed an increase in liver FAS activity following oestrogen administration to the ovariectomized rat. They suggested that in oestrogen-treated animals the plasma insulin level is raised and the effect of oestrogen on FAS may be predominantly via the increase in plasma insulin.

Afalabi et al. (178) have also found an increase in hepatic FAS activity when ovariectomized rats are treated with 17β-oestradiol, but this recovery in enzyme activity does not occur in adrenalectomized/ ovariectomized animals. They found that corticosterone is necessary for the oestrogen-induced increase in FAS. The mechanism of the oestrogen and progesterone effects on lipid and carbohydrate metabolism have been studied by Mandour et al. (70). They found that oestrogen administration to ovariectomized rats slightly decreased the portal vein basal insulin level but suppressed the alpha cells of the pancreas which results in a greater reduction of basal glucagon levels. This decreased the plasma glucose level, increased the liver FAS activity and also increased the fasting plasma triacylglycerol level.

In contrast to oestrogen, progesterone had no significant effect on the above parameters, although both portal vein basal insulin and glucagon were increased, but the relative insulin to glucagon (I/G) ratio in portal vein was unchanged. They suggest that changes in enzyme activity and fasting plasma TG level in oestrogen-treated animals may be a secondary effect which follows changes in the portal insulin/glucagon ratio..

Qurashi et al.(205) studied the regulation of pigeon liver FAS and have isolated two forms of the enzyme, by affinity chromatography, called halo$_a$ and halo$_b$. They suggested that variations in the enzyme activity due to nutritional and hormonal factors may be due to an interconversion of two enzyme forms under a phosphorylation-dephosphorylation mechanism regulated by insulin and glucagon.

5. Glucose 6-phosphate dehydrogenase (E.C.1.1.1.49) or D-glucose 6-phospho-NADP oxidoreductase (G6PDH)

The enzyme catalyzes the conversion of glucose 6-

phosphate (G6P)to 6-phosphogluconate (6-PG), in the presence of NADP. This conversion occurs in two steps as follows:

Glucose 6-phosphate+$NADP^-$ $\xrightarrow{G6PDH}$ $NADPH+H^+$+6-phospho-gluco-γ-lactone

6-phospho-gluco-γ-lactone $\xrightarrow{H_2O}$ 6-phospho-gluconate.

The second part of the process is the hydrolysis of the lactone by a specific lactonase yielding 6-PG (214,. G6PDH appears to be a regulatory enzyme (214, 211) in the direct oxidation pathway of glucose, and is also important in the generation of NADPH for utilization in de novo fatty acid synthesis (165, 207, 208, 209). The liver enzyme has been purified and the Km for G6P is $1.3x10^{-5}$M and Km for NADP is$1.3x10^{-5}$M (210, 212). Hepatic G6PDH activity is believed to be 1.08/(u/g liver) in male rats fed on normal diet. This cytoplasmic enzyme (211) is stimulated by some divalent ions such as Mg^{++} and Mn^{++} (210, 213). Liver enzyme activity is competitively inhibited by free fatty acids and acyl-CoA, Ki for octanoate is 1×10^{-3}M (215) and also by orthophosphate (Ki $=1 \times 10^{-2}$M) (212). Wititsumannakul and Kin (216) also demonstrated the inhibitory effect of palmitoyl-CoA on the rat liver enzyme activity. Palmitoyl CoA inhibits the enzyme by dissociating the tetrameric dehydrogenase to the dimeric form, which is inactive (216).

Rat liver G6PDH activity seems to be higher in young animals than in adults (165), and it is also influenced by different nutritional states. The enzyme activity in rat liver is decreased after 48 hr starvation and dramatically increased after refeeding (217, 218). Sugawa and Marita (219) fed rats which had previously been starved for 48 hr on 69% fructose or 69% corn starch diets for a four-day period. They found an increased G6PDH activity in fructose fed animals in comparison

to corn starch fed rats. Bruckdorfer et al. (62) also reported a higher liver G6PDH activity following a 150-day period when rats were fed 50% sucrose as compared to control starch-fed rats. They also found that saturated fat in the diet resulted in decreased enzyme activity. Chang et al. (221) have also found an increase in G6PDH in response to dietary carbohydrate, which is independent of the age and the strain of the animals; the highest increase was found in sucrose-fed rats. They also reported that these increased levels could be correlated to the hepatic lipid level. Other investigators have also found that carbohydrate feeding especially sucrose (207) and fructose diets, leads to increased G6PDH activity (229, 219, 208). Gibson et al. (139) also found a decreased G6PDH level in starved rats and a supranormal level of enzyme following fat-free feeding of the starved animals.

High-fat diets depress the liver G6PDH activity, whereas high-carbohydrate diets have the opposite effect. The G6PDH activity in animals fed on saturated fat is higher than in animals fed a diet containing unsaturated fat (207). Sucrose appears to be more effective than starch in increasing the hepatic enzyme activity but the starch diet resulted in a higher adipose tissue G6PDH than as comparable sucrose diet. These results suggest an increased rate of liver lipogenesis as indicated by changes in PK and G6PDH activities, and a decrease of the rate of lipid synthesis in adipose tissues following a period of 50 days on a sucrose diet.

The effect of insulin on the liver G6PDH activity has been investigated by several workers. Although insulin seems to have a role in increasing enzyme activity. Rudack et al. (222) did not find any correlation between plasma insulin

level and enzyme adaptation resulting from high-carbohydrate diets. They believe that, although the enzyme induction is observed following large doses of insulin, that the increase in enzyme activity is a secondary effect resulting from an increased consumption of food which occurs following insulin administration (223). On the other hand, Grimm (224) found a marked increase in G6PDH activity in human and rat hepatoma cells exposed to a level of 10 μU/ml of insulin for one hr. This was correlated to a 50% fall in cyclic AMP levels in the cells.

In fasting-refeeding experiments glucagon has been shown to inhibit the induction of rat liver G6PDH. The kinetics of the induction indicates that the hormone decreased the rate of enzyme synthesis without a change in the rate of enzyme degradation. Cyclic AMP will also repress the synthesis of the enzyme. The change in enzyme activity does not appear to be the result of the accumulation of an enzyme inhibitor (225, 226). It is suggested that glucagon and cAMP can regulate the levels of lipogenic enzymes by exerting an effect on protein synthesis.

Liver G6PDH activity has also been shown to be influenced by thyroxine. It has been shown that hypothyroid animals have a lower G6PDH activity than normal (227, 204) and that the normal activity can be restored by thyroxine injection (227,165). The liver G6PDH activity in normal rats fed a stock diet was 2.09 units/g, whereas in thyroidectomized rats the level was 1.47 units/g of liver. The induction of G6PDH by dietary carbohydrate does not occur in hypophysectomized rats (217). It was shown that the individual treatment of the above animals by thyroxine, somatotropin or cortisone separately does not lead to a recovery of enzyme activity, but administration of all three hormones together is effective in raising enzyme activity.

Lockwood et al. (165) found that the activity of G6PDH is higher in adult female rats than in males. They showed that the enzyme activity is lower after castration. They suggested that these changes in enzyme activity reflect enzyme regulation by male and female sex steroid hormones. Huggins & Yao (227) have also found similar sex differences. However they showed no change in enzyme activity following oestradiol injection of ovariectomized animals and a four-fold increase in G6PDH activity following the oestradiol injection of males. They also found a decreased enzyme activity following testosterone administration to female rats and the administration of both testosterone and 17β-oestradiol together resulted in a marked increase in G6PDH activity of both sexes.

6. 6-Phosphogluconate dehydrogenase (E.C.1.1.1.44) or 6-Phospho-D-gluconate-NADP oxidoreductase (6PGDH)

This cytoplasmic enzyme catalyzes the second step in pentose monophosphate cycle, the conversion of 6-phosphogluconate to D-ribulose 5-phosphate which is linked to the reduction of NADP (210).

$$\text{6-Phosphogluconate} + \text{NADP} \xrightarrow{\text{6PGDH}} \text{D-ribulose 5-phosphate} + \text{NADPH} + H^+ + CO_2$$

The purification and characteristics of liver 6PGDH have been studied by Procsal and Halton (228). The enzyme is composed of two subunits. The Km for 6PG is 7.1×10^{-5} M and for NADP is 1.3×10^{-5} M. In contrast to G6PDH, Mg^{++} and Mn^{++} have no effect on 6PGDH activity (228). NADPH is a competitive inhibitor of the enzyme, Ki = 2×10^{-5} M, suggesting that 6PGDH activity may have some role in the maintenance of the $NADP^+$:NADPH

ratio in the cell. This enzyme activity may be inhibited in vivo when this ratio is lowered (in normal rat this ratio has been calculated by Veech et al. (229) to be 0.01). Both free fatty acid and long-chain acyl-CoA can inhibit the liver 6PGDH activity, the Ki for palmitoyl-CoA is 2.5×10^{-2} M (144).

The liver 6PGDH activity can be influenced by different nutritional states. The enzyme activity is decreased during fasting and increased after refeeding the fasted animal with a high-carbohydrate or a fat-free diet (230). Procsal et al. have studied the activity of rat liver 6PGDH in response to different dietary states using pulse-labelling method in order to study the changes in the enzyme synthesis and degradation. They found that the rate of enzyme synthesis increases by 3.7 times when a pellet diet replaced with a high-carbohydrate (60% glucose for four days) and by 5.6 times greater when the fasted rat refed with a high-carbohydrate diet (60% glucose for a four-day period after two days fasting). Rudack et al. (231) also found a marked increase in rat liver 6PGDH activity following an eight-day period of feeding with 60% glucose or fructose diet, the increase was proportional to the carbohydrate consumption. In these experiments a high-fat diet feeding resulted in a decrease in enzyme activity. They showed that dietary carbohydrate causes 10-fold increase in the rate of enzyme synthesis and 6-fold increase in the rate of the enzyme degradation, whereas high-fat diet decreases the 6PGDH synthesis only. An increase in 6PGDH activity following 60% glucose or fructose diets has also been reported by Fitch and Chaikoff (208). The increase in enzyme activity was greater in the fructose fed animals. The similar changes in the enzyme activity following dietary glucose or fructose have also been observed by Angelico et al. (220) and Szepesi and Freedland (232).

The effect of insulin on 6PGDH activity has been studied by several workers. Weber and Convery (233) have suggested that insulin is an inducer for the hexose monophosphate shunt dehydrogenases. Greenberg et al. (147) found no correlation between plasma insulin level and liver 6PGDH activity in obese mice and in lean mice. Rudack et al. (231) have demonstrated that the increase in 6PGDH activity following insulin administration is the result of increased consumption of carbohydrate following this treatment rather than a direct effect of the hormone on 6PGDH.

In contrast to G6PDH, no change in the rate of enzyme synthesis due to glucagon administration has been observed (230, 225). The carbohydrate-induction of 6PGDH does not occur in hypophysectomized rat (217). Individual administration of thyroxine, somatotropin, or cortisone does not overcome the lowering of 6PGDH, but together the hormones can restore the enzyme level.

Baquer et al. found that thyroidectomy causes a decrease in hepatic 6PGDH activity. They also observed other changes in lipogenic enzyme levels which are characteristic of an insulin deficient state and, since there is no reduction in circulating insulin levels in hypothyroid animals (234), they suggest that there is a decreased sensitivity of the liver to insulin in such a state.

The enzyme activity in adult female rat liver is four-fold that in males (227). Huggins also showed a decrease in enzyme activity after ovariectomy, whereas orchidectomy does not cause any change in male 6PGDH activity. This is in contrast to changes in G6PDH which were reported by Lockwood et al. (165) to be lowered following castration.

7. Malic enzyme (E.C.1.1.1.40) or. L-malate NADP: oxidoreductase

This enzyme also seems to be involved in the production of NADPH required for de novo fatty acid synthesis. Malic enzyme (ME) catalyses the conversion of L-malate to pyruvate in the presence of NADP, by an oxidative decarboxylation of L-malate, resulting in the formation of NADPH, CO_2 and pyruvate. The enzyme from liver and adipose tissue has been studied by several groups (165, 219, 204). Malic enzyme is a cytoplasmic enzyme and its activity in rat liver has been reported to be 1.27 μ moles/min/g liver (229). ME is stimulated by Mn^{++} (235) and inhibited by citrate (236). According to Rutter and Lardy (235) Km for L-malate is 3.9×10^{-4} M and for NADP is 1.6×10^{-6} M for enzyme from pigeon liver. In rat adipose tissue, the specific enzyme activity has been reported nearly ten times higher than of liver enzyme (237). Lockwood et. al. (165) have suggested that ME plays an important role in hepatic lipogenesis from carbohydrates, whereas Kornacker and Ball (145) believe that this enzyme is not an important co-enzyme producer for fatty acid biosynthesis in liver, since G6PDH is present at a much higher level (229, 238).

It has been shown that hepatic ME activity can also be affected by different nutritional states. Two groups of workers (229, 139) found a low enzyme activity in 48 h fasted rats and a supranormal level in animals fed a diet rich in sucrose. Gibson et al. (139) also studied the nature of the change in ME activity by pulse labelling, using ^{14}C-leucine. They found that the changes in enzyme activity are due to the changes in enzyme protein synthesis. Animals fed a fructose

diet for a period of 4 days have a higher hepatic ME level than similar animals on a corn starch diet. This difference was even more marked when fasted animals were refed with the diets (219). A sharp increase in enzyme activity also occurs at weaning which again may be related to the change in diet (165).

A positive correlation between ME activity and plasma insulin levels has been observed in obese and normal mice (147). Thyroxine administration to rats leads to a 20-fold increase in ME at 18 days of age, whereas glucagon, cortisone and growth hormone are without effect (165). Baquer et al. (204) found a decrease in liver ME activity following thyroidectomy, thyroxine injection restores the enzyme activity to the normal level. It has also been shown that the increase in the rate of protein synthesis in response to a high-carbohydrate diet was exaggerated following thyroxine or insulin administration (139).

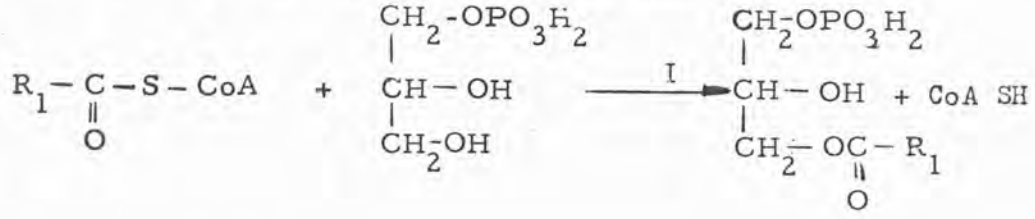
E. Enzymes involved in triacylglycerol synthesis

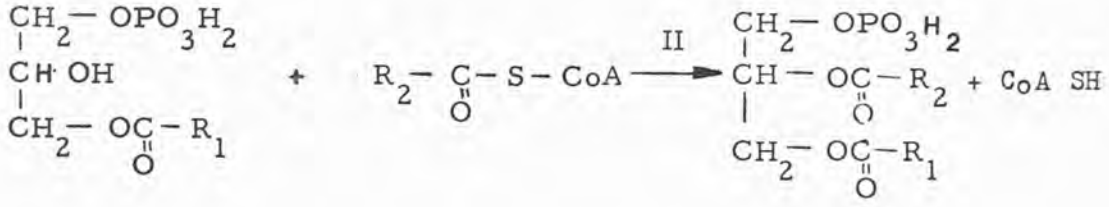
Hepatic triacylglycerol synthesis requires the availability of long-chain acyl-CoA which may be derived either by de novo fatty acid synthesis or from circulating free fatty acids by the action of the enzyme long chain acyl-CoA synthase. The glycerol component of the TG comes from glycerol 3-phosphate which can arise either from glycerol by the action of a specific glycerokinase or from DHAP by the action of glycerol 3-phosphate dehydrogenase. The following enzymes are involved in the synthesis of TG from these substrates (see Fig. 2 for pathway).

1. Glycerol 3-phosphate acyl transferase (E.C.2.3.1.15) or, Acyl-CoA: L-glycerol 3-phosphate acyl transferase (I) and Acyl-CoA: 1-acylglycerol 3-phosphate acyl transferase (II).

The first specific reaction of endogenous glycerolipid synthesis is catalyzed by the glycerol 3-phosphate acyltransferase system. This enzyme system catalyzes the conversion

of acyl-CoA and sn-glycerol 3-phosphate (G3P) to lysophosphatidate and subsequently to phosphatidate (239) as follows:





Glycerol phosphate acyltransferase (GPAT) appears to be localized in two different subcellular organelles, namely the mitochondrial and microsomal fractions. The two activities appear to be associated with two separate enzymic proteins as demonstrated by a study of kinetic properties and differential inhibition by-SH blockers. The microsomal GPAT has a Km for G3P of 0.5 mM but the acyl-CoA kinetics are complicated by the micellar nature of these molecules (240). The pH optimum is between 7.5 to 8 and sulphhydryl group protectors, such as cysteine and 2-mercaptoethanol, stimulate the enzyme activity (240, 241). Acyl-CoA with 15-18 carbon atoms are the best substrates for acylating G3P (240). Acyl-CoA at high concentrations (higher than 10×10^{-6} M) inhibit the enzyme activity by a mechanism which is thought to be due to the detergent effect of the acyl-CoA (63).

Mitochondrial GPAT (I) is localised on the outer membrane of the organelle (242), is specific for palmitoyl-CoA and unlike the microsomal enzyme is not inhibited by p-chloromercuribenzoate and DTNB (5, 5-dithiobis(2-nitrobenzoic acid))(243).

The next step (II), the formation of diacyl glycerol phosphate by the acylation of 1-palmitoyl-glycerophosphate, may not occur in mitochondria (244). There have been claims that the second acylation can occur in mitochondria but these reports can be explained by microsomal contamination of the fractions used. The DTNB resistant enzyme may be artificially found to become associated with the mitochondria during the isolation procedure (243). It has been suggested that the mitochondrial enzyme catalyses the synthesis of palmitoyl glycerophosphate which is then transported to the endoplasmic reticulum where it is utilized as a precursor for glycerolipid synthesis (243).

In contrast to the mitochondrial enzyme, microsomal GPAT shows comparable activities when incubated with a number of different acyl-CoA derivatives (243). The major product from isolated microsomes is phosphatidic acid whereas isolated mitochondria form a mixture of phosphatidic acid and lysophosphatidate (243, 245). The participation of two enzymes in the synthesis of phosphatidate by the microsomal fraction has been proven (246). An enzyme which catalyses the formation of 1-acylgycerol 3-phosphate has been solublized and partially purified from a microsomal preparation. The enzyme requires Ca^{++} for activity, other divalent cations such as Mg^{++} and Mn^{++} can replace Ca^{++}. The Km for G3P is 0.2 mM and it appears that palmitoyl-CoA is the most efficient acyl donor, the unsaturated fatty acyl-CoAs being poor donors(246). However, the sensitivity of the enzyme to -SH blocking reagents was not tested. Eibl et al.(247) have found that the capacity of rat liver for introducing the second fatty acid in phosphatidic acid was 10-fold greater than the capacity for introducing the first fatty acid, suggesting that the GPAT (I) may be a regulatory

step in the synthesis of acylglycerols. This suggestion is reinforced by the fact that lysophosphatide does not accumulate in microsomal preparations. The DTNB sensitive GPAT from rat liver microsomes utilizes palmitoyl, oleoyl and linoleoyl CoAs at comparable rates in the presence of saturating substrate levels. The acylation at the 2 position is highly selective for linoleate but the diacyl glycerolphosphate synthesised by rat liver *in vivo* is not confined to the 1-saturated 2-unsaturated diacyl glycerol phosphate because of the substrate availability (243).

Both microsomal and mitochondrial DHAP acyl transferase show similar specificity to the corresponding glycerol phosphate acyltransferases. Suggesting that the DHAP and G3P are acylated by the same enzyme (248). Schlossman and Bell (249) have provided evidence that liver microsomes have a single enzyme capable of acylating both DHAP and G3P. DHAP was a competitive inhibitor of GPAT and *vice versa*. However, consideration of the Vmax for each substrate and the relative concentration of G3P and DHAP pools in the liver led these authors to suggest that the *in vivo* ratio of G3P to DHAP acylation by liver microsomes is greater than 84:1.

There is a very rapid increase in microsomal GPAT in the neo-natal rat to a level of 8.03 n moles/min/mg protein after 24 h. and this level drops gradually to 0.95 n mol/min/mg protein in the adult animal. The neo-natal increase in enzyme activity is thought to be a response to suckling and involves the synthesis of new enzyme protein as tested by puromycin inhibition. There is also a rapid accumulation of hepatic TG which appears parallel changes in enzyme activity (250).

Liver microsomal GPAT activity has been shown to be influenced by various dietary states. The total hepatic enzyme activity has been reported to be decreased after 48 h fasting and to increase when the fasted animals are refed a fat-free or a carbohydrate diet (248, 252). However, other workers (251) have reported no change in specific activity of GPAT after a fasting period although the specific activity of the enzyme is increased following 7-day periods on a high-fat or on a high-carbohydrate diet.

Lamb and Fallon (249) have found diet-induced changes in hepatic GPAT activity in rats. Diets rich in either glucose or fructose given for three days increased the microsomal enzyme activity by 33%. This change has accompanied a change in the ratio of neutral lipid to polar lipid produced and also a 3-fold increase in the supernatant phosphatidate phosphohydrolase activity. Glenny et al. (253) have examined the effect of feeding animals fed on a diet containing 53% starch compared to groups fed on a diet in which 20% of the starch was replaced by sucrose, lard or corn oil. They found that GPAT activity was higher in the starch and corn oil fed group than in the sucrose or lard fed animals. This in constrast to the phosphatidate phosphohydrolase which is significantly higher in the sucrose and lard fed animals.

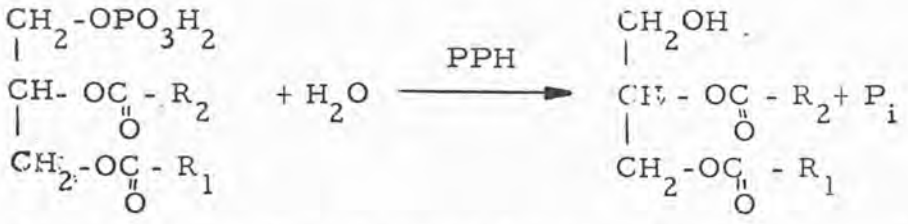
There has been very little research on the hormonal regulation of GPAT activity in the rat liver. Insulin and adrenaline have been reported to influence GPAT activity in short-term experiments using isolated adipocytes (254).

Coleman et al. (255) have reported that the hypertriglyceridaemia produced in chicks as a result of oestrogen treatment for five days is the result of an increase in the total activities of GPAT

and some other enzymes involved in triglyceride synthesis. This reflects the increased size of the liver which occurs after the treatment. The specific activity of the enzyme remains unchanged.

2. Phosphatidate phosphohydrolase (E.C.3.1.3.4) or, L-α-Phosphatidate phosphohydrolase (PPH)

Diacylglycerol formation is catalysed by PPH as follows:



This enzyme is thought to be a regulatory enzyme in the synthesis of triglycerides (256, 252, 254). PPH has been found in several different animal tissues and has been found in the microsomal, mitochondrial and particle-free supernatant fractions (257, 256). Smith et al. (257) found that the activity of soluble PPH is higher than the particlulate enzyme. However, membrane bound phosphatidate is thought to be the best substrate for PPH (257). Caras and Shapiro (265) have found that the microsomes from rat liver have at least two active forms of PPH, F_A and F_B. The F_A form is non-specific with a high Km (0.3×10^{-3} M) for phosphatidate and the F_B form is specific for phosphatidate with a low Km (0.03×10^{-3} M) for the substrate. The V max, using phosphatidate as substrate, is higher for F_B than F_A. Unlike the F_A, the F_B form can be inhibited non-competitively by diacylglycerol. The F_B form is disaggregated and activated by high concentrations of monovalent cations. Divalent ions such as Ca^{++}, Mn^{++} and Mg^{++} are not required for the activity of either F_A or F_B and are inhibiting

at concentrations greater than 1 mM. Lamb and Harold (256) also found a similar inhibition, by divalent cations, for the particulate enzyme and have found that the soluble PPH is activated.

The effect of glucose, sucrose, glycerol, sorbitol and ethanol intubation on hepatic PPH activity in rat have been studied by Sturton et al. (258). The animals were killed six hours after intubation, and the specific activity of PPH was found to be increased in all groups, except glucose-treated animals, in comparison to control animals intubated with 0.15 M NaCl. The greatest increase was observed in both soluble and microsomal PPH activity of rats treated with ethanol. Both 75% glucose or 75% fructose given in diets for a period of 60 h results in a 3-fold increase in PPH activity in both the microsomal and supernatant fractions in comparison with chow fed animals ( ). These changes can be correlated to the increase in TG synthesis which occurs on carbohydrate feeding and to the change in the rates of neutral lipid to polar lipid observed using rat liver homogenates or microsomal fractions. It is suggested that PPH activity can regulate the rate of neutral lipid synthesis. Glenny et al. (253) also found that rat liver soluble PPH activity was influenced by dietary factors. They showed an increased PPH activity after rats were fed on either a sucrose or a lard diet in comparison to starch fed animals. They also suggested that PPH is a rate-limiting enzyme in hepatic TG synthesis.

An increased supply of saturated and monounsaturated fatty acids to the liver, such as in starvation, stress and obesity, is accompanied by an increase in the soluble PPH activity. Similarly, Glenny et al.(253) suggest that ingestion of diet rich in

fructose or glucose results in an increased synthesis of saturated and monounsaturated fatty acids which in turn may give rise to an increased PPH activity.

Soluble PPH is thought to be a major regulator of de novo TG synthesis in the liver. There are a number of reports that substantiate the claim. For example, the reaction rate of phosphatidate phosphohydrolase in microsomes approximate to the rate of triglyceride symthesis by the same fraction. However, the activity of GPAT is 5-7 fold higher. Addition of the soluble supernatant results in a 5-20 fold increase in triacylglycerol synthesis (256).

The increased hepatic triacylglycerol synthesis after administration of a high fructose diet is accompanied by an increase in PPH activity and a decrease in microsomal phosphatidate levels (259). An increase in liver triacylglycerol levels observed after partial hepatectomy is also accompanied by an increase in PPH but no change in GPAT and DGAT activity (260). Lamb et.al. (261) have shown that 1, 3 bis (substituted phenyoxy)2-propanones inhibit microsomal PPH activity and, to a greater extent, GPAT in vitro These drugs have a hypolipidaemic effect when given in vivo.

A study of the products of microsomal synthesis of glycerolipid from [^{14}C] G3P showed a rapid incorporation into phosphatide but a much slower increase in neutral lipid radioactivity suggesting that microsomal PPH may be rate-limiting (256, 265). In addition, the increased capacity for triacylglycerol synthesis in obesity is accompanied by a rise in microsomal PPH activity and an increase in the ratio of neutral lipid to phosphatidic acid (263) also supports this view. However, there are some

contradictory reports. For example, actinomycin-D administration results in a lowering of enzyme activity in the partially hepatectomized animal but nevertheless triacylglycerol accumulation occurs (260). An increase of between 180 and 320% in hepatic soluble PPH activity has been observed after 40 h fasting. In contrast, GPAT levels are lower following food deprivation. Glyceride synthesis by liver homogenates was found to be unchanged or slightly increased in the starved animals (252).

Until recently very little work has been published on the hormonal regulation of hepatic PPH. However, it has been shown that the particulate PPH is inhibited by incubation of adipocyte with noradrenaline but it is not clear if this is a direct effect of the hormone on the enzyme or an indirect effect via the stimulation of lipolysis (264).

The effect of dibutyryl cyclic AMP (Bt_2cAMP) on the microsomal PPH, DGAT and GPAT have been studied by Soler-Argilaga *et al.* (157). They found an increase in PPH and DGAT activities in microsomal isolated from liver which had been perfused for 60 min with 20 μM Bt_2 cAMP. These effects were associated with a decrease in microsomal TG, DG and phosphatidate synthesis from [U-^{14}C] glycerol 3-phosphate. The output of TG was decreased by the cyclic nucleotide and ketogenesis and glucose output were stimulated. These authors suggest that Bt_2cAMP inhibits hepatic microsomal synthesis of TG at a step prior to the formation of phosphatidate presumably at the GPAT step.

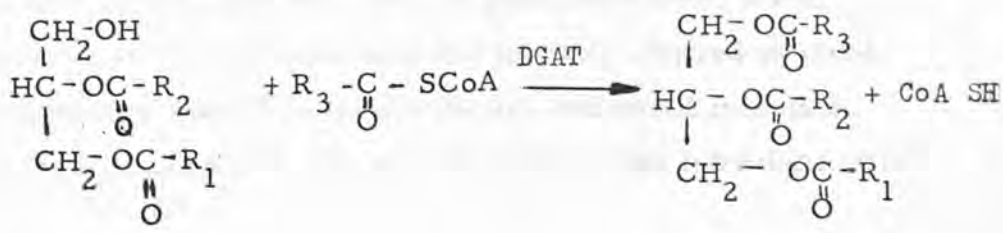
Brindley's group (106, 253, 268) have provided evidence that PPH activity undergoes changes in response to the physiological state of the animal which parallel the observed rate of

of triacylglycerol synthesis. They have suggested the involvement of hormone in the regulation of PPH activity. For example, cortisol administration results in an increased hepatic PPH level and an increase in the relative proportion of [^{14}C] glycerol incorporated into triacylglycerol. Thyroxine was also reported to increase the relative rate of triacylglycerol synthesis (106). Lehtonen et al. (269) have recently described the induction of soluble hepatic PPH by cortisol both in vivo and in an isolated perfused liver system. Glucagon and insulin had no significant effect on the enzyme activity. Knox et al. (270) have shown diurnal variation in hepatic PPH activity and in plasma corticosterone levels. The latter reaches a peak 4 h prior to the former and they suggest a relationship between the two factors.

Brindley et al. (271) have also examined the relationship between diet, glucocorticoids, PPH activity, and TG synthesis. Feeding rats with fructose, sorbitol, glycerol and ethanol increases the plasma corticosterone levels without affecting insulin concentration. There was an associated increase in soluble PPH activity and an enhanced hepatic synthesis of TG. The ethanol effect was observed in adrenalectomized animals (271).

3. Diacylglycerol acyl transferase (E.C.1.3.1.20) or Acyl-CoA: Diacylglycerol phosphate acyl transferase (DGAT)

The enzyme has been isolated from both microsomal and mitochondrial fractions of different mammalian tissues. It catalyzes the last step of the formation of triglyceride, as follows:



The pH optimum is 7.0 and its activity estimated to be 0.4 n mol / min/ mg protein in rat liver microsomal fraction (262). This is of the same order of magnitude as the rate of microsomal TG synthesis (263).

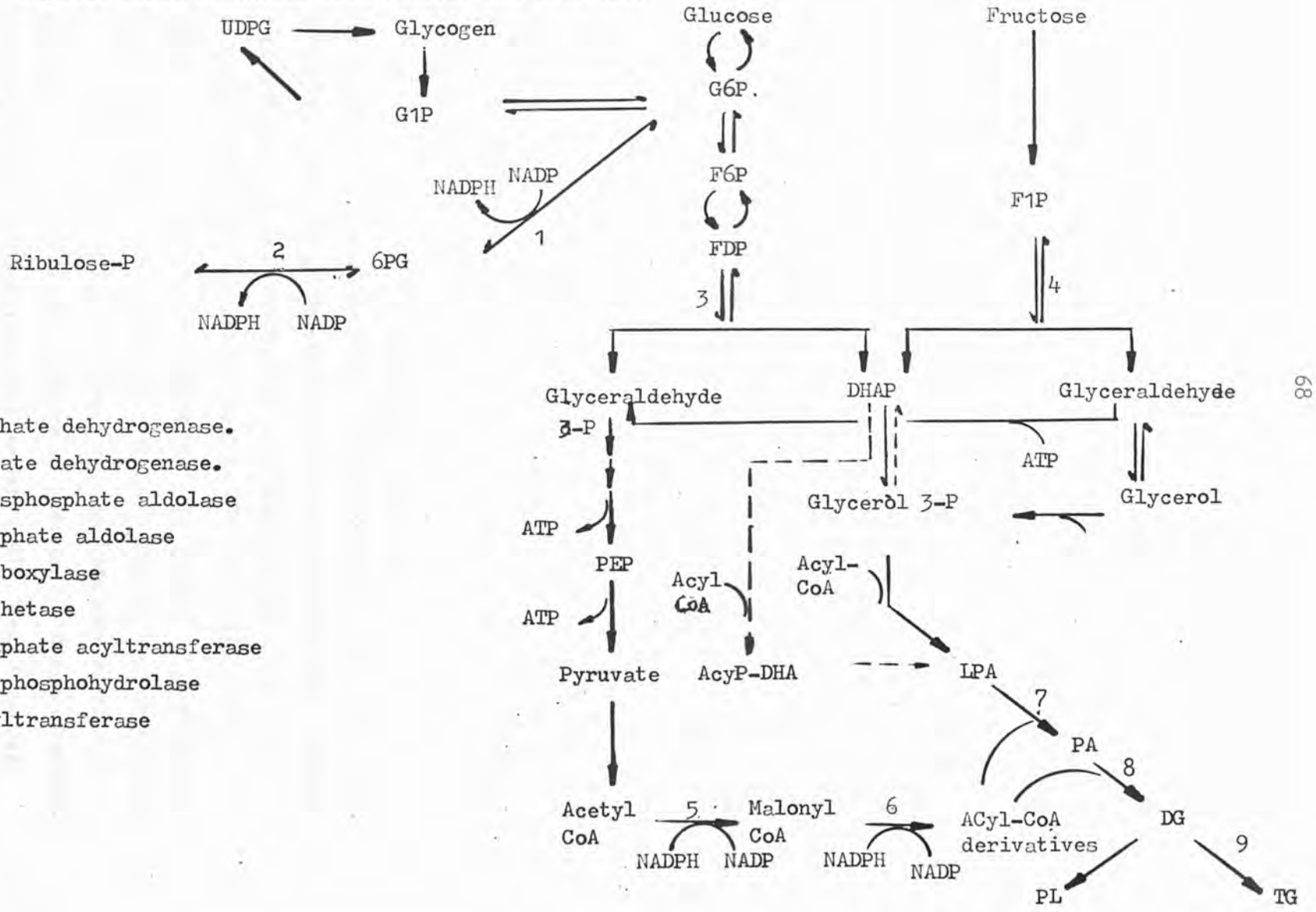
The enzyme activity can be affected by different nutritional states. Vavrecka et al. (252) found that rat liver homogenate DGAT activity was increased by fasting. This increase in enzyme activity correlated with an increase in TG/DG ratio, from 0.7 to 1.0. Fallon et al. (262) have reported a two fold increase in microsomal enzyme activity following fructose feeding for 11 days but a simultaneous accumulation of diacylglycerol occurs. This may be because the rise in microsomal DGAT activity does not compensate for the increased rate of diacylglycerol synthesis under these conditions. Glenny et al. (253) have found that sucrose, corn oil and lard feeding for a period of 14 days results in an increase in hepatic DGAT activity in rats when compared to a starch fed control. Fat feeding was more effective than sucrose in raising enzyme activity. Subcutaneous injection of oestrogen to chicks for five days, causes an increase in total liver enzyme activity but the specific enzyme activity did not change significantly (255).

II. Hepatic carbohydrate metabolism.

It is well known that the major carbohydrates in human and animal diets are sucrose, starch, glycogen and lactose. These carbohydrates are absorbed by gastrointestinal tract, disaccharides and polysaccharides are initially cleaved to yield monosaccharides mainly in the duodenum and small intestine. The monosaccharides are absorbed by cells of the intestinal wall

(brush border mucosal cells), taken up by mesenteric veins, enter the portal vein and are absorbed and utilized by the liver. Dietary glucose is absorbed through the hepatic portal system and enters the parenchymal cells where it may be laid down in the form of the storage polysaccharide, glycogen. Secondly it may undergo glycolysis to yield pyruvate and acetyl-CoA which in turn can be utilized to produce energy via the tricarboxylic acid cycle, used for the synthesis of lipids (Fig. 4) (53). Thirdly, glucose can be metabolized via the hexose monophosphate shunt to yield the pentoses and NADPH. It is postulated that the primary control of the glycolytic flux (glucose utilization) is located at glucose phosphorylation step (238). Glucose may be synthesized from non-carbohydrate sources by the liver parenchymal cells by the process known as gluconeogenesis which is essentially the reverse of glycolysis. The processes of glycolysis via the Embden-Meyerhoff pathway, and of gluconeogenesis have been widely investigated. It is postulated that there are two types of enzyme involved in this pathway. The first type called bifunctional enzymes, which catalyze certain reversible reactions and are common to both glycolysis and gluconeogenesis. The direction of the reaction catalyzed by these enzymes is determined by the requirement of the cell at that time. In general, the level of these enzymes is not markedly influenced by hormones or by dietary factors (215). The second type of enzyme is called a regulatory enzyme which is influenced by the dietary and hormonal states of the animal (215). These reactions are generally not reversible under physiological conditions and normally regulated by a number of different mechanisms involving both enzyme synthesis and the regulation of enzyme activity by metabolites. The regulatory enzymes for a particular pathway are unique to that pathway and the reversal of the pathway involves a number

Fig. 4 Hepatic lipid synthesis from glucose and fructose.



1 - Glucose 6-Phosphate dehydrogenase.
2 - 6-Phosphogluconate dehydrogenase.
3 - Fructose 1,6-bisphosphate aldolase
4 - Fructose 1-phosphate aldolase
5 - Acetyle-CoA carboxylase
6 - Fatty acid synthetase
7 - Glycerol 3-phosphate acyltransferase
- Phosphatidate phosphohydrolase
9 - Diglyceride acyltransferase

of totally different key enzymes. For example the key enzymes in glycolysis are thought to be hexokinase, glucokinase, phosphofructokinase (HK, GK, PFK) and pyruvate kinase (48 , 276, 277). The regulation of gluconeogenesis is thought to be by control of glucose 6 - phosphatase (G6Pase), fructose 1,6-bisphosphatase (FDPase), pyruvate carboxylase (PC) and phosphoenoylpyruvate carboxykinase (PEPCK).

A. Fructose metabolism

The metabolism of fructose by the liver is thought to differ from that of glucose in several important respects (Fig. 4). The rate of absorption of the ketose by the small intestine is thought to be slower than that of glucose (339). The sugars are transported to the liver in the hepatic portal vein. Topping and Mayes (56) have found that the fructose level in the portal vein is between 1.4 and 2.5 mM in rats absorbing fructose. Fructose and glucose uptake by isolated hepatocytes has been shown to involve a carrier-mediated system but the ketose uptake is much slower than the uptake of glucose (340).

However, the metabolism of fructose by the liver is thought to be much more rapid than that of glucose (21, 279, 280). Fructose is normally phosphorylated by a ketohexokinase which yields fructose 1-phosphate, which is then cleaved by aldolase to give DHAP and glyceraldehyde (48, 276, 277, 283, 284). The latter is converted to glyceraldehyde 3-phosphate by triokinase (TK) (279, 276). Thus KHK and TK are unique to the metabolism of fructose (284). Glyceraldehyde 3-phosphate and DHAP are involved in the glycolytic sequence of reactions and are common to both glucose and fructose metabolism (18). Thus fructose enters the glycolytic pathway at the triose phosphate level

and its entry does not appear to involve any regulatory enzymes (18). This pathway accounts for the fact that fructose is much more readily metabolised than glucose (21, 279, 280). The only key regulatory enzyme which is common to the glycolysis of both glucose and fructose is pyruvate kinase (285). Thus the fate of fructose in the hepatic parenchymal cells is to be converted to pyruvate and lactate or alternatively to be converted to glucose and/or glycogen via the gluconeogenic pathway (287) (Fig. 4). Hue et al. (287) have shown that both glucose and lactate are synthesised when isolated hepatocytes are incubated with fructose and the ratio of glucose/lactate synthesised can be increased by adding glucagon or phenylephrine to the cells.

Although fructose can be phosphorylated by hexokinase to fructose 6-phosphate the low activity of this enzyme in rat liver and the high Km (2-5 mM) for fructose as compared with the Km (0.1 mM) for glucose makes it unlikely that the phosphorylation of fructose by this enzyme is significant under physiological conditions (277)

Ketohexokinase is specific for ATP but can utilize monosaccharides other than fructose, such as L - sorbose, L-arabinose, D-xylose and D-tagatose. The activity of the enzyme in rat liver (2-2.5 μ moles/min/g liver) (13) equates well with the rate of extraction of fructose during liver perfusion (288). This together with the low Km (0.4-0.8mM) of the enzyme for fructose accounts for the rapid metabolism of the ketose. In contrast, the activity of hexokinase and glucokinase in rat liver is about 50% of the ketohexokinase activity (289).

It has been suggested that males are able to metabolise fructose at a faster rate than females by virtue of a higher KHK activity in the former (290) but Pollard (291) and Mahmoud (292) have not been able to demonstrate sex differences

in hepatic KHK activities. In addition Tay (81) was unable to show any sex differences in FIP levels in male and female rat liver following sucrose feeding.

Ketohexokinase activity in rat liver is affected by diets. Heinz (297) has shown that hepatic KHK activity in rats is raised by fructose feeding for 21 days but Mahmoud (292) confirmed that high carbohydrate diets did not raise level of KHK, and he could find no differences between the effects of dietary sucrose and glucose.

The major fate of the fructose 1-phosphate produced by KHK, is breakdown by aldolase (see Section II. B) to yield DHAP and glyceraldehyde. The latter is an unique product which arises as a result of fructose metabolism. Glyceraldehyde metabolism is thought to occur mainly via an ATP dependent triokinase which results in the formation of a normal glycolytic intermediate, glyceraldehyde 3-phosphate.

The Km of the enzyme for glyceraldehyde has been reported to be between 8 and 35 x 10^{-6} M according to experimental condition (13). Sillero et al., (294) believe that because of this low Km phosphorylation is the major route of the metabolism of the triose. On the other hand, the Km for ATP-Mg^{++} is high and the enzyme is inhibited by ADP (295) so it is suggested that when the cytoplasmic ATP/ADP ratio is low, e.g. after an intravenous fructose load, then TK will be less active and alternative routes for glyceraldehyde metabolism may become more important. The D-glyceraldehyde may be converted to glycerol by alcohol dehydrogenase or converted to glyceric acid by a non-specific aldehyde dehydrogenase (276, 296, 277, 280, 284).

The activity of TK in rat liver is comparable to the activities of the other enzymes involved in fructose metabolism, KHK and aldolase. This suggests that there is sufficient TK present to catalyze the converstion of D-glyceraldehyde to glycolytic intermediates (13).

Triokinase has been reported to be subject to dietary regulation. Heinz (297) observed that the enzyme activity is raised by feeding a high carbohydrate diet. A high fructose diet increased TK activity 4-fold but a glucose diet was less effective. Similarly, Veneziale (298) has found that high fructose and glucose diets, in comparison to chow, can raise TK activity in rat liver over a period of 48 h. In contrast, Adelman (13) found little difference in TK activity in animals fed a high carbohydrate diet for 24 h. In a study similar to that reported in this thesis, Mahmoud (292) found that long term administration of a sucrose supplemented diet over a period of 110 days resulted an increase in TK activity in both male and female animals. However, glucose supplementation of the diet had little effect on enzyme activity. No sex differences were observed.

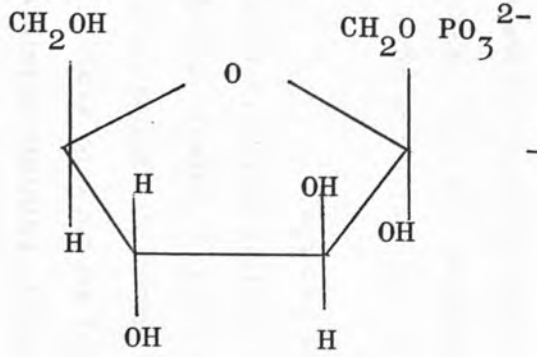
There have been few studies on possible hormone regulation of the enzyme. Streptozotocin diabetic rats do not have significantly lower enzyme activity than normal animals. Glucagon administration does not appear to affect enzyme activity in the short-term (298). Adrenalectomy appears to have little effect on enzyme activity, but administration of hydrocortisone to these animals resulted in a two fold increase in enzyme activity (13).

B. Aldolase (E.C.4.1.2.13), fructose 1,6-bisphosphate D-glyceraldehyde 3-phosphate ligase and fructose 6-phosphate D-glyceraldehyde 3-phosphate lyase

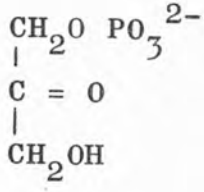
The enzyme catalyzes the conversion of either fructose 1-phosphate or fructose 1,6-bisphosphate to dihydroxyacetone phosphate and either glyceraldehyde or glyceraldhyde 3-phosphate respectively. (see Fig. 5).

There appears to be three types of mammalian aldolase. Type A enzyme has been found in muscle, Type B found in liver and Type C in brain (300). Type B is also found in the intestine (301). Type A and C catalyze mainly the conversion of fructose bisphosphate (FDP), but for type B, both fructose 1-phosphate (FIP) and FDP are substrates (302). The ratio of FDP:FIP utilization by type A, B and C is 50, 1 and 10 respectively (303). The hepatic enzyme is located in cytoplasm (303) in both parenchymal and non-parenchymal cells (304). Rutter et.al. (302) believed that, in the adult rat liver and rabbit liver, there are both A and B types, but type B is predominant. In foetal liver types A, B and C are present but type B becomes predominant just before birth. Rat liver enzyme activity believed to be 3.46 u/g liver (119). Type B aldolase from rat liver is a reversible enzyme with a Km for FDP, 1×10^{-6} M; for FIP, 9×10^{-4} M; for dihydroxyacetonephosphate 4×10^{-4} M; for glyceraldehyde 3-phosphate; 3×10^{-4} M (306, 307). Liver aldolase has been purified and crystallized by Edward and Rutter (306). Aldolase B is is competitively inhibited by AMP and ADP but not by ATP. Adelman (284) believes that this inhibition may play a physiological role in in vivo controlling of the activity of aldolase.

Fig. 5 Cleavage of F1P and FDP catalysed by aldolase.



F1P Aldolase →



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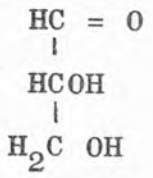
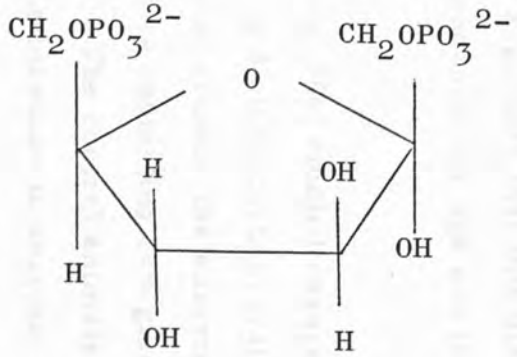
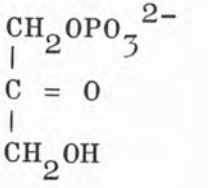




FDP Aldolase →



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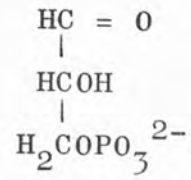

The inhibition of aldolase B by ATP degradation products such as IMP and uric acid have been described by Heinz (119). The effect of dietary carbohydrate on aldolase B in liver has been studied by several investigators (221, 284, 13). There is a decrease in total enzyme activity in fasted rats which can be restored to normal by refeeding with glucose, fructose or sucrose. Adelman (13) showed a reduction of rat liver total enzyme activity after 48-72 h fasting. When fasting animals given diets rich in glucose or fructose, the activity of enzyme is restored and a supranormal activity appears when diet is administered for three weeks. Both a high-fat diet or a diet rich in protein will cause a decrease in enzyme activity. Adelman (13) found no change in specific activity of liver aldolase due to fasting, carbohydrate or high-fat diet. Chang et al. (221) also determined enzyme activity in different nutritional states in three ages and two species of rat. In contrast to Adelman et al. they found changes in enzyme activity due to the type of diet. The highest enzyme activity was observed when a high-fat chow diet supplemented with sucrose was given for periods up to four weeks, and the lowest activity in animals fed on a starch supplemented diet. They suggested that this diet-induced change in enzyme activity is independent of the age and the strain of the animals.

Espinoza and Rosensweig (308) studied changes in rat liver aldolase activity in response to dietary carbohydrate. They demonstrated that in different ages of animal, the enzyme activity is increased following feeding of diets containing 68% glucose, fructose or sucrose for three days. The control animals were fed a diet containing 40% glucose. This increase in enzyme activity

occurred in animals of 3, 8 and 52 weeks of age but this response was somewhat less as the animal aged. Fitch and Chaikoff (208) have reported a similar adaptation to diet for a 7-day period but fructose was apparently more effective in restoring enzyme activity than glucose.

Espinoza et al. (309) have shown an increased aldolase activity in the duodenum and other parts of the small intestine of rats in response to dietary sugar, particularly when fructose is given. Greene et al. (310). showed that human jejunal aldolase activity is increased by the oral administration of glucose and also, to a lesser extent, by intravenous glucose administration. In hypogonadal males jejunal aldolase activity has been shown to be reduced in comparison to that in normal men (311). However, testosterone administration did not significantly affect the activity of the enzyme. The short-term effect of insulin and glucagon on aldolase activity in perfused rat liver has been studied by Taunton et al. (312, 313). In contrast to other glycolytic enzymes such as PFK and PK, aldolase activity is not subject to regulation by these hormones.

C. Pyruvate kinase (E.C.2.7.1.40) or, ATP: Pyruvate Phosphotransferase (PK)

This enzyme catalyzes the conversion of phosphoenoyl-pyruvate to pyruvate, in the presence of ADP. It is postulated that pyruvate kinase is a key enzyme in the glycolytic pathway, hence lipogenesis from both fructose and glucose may be markedly affected by PK activity.

Liver type PK is localized in cytoplasm of parenchymal cells of the liver (314, 304, 315). The Km for PEP is 0.5×10^{-3} M (316) and for ADP is 0.21×10^{-3} M (317). Divalent ions such as Mg^{++} and Mn^{++} are required for maximum velocity (285). A number of monovalent ions such as K^{+} or NH_4^{+} activate the liver PK activity (315).

The specific activity of liver PK seems to be 1 μ mole/min/mg protein (319). The L-type enzyme appears to occur as a tetramer and is subject to allosteric regulation by fructose 1, 6-bisphosphate, phosphoenolpyruvate, ATP, Mg^{++} and some monovalent cations (K^{+}, NH_4^{+}) (279, 317). FDP and PEP are positive effectors which stimulate the enzyme activity, or decrease the inhibitory effect of ATP and alanine (322, 321, 323) (see Fig. 6). There is no evidence that muscle type enzyme is affected by FDP (221, 322). Liver type enzyme is also activated by FIP (324).

The inhibitory effect of ATP and alanine on the PK activity in rat liver has been demonstrated by several workers (321, 325, 326, 315, 334). The Ki for ATP is 0.16×10^{-3} M and for alanine is 0.41×10^{-3} M. Acetyl-CoA, medium and long-chain fatty acids have been reported to be liver PK inhibitors (327, 322). In contrast, Foster and Blair (286) showed that although incubation of isolated hepatocytes with oleate results in a decrease in the formation of pyruvate and lactate from PEP, dihydroxyacetone and fructose the PK activity is unaffected.

Lockwood et al., (165) have studied the changes in hepatic PK activity during development of the rat. They found that the activity of PK at all ages is higher than for some other glycolytic enzymes, such as PFK and aldolase (165, 328). Liver

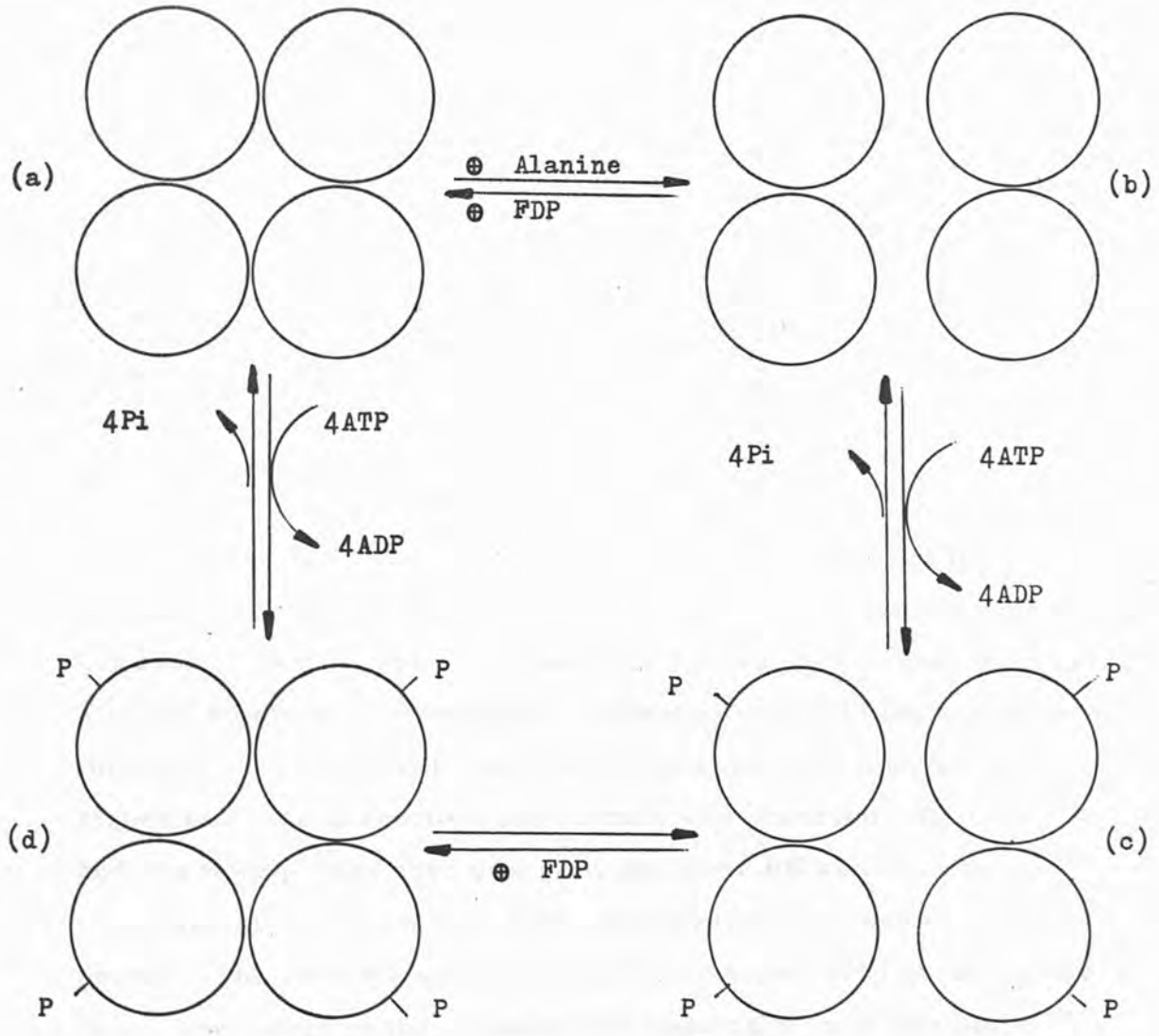

Fig. 6. A model for the regulation of L-type pyruvate kinase by phosphorylation/dephosphorylation and allosteric effectors (modified from Eigenbedt and Shoner (323)). In this model (a) represent as the active form, (c) least active form and (b) and (d) are intermediates.

PK activity increases after weaning, from 11 u/g at 21 days to 33 at 32 days of age. The effect of prolonging the high-fat diet past the normal weaning time is to suppress the PK activity. Thus, these workers suggest that the changes in activity which occur at weaning are related to changes in the diet. This can be correlated to the pronounced increase in the conversion of carbohydrate to lipid which occurs at this time. Changes in total liver PK activity during development of the rat, but in the absence of solid food have also been investigated by Walker et al.(328). They found that the increase in PK activity in the late suckling period was not dependent upon the provision of solid food, but rather on the carbohydrate (glucose) composition of the diet. These workers also commented on the absence of diurnal variation on the levels of PK after weaning in contrast to the diurnal variation in GK observed.

The effect of various dietary carbohydrates on PK activity has been studied by several investigators (61, 62, 313, 146, 207, 308). It has been shown that rats fed a 50% sucrose diet for 150 days have a higher liver PK activity in comparison to animals fed a 50% starch diet over the same period. In contrast, the adipose tissue PK is not affected by dietary carbohydrate. Förster et al. (61) found a slight increase of hepatic PK in rats fed 70% glucose for 12 days and a higher increase in fructose fed animals was observed. Espinoza and Rosenweig (308) have observed that liver PK activity can be increased in rats fed a high (68%) carbohydrate diet over a 6-day period. The controls were animals fed on a low (40%) carbohydrate diet. The nature of the carbohydrate appears to be important. Fructose and sucrose caused a higher increase than glucose in animals of 3, 8 and 52 weeks of age. Indeed, in the latter age group no increase in enzyme activity was found when the animals

were fed on glucose. Bruckdorfer et al. (62) have also found that sucrose feeding over a 50-day period results in a higher enzyme activity than starch feeding. Naismith and Rana (207) also found an increase in liver PK activity in sucrose fed rats. In this experiment animals were fed for ten days on a high-fat or a high-carbohydrate (starch or sucrose) diet. They observed a decrease in liver PK activity following high-fat feeding and the increase in PK in sucrose-fed animals was double that found in starch-fed animals. Gunn and Taylor (331) studied the total hepatic PK activity in different nutritional states in normal and diabetic rats. A decrease in enzyme activity was found following starvation which increased after refeeding with diets enriched with glucose, sucrose or glycerol for a 48-h period. However, the time course for the induction was different for the different diets. From these three diets, sucrose and glycerol had a more rapid effect on PK activity than glucose. The authors suggest that changes in PK activity are the result of changes in the level of a particular metabolite, which may be common to the metabolism of fructose, glucose and glycerol. They suggest that there is a possible relationship between DHAP levels and PK levels. Enzyme adaptation has also been reported in duodenum and other parts of the small intestine after glucose or fructose administration. The increase in PK is more apparent when fructose is given (313). Pyruvate kinase activity from intestinal mucosa is increased in humans given diets rich in glucose for five days (312). Green et al. (310) found that no change in enzyme activity occurred on a carbohydrate-free diet or when glucose was given by intravenous injection.

The acute and chronic effects of various hormones on liver PK have been studied by several investigators. Taunton et al. (313, 312) have reported rapid changes in PK activity in rat liver

following the administration of both insulin and glucagon via the portal vein. Insulin raises PK levels 5 min after the injection of the hormone, the effect was maximal after 10 min. Glucagon lowers PK activity over a similar time-scale. The effect of the latter hormone appears to be mediated via an increase in cAMP level, but insulin had no effect on the level of the cyclic nucleotide. Pretreatment of the rats with either puromycin or actinomycin-D did not alter the responses to the hormones, suggesting that the synthesis of new enzyme protein is not involved in this regulatory mechanism.

Blair et al.(333) have observed similar effects using a perfused rat liver system and have further shown that the changes in enzyme activity are a result of the reversible interconversion of two kinetically distinct forms of hepatic PK (see Fig. 6).

Pilkis et al. (316) in an in vitro study using hepatocyte homogenates have provided evidence that the cAMP induced inactivation of PK is the result of the phosphorylation of the enzyme by a cAMP dependent protein kinase. They also suggested that the presence of PEP or FDP can reduce the inactivation produced by cAMP.

Van Berkel et al.(334) have examined the effect of both glucagon and starvation on the L-type PK from rat liver and have also concluded that the enzyme can be regulated by glucagon or cAMP via a phosporylation/dephosphorylation mechanism in addition to the regulation via FDP, PEP and alanine.

Foster and Blair (286) have shown a reciprocal relationship between the levels of glucagon, adrenalin and cAMP and PK in isolated rat hepatocytes. In addition there was a good correlation between the enzyme activity and the rate of pyruvate

and lactate production from dihydroxyacetone in hepatocytes isolated from fasted rats. Adrenalin has also been reported to cause an acute inhibition of enzyme activity (313) and of glycolytic flux (286). The effect of this hormone appears to be potentiated by theophilline (286). Foster and Blair have further shown that the inhibiton of PK by adrenalin in isolated hepatocytes may involve both a cAMP-dependent protein kinase and an α-receptor mechanism not involving the cyclic nucleotide.

Early work by Weber et al. (335) suggested that insulin is an inducer of hepatic PK. The enzyme activity is markedly decreased in alloxan-diabetic rats over a period of three days and restored in 24 h by insulin injection. The increase in activity is prevented by injection of actinomycin-D, suggesting that RNA synthesis is regulated.

Gunn and Taylor (331) also showed that insulin administration to diabetic animals also resulted in an increase in enzyme activity after 24 and 48 h. Refeeding diabetic animals with sucrose or glycerol also caused an increase in PK activity after 48 h but glucose feeding was ineffective in this report. These authors suggest that insulin may exert its effect via changes in metabolite concentrations rather than by a direct effect of the hormone.

The effect of chronic hyperinsulinaemia over a 6-day period on various lipogenic and carbohydrate metabolism enzymes has been studied (146). Insulin-treated rats have increased PK activity at high substrate (PEP) levels, whereas at low substrate levels no change was observed. This is in contrast to the data study of the acute effects (60 min) of insulin onthe perfused liver. This suggests that in the short term, insulin regulates enzyme

activity by altering the interconversion of the kinetically distinct forms of the enzyme, but that insulin may also be a long-term inducer of enzyme activity.

Baquer et al. (204) have found a decreased hepatic PK activity in thyroidectomized rats in comparison to controls. Since there is no reduction in the circulating insulin level in hypothyroidism (234), they suggest that this represents a decreased insulin sensitivity of the tissue.

Lufkin et al. (311) have examined jejunal PK activity in normal and hypogonadal men, and have found that the latter have a lower activity of the enzyme and that oral testosterone treatment over a period of two days results in an increase in enzyme activity in both classes. They suggest that these changes are due to a regulation of protein biosynthesis.

III. RESULTS AND DISCUSSION

AIMS OF PRESENT STUDY

Dietary carbohydrate in the human diet can increase the fasting plasma triacylglycerol levels (329, 293). This increase is more pronounced in men and in post-menopausal women than in pre-menopausal women (336, 329, 341). Sucrose and fructose feeding also cause fasting hypertriglyceridaemia in both male and female rats (15, 82) and it has been shown that plasma TG levels in male rats is higher than in female rats (80).

Plasma TG levels are a function of the rate of TG synthesis and secretion and the rate of clearance from the blood. This work is concerned mainly with the enzymes involved in lipid synthesis and secretion and the rate of clearance from the blood. This work is concerned mainly with the enzymes involved in lipid synthesis and is an attempt to explain the sex differences in TG levels in rats.

This thesis reports part of a study on the possible sex differences in hepatic lipid and carbohydrate metabolism in animals fed on diets enriched with glucose or sucrose. The rat was used as the experimental animal in the study because it had previously been shown that male rats fed on a diet supplemented with fructose or sucrose had raised liver and serum TG levels in comparison with animals fed on glucose or starch supplemented diets (see section I.A.).

The aim of the present study was to investigate the possibility that sex and diet related differences in plasma and liver TG levels were related to differences in the enzyme levels involved in lipid metabolism. Similar studies were carried out simultaneously on key hepatic metabolite levels (81) and on some enzymes involved in carbohydrate metabolism (292).

A. Effect of long-term ingestion of various diets on hepatic enzyme activities in male and female rats

In this study the effects of sucrose and glucose supplementation of the diet of rats for 110 days on the level of some enzymes involved in lipid metabolism have been examined. Control experiments were carried out in which unsupplemented chow diets were fed.

Previous work in this laboratory using male and female rats kept on similar dietary regimes have shown that adult male rats have generally a higher serum TG level than comparable females (81). The sex differences were especially marked in animals fed on the glucose supplemented diet. The serum TG of the female was markedly lower in the female animals fed on this diet than in any of the other groups. Prolonged feeding of the sucrose supplemented diet led to a higher serum TG level in both sexes when compared to animals fed on either the glucose supplemented diet or the chow controls. The hepatic TG levels were higher in animals fed glucose than in rats on the sucrose supplemented diet. Significant sex differences (♀>♂) in liver TG, phospholipid and cholesterol levels were observed only in animals fed on the sucrose supplemented diet.

In addition female rats on sucrose diets had significantly lower DHAP, fructose-1,6 bis-phosphate and glycerol 3-phosphate and [lactate/pyruvate] than corresponding males. A sex difference in DHAP level was the only difference observed in animals fed chow diets and no significant sex differences in any of these metabolites were found in glucose fed animals (81).

In the study 21 day old male and female Wistar rats with approximately the same initial body weight (50 ± 5 g) were used in order to investigate the possible effects of diet on sex-related differences in enzyme activities age-matched male and female rats were used for the study. Groups of four or five rats of the same sex were caged together and fed ad lib. on CDD [R] diet and other similar groups were fed on the same diet supplemented with either 5% (w/v) sucrose or 5% (w/v) glucose in the drinking water. The levels of supplementary carbohydrate used were significantly lower, and also the feeding was over a longer period, than most previous studies.

Separate groups of animals were fed on Dixon-86. This latter diet is low in fat when compared to CDD [R] and although the digestible carbohydrate content of .86 is only about 20% greater than that of CDD [R] a significant proportion (about 5% w/w) is molasses which is largely composed of sucrose. The temperature of the animal house was 22° ± 2°C and the animals were kept under a 12 h dark/12 h light regime with the lights on from 08.00 to 20.00 h daily. The duration of the feeding period was 110 days after which the rats were sacrificed by cervical dislocation, between 9.00 and 10.00 to minimise differences occurring as a result of diurnal variation which have been reported by several authors (270). The livers were excised and freeze-clamped within 45 sec of death and the tissues were stored at -70°C until required.

A relatively large number of animals were used in these studies in order to apply meaningful statistical analysis of the enzyme levels. As a consequence no attempt was made to measure the intake of chow by the animals because of lack of suitable animal house facilities. However, the consumption of

both glucose and sucrose supplement was measured and shown to be similar for both sexes and both sugars (7-8 g/rat/day in adult animals).

An unpublished study in this department by Pollard (291) indicated that male animals consumed approximately 20% more of the CDD [R] diet than female. This difference in food intake was reflected in the difference in body weight between male and female animals (see table I).

It is likely, therefore, that the intake of the chow diet is roughly proportional to the increase in body weight in male and female animals. However, the effects of the supplementation of the diet with glucose and sucrose may have differentially influenced the chow intake. For example, although an animal may consume equicaloric amounts of the sugars the greater sweetness of sucrose may result in a decreased chow consumption (195).

In view of these factors some caution should be exercised in the interpretation of the sex and diet-related differences observed in this and similar studies. A comparison of male and female animals is always problematical because of the differential food consumption and body weight gain which occurs in such a long-term study.

The feasibility of using age-matched or weight-matched animals for such comparisons was considered and it was decided that the former method was more valid. Similarly, it was decided to feed the animals ad lib. rather than to meal-feed the rats since the latter method would result in either a partial starvation of the male or an overfeeding of the female.

It is not possible to state unequivocally the specific

effects of the glucose or sucrose supplement although, in general, there do not appear to be any diet related variations in the sex differences.

1. The effect of diet on body and liver weights

The effect of diet on the growth of the animals is shown in table I. The results show that at the end of the feeding period the body weight and liver weight of male animals were generally higher ($p < 0.01$) than that of the females. However, the liver weight expressed as a percentage of the body weight is higher in females on all diets. Sucrose supplementation of the diet as compared to chow or glucose feeding resulted in a significant ($p < 0.1$) increase in liver weight in the female and also an increase in the liver weight expressed as a percentage of the body weight. Significant differences in liver weight were not observed in the male.

Sex differences in the growth of rats are well documented and are related to differences in the intake of food. Oestrogens have been reported to depress food intake and subsequent growth of both male and female rats. Body weight increases in ovariectomized females and is lowered in castrated males (172, 206).

Sucrose feeding has been variously reported to reduce the rate of growth in male and female rats fed for one month on various diets containing 77% carbohydrate (82) or to increase the rate of growth when male rats were fed a 70% sucrose diet for a similar time period (282). In the latter case the weight gain was related to the food consumption which was higher in the sucrose fed rats. The increased liver weight in female rats fed sucrose has not been observed previously in similar feeding experiments (82).

Table I The effect of different dietary regimens on body and liver weights of male and female rats.

Diet and sex	Body weight (g)	Liver weight (g)	Liver weight / Body weight
CDD [R]			
Male (n= 9)	412 ± 23	12.2 ± 1.0	2.9
Female (n= 9)	268 ± 12	9.3 ± 0.6	3.4
CDD [R] + sucrose			
Male (n= 9)	435 ± 40	13.0 ± 1.9	3.0
Female (n= 9)	285 ± 35	11.3 ± 0.9	3.9
CDD [R] + glucose			
Male (n= 9)	405 ± 5.0	12.2 ± 0.5	3.0
Female (n= 9)	277 ± 14	10.0 ± 0.3	3.6

The values given are mean ± S.D. with 9 rats of each sex on each diet.

A statistical analysis of the data is given in Table Ia.

Rats (initial body weight 50 ± 5 g) were fed for 110 days ad lib. on CDD [R] diet and CDD [R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water. Water and carbohydrate solution were also available ad lib.

The yield of soluble protein (100,000 g supernatant) from the livers of rats fed on the different diets is shown in table II. The only significant sex difference ($p < 0.05$) was found in chow fed animals but glucose supplementation of the diet resulted in an increase ($p < 0.01$) in the soluble protein levels in livers from both male and female animals in comparison to both the chow and sucrose fed animals. A similar but significant increase ($p < 0.01$) was observed in male rats fed on sucrose compared to animals fed on chow. These diet-induced differences may result from differences in the insulin response of animals fed various diets. The average plasma insulin levels would be expected to be greater in the glucose fed rats than in the sucrose fed animals which would in turn be greater than those in CDD[R] fed animals. Insulin has been reported to have no effect on the rate of total protein synthesis by the perfused liver but protein catabolism is inhibited by the hormone (267). It has been suggested that the effect of the hormone is related to an inhibition of autophagic vacuole formation in rat liver and thus to a reduced rate of proteolysis (273).

2. Acetyl . CoA Carboxylase

This enzyme is thought to be a rate-limiting enzyme in hepatic fatty acid synthesis (155). In this study the enzyme was assayed by following the incorporation of radioactive isotope from $H^{14}CO_3^-$ into the acid stable product, malonyl-CoA. In an initial study the properties of the enzyme in the 100,000 g supernatant of liver obtained from male rats were examined. It was found that addition of 20 mM citrate was required for the full activation of the enzyme. This is a well known phenomenon which has been described in the literature (see Section I. D. 3). The effect of increasing protein concentration in the assay mixture was examined and it was found that at low concentrations of protein there was a proportional

Table II The effect of different dietary regimens on the soluble protein content of rat liver.

Diet and sex	mg soluble protein/ g liver
CDD [R]	
Male (n = 9)	182 ± 21
Female (n = 9)	209 ± 29
CDD [R] + sucrose	
Male (n = 9)	223 ± 22
Female (n = 9)	231 ± 29
CDD [R] + glucose	
Male (n = 9)	273 ± 33
Female (n = 9)	268 ± 20

The values given are means ± S.D. with 9 rats of each sex on each diet.

A statistical analysis of the data is given in Table IIa.

Rats (initial body weight 50 ± 5 g) were fed for 110 days ad lib. on CDD [R] diet and CDD [R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The 100,000 g supernatant of rat liver homogenate was prepared as described in the methods Section IV. Protein content was assayed by the Biuret method (344).

Table Ia Statistical analysis of the data in Table I

Group Comparison			Significance	
			Body weight	Liver weight
Male				
CDD [R]	vs	CDD [R] + sucrose	NS	NS
CDD [R]	vs	CDD [R] + glucose	NS	NS
CDD [R] + sucrose	vs	CDD [R] + glucose	$p < 0.05$	NS
Female				
CDD [R]	vs	CDD [R] + sucrose	NS	$p < 0.01$
CDD [R]	vs	CDD [R] + glucose	NS	$p < 0.05$
CDD [R] + sucrose	vs	CDD [R] + glucose	NS	$p < 0.01$

Table IIa Statistical analysis of the data in Table II

Group Comparison				Significance
CDD [R] male	vs	female		$p < 0.05$
CDD [R] + sucrose male	vs	female		NS
CDD [R] + glucose male	vs	female		NS
CDD [R] male	vs	CDD [R] + sucrose	male	$p < 0.01$
CDD [R] male	vs	CDD [R] + glucose	male	$p < 0.01$
CDD [R] + sucrose male	vs	CDD [R] + glucose	male	$p < 0.02$
CDD [R] female	vs	CDD [R] + sucrose	female	$p < 0.01$
CDD [R] female	vs	CDD [R] + glucose	female	$p < 0.01$
CDD [R] + sucrose female	vs	CDD [R] + glucose	female	$p < 0.02$

relationship which was not found at higher concentrations of protein. In all subsequent studies optimal levels of protein were used in assay media.

The effect of changing individual substrate concentrations was examined in this study. It was found that at optimum concentrations of the other two substrates, the Km for ATP was 0.67×10^{-3} M, for $NaHCO_3$ was 9.3×10^{-3} M and for acetyl-CoA was 5.0×10^{-5} M. These values are comparable to those found in the literature (see section I.D.3) in which workers used either purified enzyme or crude liver extracts. In this study optimum levels of each of the substrates were used in the subsequent assays. Finally the effect of varying the pH of the assay medium was examined and the optimum was found at pH 7.5. Thus in assaying the 100,000 g supernatant for acetyl-CoA carboxylase activity optimum conditions were used throughout. It was assumed that there are no differences between the properties of the enzyme from male and female rats. There is no evidence in the literature for such a sex difference.

The level of enzyme activity was examined in male and female rats fed on various dietary regimens (see table III). When animals fed on the same diet were examined it was found that invariably specific activities were higher ($p < 0.02$) in female rats than in male animals. A similar sex difference was found ($p < 0.05$) when the results were expressed on the basis of activity per g liver. The activity per liver was also found to be higher in females than in males in all cases.

Sex differences in hepatic acetyl-CoA carboxylase activity have not previously been reported but one report (66) suggests that de novo biosynthesis of TGFA by perfused liver from

Table III The effect of different dietary regimens on hepatic acetyl-CoA Carboxylase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)		Activity per g liver (μ mole min^{-1})	
	Male	Female	Male	Female
CDD [R]	0.38 ± 0.06	0.57 ± 0.10	0.06 ± 0.01	0.11 ± 0.03
CDD [R] + sucrose	0.90 ± 0.15	1.58 ± 0.44	0.19 ± 0.03	0.34 ± 0.11
CDD [R] + glucose	0.75 ± 0.14	1.34 ± 0.33	0.19 ± 0.04	0.35 ± 0.09

The values given are mean ± S.D. with 5 rats of each sex on each diet.

A statistical analysis of the data is given in Table IIIa.

Rats (initial weight 50 ± 5 g) were fed for 110 days ad lib on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by the method of Inoue and Lowenstein (151) as described in the method (Section IV B.2.a.)

Specific enzyme activity is expressed as n mole malonyl-CoA produced min^{-1} mg^{-1} protein.

Table IIIa Statistical analysis of the data in Table III

Group comparison*					Significance	
					Specific activity	Enzyme activity per g liver
CDD[R]	male	vs	female		$p < 0.02$	$p < 0.01$
CDD[R] + sucrose	male	vs	female		$p < 0.02$	$p < 0.05$
CDD[R] + glucose	male	vs	female		$p < 0.01$	$p < 0.05$
CDD[R]	male	vs	CDD[R] + sucrose	male	$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + glucose	male	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	male	vs	CDD[R] + glucose	male	NS	NS
CDD[R]	female	vs	CDD[R] + sucrose	female	$p < 0.01$	$p < 0.01$
CDD[R]	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.02$
CDD[R] + sucrose	female	vs	CDD[R] + glucose	female	NS	NS

NS = Not significant

* = 5 rats in each group

female rats may be higher than that from males. Further evidence that sex hormones are involved in the regulation of ACC comes from the finding that ovariectomized female rats have a lower hepatic ACC activity compared to normal females and treatment of the former with oestradiol restores the enzyme activity (178, 29). It has been suggested that this oestrogen effect is mediated via changes in the insulin/ glucagon ratio (70).

Table III also shows the effect of different dietary regimes on ACC activity in both male and female rats. Both dietary supplements raised the ACC level in both sexes ($p < 0.01$) but no significant difference was observed when the glucose fed animals were compared with the sucrose fed animals. Cohen et al. (275) have also found that dietary carbohydrate can increase ACC activity in male rats fed for 12 months on diets containing 72% sucrose or starch. In this case the sucrose diet appeared to increase ACC activity more than the starch diet.

A possible mechanism for the changes in ACC activity by diet is suggested by the work of Jeanrenaud et al. (109). These workers have observed a correlation between the rate of lipogenesis, plasma insulin levels and hepatic ACC activity. Nakanishi et al. (171) have found that rates of hepatic ACC synthesis in diabetic rats are lower than in normal rats and that insulin treatment restores both the level of enzyme activity and the rate of synthesis of ACC. It may be suggested, therefore, that the dietary differences found in the present study may be related to a higher rate of insulin secretion in the animals fed the carbohydrate supplement. Nakanishi et al. (171, 167) have also found that the rate of ACC synthesis is correlated to the dietary state of the animal. They found that fasting results in a lowering of ACC synthesis and that refeeding

a fat-free diet results in an increased rate of enzyme synthesis above the normal level. However, the mechanisms underlying these changes in ACC activity are not known. The nature of the inducer of the enzyme has not yet been determined.

However, recently Geelen et al. (87) have demonstrated a correlation between ACC activity and lipogenesis from ^3H_2O by isolated hepatocytes, and that insulin can stimulate ACC activity and glucagon inhibits ACC activity in short-term experiments suggesting that direct hormonal stimulation of the enzyme can occur at least in short-term experiments.

Assuming that a correlation does exist between rates of hepatic lipogenesis and ACC activity, it is clear that rates of lipogenesis by female rat liver would be 70-90% greater than in the male irrespective of diet. It is also apparent that carbohydrate supplementation of the diet results in a 3-fold increase in rate of hepatic lipogenesis in both males and females but no differences between the effects of glucose and sucrose were observed.

3. Fatty acid synthetase

It has been suggested that this enzyme complex may also regulate hepatic lipogenesis (55). In the present study the enzyme was assayed by a spectrophotometric method, which follows the rate of oxidation of NADPH in the presence of acetyl-CoA and malonyl-CoA. In an initial study the properties of the enzyme in the soluble 100,000 g supernatant from male rat liver were examined. A linear relationship between protein concentration and enzyme activity was observed at lower protein levels but at higher protein concentrations some inhibition was observed. In all subsequent assays optimal levels of protein were used in the assay medium.

The effects of varying individual substrate concentrations were examined. It was found that at optimum levels of the other two substrates, the Km for acetyl-CoA was 2.1×10^{-6} M, the Km for malonyl-CoA was 1.7×10^{-6} M and for NADPH the Km was 3.3×10^{-4} M. These values are comparable with those obtained by other workers using purified FAS (see section I.D.4). The two coenzyme A derivatives were found to be inhibitory at higher concentration. In the present study the substrate concentrations used ensured that the maximal FAS activity was observed in each case. Finally, the effect of varying pH on enzyme activity was examined. The pH optimum, 6.8, was used in all subsequent assays. There is no evidence from the literature to suggest a sex difference in the properties of the enzyme.

The levels of hepatic enzyme activity were examined in male and female rats fed various dietary regimes (see table IV). When animals on the same diet were examined it was found that the specific activity and the activity per g. liver of the enzyme were invariably higher ($p < 0.01$) in the female than in the male. The activity per liver was also found to be higher in females than in males. Only one comparison of FAS levels in male and female rats has been reported (80) but no significant difference was observed in this case. However, oestradiol administration to ovariectomized rats has been reported to result in an elevation of hepatic FAS levels accompanied by a corresponding increase in plasma triacylglycerol (70, 178). It is therefore possible that the elevated enzyme activity is related to the high levels of plasma oestradiol found in female rats compared to males (281).

The effects of the various dietary regimes on hepatic FAS activity are also shown in table IV. Both dietary supplements raised the FAS level compared to the control ($p < 0.01$) and sucrose feeding raised the enzyme level 2-fold in comparison to the glucose

Table IV The effect of different dietary regimens on hepatic fatty acid synthetase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)		Activity per g liver (u mole min^{-1})	
	Male	Female	Male	Female
CDD [R]	2.7 ± 0.6	5.0 ± 0.8	0.4 ± 0.1	0.9 ± 0.1
CDD [R] + sucrose	8.0 ± 2.2	21.2 ± 3.6	1.7 ± 0.5	4.5 ± 0.7
CDD [R] + glucose	5.0 ± 1.4	9.6 ± 0.2	1.2 ± 0.1	2.5 ± 0.2

The values given are mean ± S.D. with 5 rats of each sex on each diet.

A statistical analysis of the data is given in Table IVa.

Rats (initial weight 50 ± 5 g.) were fed for 110 days ad lib on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by the method of Lynen (150) as described in the method (Section IV B.2.b.)

Specific enzyme activity is expressed as n mole malonyl-CoA consumed min^{-1} mg^{-1} protein.

Table IVa Statistical analysis of the data in Table IV

Group comparison*					Significance	
					Specific activity	Enzyme activity per g liver
CDD[R] male	vs	female			$p < 0.01$	$p < 0.01$
CDD[R] + sucrose male	vs	female			$p < 0.01$	$p < 0.01$
CDD[R] + glucose male	vs	female			$p < 0.01$	$p < 0.01$
CDD[R] male	vs	CDD[R] + sucrose	male		$p < 0.01$	$p < 0.01$
CDD[R] male	vs	CDD[R] + glucose	male		$p < 0.05$	$p < 0.01$
CDD[R] + sucrose male	vs	CDD[R] + glucose	male		$p < 0.1$	NS
CDD[R] female	vs	CDD[R] + sucrose	female		$p < 0.01$	$p < 0.01$
CDD[R] female	vs	CDD[R] + glucose	female		$p < 0.01$	$p < 0.01$
CDD[R] + sucrose female	vs	CDD[R] + glucose	female		$p < 0.01$	$p < 0.01$

NS = Notsignificant

* = 5 rats in each group

supplemented diet ($p < 0.01$) in the female rats. In the male however, the differences were not as clear cut between the glucose and sucrose supplemented diet. Sucrose feeding raised the specific activity of the FAS compared to the control ($p < 0.01$) as did glucose feeding ($p < 0.05$) but the difference between the glucose and sucrose diets was only marginal ($p < 0.1$) in this study.

An elevation of FAS by fructose and sucrose feeding (compared to glucose or starch diets) of male rats has been reported by Bruckdorfer et al. (55, 44). This increase was also correlated to an increased plasma TG level but a low plasma insulin level was found in animals fed on the fructose and sucrose diets.

Diet-induced changes in FAS levels have been shown to be due to changes in the rate of synthesis or degradation of the enzyme and not to changes in enzyme activity (75). Although the enzyme level falls in diabetes and is raised by insulin administration the liver is not dependent on the hormone for maintenance of normal synthetase activity, since fructose, but not glucose, feeding has been shown to elevate the enzyme activity in diabetic rats. It has been suggested that the fructose affects enzyme synthesis by virtue of the glucokinase independent metabolism of this sugar (203). Yen et al. (147) have also observed a lack of correlation between insulin levels and FAS activities.

The data reported in this study confirms that hepatic FAS level is raised by carbohydrate feeding and that sucrose raises the level of the enzyme to a greater extent than glucose. This indicates that animals fed on sucrose-containing diets have higher rates of lipogenesis than animals fed on either chow or glucose supplemented diets. This is in contrast to the effect of dietary carbohydrate on ACC level (see section 1.D.3) where the type of carbohydrate fed did not appear to significantly affect the magnitude

of the increase in activity observed.

The explanation for the increased FAS level which occurs on fructose feeding is problematical. One theory (55) is that fructolysis leads to an increased glycerol 3-phosphate level which may stimulate utilization of acyl-CoA for the synthesis of tricylglycerol. This may result in an adaptive increase in FAS activity. However, Tay (81) in a similar study to that reported in this thesis could find no differences in hepatic glycerol 3-phosphate levels between sucrose and glucose fed animals and, in addition, he observed a sex difference in the metabolite level (male $>$ female) in the sucrose fed animals which suggests that triacylglycerol synthesis should be faster in the male than in the female if the above theory is correct.

4. Glucose 6-phosphate dehydrogenase

It has been suggested (230, 207, 208, 209) that hepatic lipogenesis may be limited by the supply of cytoplasmic NADPH required in the fatty acid synthetase reaction. It was therefore decided to examine the activity of the enzymes of the pentose phosphate pathway which are thought to be primarily responsible for the maintenance of the cytoplasmic NADPH levels (207, 208, 209, 165, 228).

In this study the combined glucose 6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) activities were measured using a spectrophotometric method in which the reduction of NADP was followed in the presence of glucose 6-phosphate and 6-phosphogluconate. The activity of the latter enzyme was then subtracted from the total activity to give the activity of G6PDH.

The properties of G6PDH in a soluble 100,000 g supernatant from a homogenate of male rat liver were examined. A linear relationship between enzyme activity and protein concentration was observed at all protein levels examined. The effect of varying substrate concentration on enzyme activity was also examined. The enzyme displayed normal Michaelis-Menten kinetics with both substrates. It was found that at optimum NADP concentration the Km for glucose 6-phosphate was 1.3×10^{-5} M. The optimal level of NADP required for the maximal velocity of the combined enzymes were determined and saturating NADP concentration was used in all subsequent enzyme assays.

The enzyme levels in male and female rats fed various dietary regimes were examined (see table V). When the male and female animals on the carbohydrate supplemented diets are compared both the specific activity and the activity per g liver of G6PDH were greater in female than in male ($p < 0.01$). But in the animals on the CDD[R] diet the sex differences was less apparent ($p < 0.05$ for specific activity; $p < 0.1$ for activity per g liver) mainly because of the lower enzyme activity observed in the animals on this diet. In common with the other lipogenic enzymes examined the activity per liver was greater in the females. Other workers (274, 210, 227) have also found similar differences in G6PDH activity in liver from male and female rats. However, these workers found that oestradiol administration did not raise G6PDH levels in ovariectomized rats but the steroid will raise the enzyme level in castrated males. Administration of dimethyltestosterone to intact females also resulted in a lowering of enzyme activity. This evidence points to the involvement of sex steroids in regulation of G6PDH activity.

Table V The effect of different dietary regimens on hepatic glucose 6-phosphate dehydrogenase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)		Activity per g liver (μ mole min^{-1})	
	Male	Female	Male	Female
CDD [R]	2.1 ± 0.6	3.2 ± 0.5	0.56 ± 0.06	0.67 ± 0.07
CDD [R] + sucrose	31.2 ± 7.5	88.8 ± 26.4	6.62 ± 1.0	18.9 ± 4.3
CDD [R] + glucose	13.4 ± 5.6	67.2 ± 13.3	3.41 ± 0.8	17.6 ± 3.4

The values given are mean ± S.D. with 5 rats of each sex on each diet.

A statistical analysis of the data is given in Table Va.

Rats (initial weight 50 ± 5g.) were fed for 110 days ad lib on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by the method of Rudack et al. (222) as described in the method (Section IV B.2.d.)

Specific enzyme activity is expressed as n mole of NADP reduced min^{-1} mg^{-1} protein.

Table Va Statistical analysis of the data in Table V

Group comparison*					Significance	
					Specific activity	Enzyme activity per g liver
CDD[R]	male	vs	female		$p < 0.05$	$p < 0.1$
CDD[R] + sucrose	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R] + glucose	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + sucrose	male	$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + glucose	male	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	male	vs	CDD[R] + glucose	male	$p < 0.01$	$p < 0.01$
CDD[R]	female	vs	CDD[R] + sucrose	female	$p < 0.01$	$p < 0.01$
CDD[R]	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	female	vs	CDD[R] + glucose	female	NS	NS

NS = Not significant

* = 5 rats in each group

The observed sex differences in this study may be the result of a direct effect of the sex steroids on hepatic G6PDH or an indirect effect which may be mediated by some other factors.

The effect of diet on G6PDH activity in male and female rats was examined (Table V). It was found that carbohydrate supplementation of the diet resulted in a large increase in the enzyme activity in both male and female rats compared to the control animals on the CDD[R] diet.

Sucrose feeding appeared to increase ($p < 0.01$) the specific enzyme activity to a greater extent than the glucose supplement in male animals only. A similar diet-induced difference ($p < 0.01$) was observed in males when the activities are expressed per g. liver. No significant differences were observed in females.

The effects of carbohydrate supplementation of the diet on G6PDH activity are in agreement with other observations (221, 207, 62, 220, 138, 208). Rudack et al. (222) have found that changes in enzyme activity following carbohydrate feeding are due to changes in the rate of enzyme synthesis and their data also indicate that insulin is not a direct regulator of enzyme level. This latter observation may be related to the finding that, in the male rat, sucrose feeding has a greater effect on enzyme activity than glucose despite the fact that the insulin response would be greater in the case of the monosaccharide. In the female, however, the differences between the effects of glucose and sucrose supplementation of the diet are not significant suggesting that there are sex differences in the response of the animals to various dietary carbohydrate.

There have been no studies on the effect of dietary carbohydrate on G6PDH activity in female rats but there are

several reports that sucrose has a greater effect on enzyme activity than starch in male rats (207, 220). The rate of synthesis of G6PDH is increased in proportion to the caloric intake of carbohydrate whether or not the carbohydrate is able to stimulate insulin release (222). Thus the differences observed in animals on the sucrose and glucose diets may be a result of differences in the consumption of carbohydrate.

If the supply of cytoplasmic NADPH regulates the rate of hepatic lipogenesis (see section I.D.5)then the study provides further evidence that the lipogenic capacity of female rat liver is greater than that in the male and that carbohydrate feeding raises lipogenic ratesin both male and female.

5. 6-Phosphogluconate dehydrogenase

Another enzyme which may limit the supply of NADPH (144) for hepatic lipogenesis is the second enzyme in the pentose phosphate pathway, 6PGDH. This enzyme was assayed in the 100,000 g supernatant from rat liver homogenate by following the reduction of NADP in the presence of 6-phosphogluconate. A linear relationship between enzyme activity and protein concentration was observed under the conditions used in this study. The effects of varying substrate concentration on enzyme activity was also examined. The enzyme displayed normal Michaelis-Menten kinetics. At optimum NADP levels the Km for 6PG was 2.7×10^{-5} M. Optimal levels of both substrates were used for all subsequent studies.

The enzyme levels in male and female rats fed various dietary regimes were examined (table VI) and it was found that the specific enzyme activity of 6PGDH in the female was considerably higher than in the male ($p < 0.01$) when animals fed on the same dietary regime are compared. The activity per g. liver was also

Table VI The effect of different dietary regimens on hepatic 6-phosphogluconate dehydrogenase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)		Activity per g. liver (µ mole min^{-1})	
	Male	Female	Male	Female
CDD [R]	6.8 ± 1.7	22.5 ± 5.1	1.5 ± 0.1	5.02 ± 0.6
CDD [R] + sucrose	90.8 ± 22.2	176.6 ± 14.9	19.6 ± 4.4	38.2 ± 4.4
CDD [R] + glucose	100.0 ± 23.5	321.6 ± 43.7	26.2 ± 4.4	84.1 ± 10.5

The values given are mean ± S.D. with 5 rats of each sex on each diet.

A statistical analysis of the data is given in Table VIa.

Rats (initial weight 50 ± 5 g.) were fed for 110 days ad lib on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by the method of Ruduck et al. (222) as described in the method (Section IV B.2.c.)

Specific enzyme activity is expressed as n mole of NADP reduced min^{-1} mg^{-1} protein.

Table VIa Statistical analysis of the data in Table VI

Group comparison*					Significance	
					Specific activity	Enzyme activity per g liver
CDD[R]	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R] + glucose	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + sucrose	male	$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + glucose	male	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	male	vs	CDD[R] + glucose	male	NS	NS
CDD[R]	female	vs	CDD[R] + sucrose	female	$p < 0.01$	$p < 0.01$
CDD[R]	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.01$

NS = Not significant

* = 5 rats in each group

greater ($p < 0.01$) in the female than in the male as was the total activity per liver. There are two previous reports that the activity per g liver is higher in females than in males (210, 227) and that ovariectomy results in the lowering of 6PGDH activity. Oestradiol treatment results in an increase of enzyme activity both in ovariectomized females and castrated males. This again suggests involvement of sex steroids in the regulation of the activity of this enzyme, but the mechanism involved is not clear.

The results in table VI also show that either glucose or sucrose supplementation of the diet of both sexes results in a large increase ($p<0.01$) in specific enzyme activity compared to the control CDD[R] fed animals. Unlike the previous enzymes examined the female animals on the glucose supplement had a higher ($p < 0.01$) 6PGDH activity than females on the sucrose diet. However, this difference was not apparent in the males. These results suggest different mechanisms for the regulation of G6PDH and 6PGDH. There is evidence to suggest that the activities of two enzymes may be independently regulated, glucagon will inhibit G6PDH synthesis but under similar conditions the rate of synthesis of 6PGDH is unaffected (225, 230).

The sex difference in the response to dietary sucrose and glucose in the present study may be related to the observation of Mandour et al. (70) who found that oestradiol inhibited glucagon release. This may result in the high enzyme activity observed in females fed on glucose.

Another report has suggested that soluble carbohydrates in the diet can raise 6PGDH activity but that sucrose is more effective than glucose and starch in this respect (220). Rudack et al. (231) found that the rate of synthesis of this enzyme is also related to the caloric uptake of carbohydrate and they could show

no differences between glucose and fructose diets in their effect on enzyme synthesis. This is in contrast to the present study in which sucrose/glucose differences were observed and in addition glucose was more potent in raising 6PGDH levels than sucrose. Further evidence for independent regulation implies that some factor other than caloric intake is involved.

6. Glycerol 3-phosphate acyltransferase

It has been suggested that GPAT is a rate limiting enzyme in hepatic triacylglycerol synthesis (251). It was of interest therefore to examine the effects of various dietary regimes on the activity of GPAT to find out if it was subject to the changes similar to those observed in lipogenic enzymes.

The enzyme was assayed by the method of Husbands and Lands (241) in which the incorporation of sn-[^{14}C] glycerol 3-phosphate into total lipid by a microsomal fraction was followed. Stearoyl-CoA and palmitoyl-CoA have been reported to be better acyl donors than other acyl-CoA derivatives. The former was used as an acyl donor in this study.

In contrast to previous enzymes assayed in the study the enzyme levels were not subject to large differences between various groups of animals. The enzyme levels in male and female animals fed on various dietary regimes were examined (Table VII). No sex differences in GPAT specific activity in any of the groups of animals on the same diet were observed. There are no reports in the literature which compare male and female GPAT levels but Soler-Argilaga et al. (69) have reported sex differences (♀ > ♂) in glycerolipid synthesis from palmitate, ATP and glycerol 3-phophate by a microsomal fraction from rat liver. However, these workers

Table VII The effect of different dietary regimens on hepatic glycerol 3-phosphate acyl transferase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)	
	Male	Female
CDD [R]	0.61 ± 0.10	0.50 ± 0.05
CDD [R] + sucrose	0.73 ± 0.07	0.71 ± 0.07
CDD [R] + glucose	0.56 ± 0.04	0.54 ± 0.08

The values given are mean ± S.D. with 5 rats of each sex on each diet.

A statistical analysis of the data is given in Table VIIa.

Rats (initial weight 50 ± 5 g) were fed for 110 days *ad lib.* on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by method of Husband and Lands (241) as described in the method (Section IV. B.2.e.) Specific enzyme activity is expressed as nmoles sn-[^{14}C]-glycerol 3-phosphate incorporated min^{-1} mg^{-1} protein.

Table VIIa Statistical analysis of the data in Table VII

Group comparison*					Significance
CDD[R]	male	vs	female		NS
CDD[R] + sucrose	male	vs	female		NS
CDD[R] + glucose	male	vs	female		NS
CDD[R]	male	vs	CDD[R] + sucrose	male	$p < 0.02$
CDD[R]	male	vs	CDD[R] + glucose	male	$p < 0.05$
CDD[R] + sucrose	male	vs	CDD[R] + glucose	male	$p < 0.01$
CDD[R]	female	vs	CDD[R] + sucrose	female	$p < 0.01$
CDD[R]	female	vs	CDD[R] + glucose	female	NS
CDD[R] + sucrose	female	vs	CDD[R] + glucose	female	$p < 0.01$

NS = Not significant

* = 4 rats in each group

suggest that these sex differences are related to differences in the uptake of Ca^{2+} by the microsomes which may in turn regulate triacylglycerol synthesis. Oestrogen treatment has also been reported to raise GPAT levels in chick (255).

If the sex differences in the lipogenic enzymes are related to differences in plasma insulin levels between males and females this would suggest that the activity of this enzyme is not influenced by insulin in the long term. Two publications suggest that this is the case (265, 77) but a third report (272) claims that microsomal GPAT is raised in the diabetic rat.

An examination of the animals on different dietary regimes shows that sucrose supplementation of the diet in both male and female animals results in an increased GPAT activity compared to the control CDD[R] and to glucose supplemented groups ($p < 0.01$) except in the case of sucrose vs CDD[R] in the male where p is < 0.02. The enzyme activties in the latter groups are not significantly different in either sex. It appears to be generally accepted that soluble phosphatidate phosphohydrolase(PPH) is the main regulatory enzyme in the synthesis of triacylglycerol but there is some evidence to suggest that triacylglycerol is formed entirely from recycled phosphatidate and presumably does not involve the soluble phosphatidate phosphohydrolase but a microsome-bound enzyme (332). There is also some evidence to suggest that GPAT levels are altered by dietary changes (257) and by age-related changes (250).

Lamb and Fallon (257) have reported that both fructose and glucose in the diet increases the activity of both GPAT and PPH associated with liver microsomes but the high ratio of neutral lipid

to phospholipid formed by the organelles from animals fed these high carbohydrate diet suggests that the effect on the PPH is predominant. Glenney et al. (253) on the other hand found that GPAT levels were not significantly affected by dietary modification, including sucrose feeding. They conclude that the enzyme is not rate limiting in hepatic triacylglycerol synthesis nor does its activity correlate with rates of triacylglycerol synthesis. Both these studies were carried out over relatively short periods (up to 14 days) and the apparent contradiction between these findings and those in the present study may arise because of a long-term adaptation to diet which may take longer than two weeks. Again there is no evidence that insulin is involved in the long term regulation of microsomal GPAT levels since glucose supplementation of the diet has no effect on enzyme activity.

7. Fructose 1, 6 bisphosphate aldolase

The enzymes reported in the previous sections have also been postulated to be key enzymes in lipogenesis and triglyceride synthesis. It was of interest therefore to compare the changes which occur in these enzymes with a bifunctional glycolytic enzyme, aldolase. This enzyme is important in fructose metabolism. Since the aldolase B from liver utilizes both fructose 1-phosphate and fructose 1, 6-bis-phosphate as substrate (see IIB.). Fructose is metabolised in the liver mainly via the former metabolite therefore the importance of hepatic aldolase is clear. The levels of ketohexokinase and aldolase are such that accumulation of fructose 1-phosphate following fructose administration would not be expected but when rat liver is perfused with 10mM fructose, the ketose phosphate accumulates by a mechanism which involves the depletion of ATP and the resulting accumulation of IMP (320). IMP is reported to be an inhibitor of aldolase (see IIB.).

Aldolase B is of interest in fructose metabolism in that it is the major route by which the hexose can be converted to glycolytic intermediates and, also, since much of the absorbed fructose is converted to glycogen (see IA.) then the enzyme is utilized twice in gluconeogenesis from fructose ie. it is also involved in the synthesis of FDP from DHAP and GAP.

Mahmoud (292) has observed no sex differences in ketohexokinase activity on diets similar to those used in this study. Sucrose and glucose feeding resulted in a two-fold elevation of the enzyme activity compared to the controls in each case. It is clear therefore that possible sex differences in the response of the animal to dietary fructose are not the result of differences in the rates of phosphorylation of the ketose. In the present study the hepatic activities of fructose 1-phosphate aldolase and fructose 1,6-bisphosphate aldolase in male and female rats fed on different dietary regimes were examined (Tables VIII and IX).

The activity towards each substrate was assayed in a coupled enzyme system consisting of glycerol 3-phosphate dehydrogenase and triose phosphate isomerase and the oxidation of NADH was followed by a spectrophotometric method. Optimal levels of both fructose 1-phosphate (10mM) and fructose 1,6-bisphosphate (2mM) were used in all studies. It has been claimed that the activities towards the two substrates result from different isoenzymes but most of the evidence points to the conclusion that a single form of the enzyme displays activity towards both substrates. The evidence in this study tends to confirm this latter view. The ratios of the FIPA/FDPA activities in similar groups are approximately 1 with one exception that is females fed on CDD[R] diet where the ratio is 0.77. Heinz (119) has reported a ratio of 0.38 but other workers (308, 303) have reported a ratio similar to that obtained in this study.

Table VIII The effect of different dietary regimens on hepatic fructose bisphosphate aldolase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)		Activity per g. liver (µ mole min^{-1})	
	Male	Female	Male	Female
CDD [R]	17.5 ± 1.7	22.0 ± 2.9	3.32 ± 0.2	4.83 ± 1.0
CDD [R] + sucrose	26.9 ± 1.4	37.0 ± 2.0	6.22 ± 0.6	9.37 ± 0.5
CDD [R] + glucose	22.7 ± 0.3	26.7 ± 2.3	6.47 ± 0.4	7.20 ± 0.3

The values given are mean ± S.D. with 4 rats of each sex on each diet.

A statistical analysis of the data is given in Table VIIIa.

Rats (initial weight 50 ± 5g.) were fed for 110 days ad lib on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by the method of Rajkumar & Rutter (343) as described in the method (Section IV B.2.f.)

Specific enzyme activity is expressed as n mole D-fructose 1,6-bisphosphate cleaved min^{-1} mg^{-1} protein.

Table VIIIa Statistical analysis of the data in Table VIII

Group comparison*					Significance	
					Specific activity	Enzyme activity per g liver
CDD[R]	male	vs	female		$p < 0.01$	$p < 0.05$
CDD[R] + sucrose	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R] + glucose	male	vs	female		$p < 0.05$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + sucrose	male	$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + glucose	male	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	male	vs	CDD[R] + glucose	male	$p < 0.01$	NS
CDD[R]	female	vs	CDD[R] + sucrose	female	$p < 0.01$	$p < 0.01$
CDD[R]	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.01$

NS = Not significant

* = 4 rats in each group

Table IX The effect of different dietary regimens on hepatic fructose 1-phosphate aldolase activity in male and female rats.

Diet	Specific activity (n mole min^{-1} mg^{-1} protein)		Activity per g liver (μ mole min^{-1})	
	Male	Female	Male	Female
CDD [R]	17.2 ± 2.2	16.9 ± 1.5	3.2 ± 0.7	3.6 ± 0.7
CDD [R] + sucrose	26.4 ± 2.2	35.4 ± 2.7	6.1 ± 0.7	8.5 ± 0.9
CDD [R] + glucose	24.4 ± 1.3	28.5 ± 2.5	6.9 ± 0.1	7.9 ± 1.0

The values given are mean ± S.D. with 4 rats of each sex on each diet.

A statistical analysis of the data is given in Table IXa.

Rats (initial weight 50 ± 5 g.) were fed for 110 days ad lib on Dixon CDD [R] and Dixon CDD [R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

The enzyme was assayed by the method of Rajkumar & Rutter (343) as described in the method (Section IV B.2.g.)

Specific enzyme activity is expressed as n mole D-fructose 1-phosphate cleaved min^{-1} mg^{-1} protein.

Table IXa Statistical analysis of the data in Table IX

Group comparison*					Significance	
					Specific activity	Enzyme activity per g liver
CDD[R]	male	vs	female		NS	NS
CDD[R] + sucrose	male	vs	female		$p < 0.01$	$p < 0.01$
CDD[R] + glucose	male	vs	female		$p < 0.05$	NS
CDD[R]	male	vs	CDD[R] + sucrose	male	$p < 0.01$	$p < 0.01$
CDD[R]	male	vs	CDD[R] + glucose	male	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	male	vs	CDD[R] + glucose	male	NS	NS
CDD[R]	female	vs	CDD[R] + sucrose	female	$p < 0.01$	$p < 0.01$
CDD[R]	female	vs	CDD[R] + glucose	female	$p < 0.01$	$p < 0.01$
CDD[R] + sucrose	female	vs	CDD[R] + glucose	female	$p < 0.01$	NS

NS = Not significant

* = 4 rats in each group

The enzyme levels in male and female rats fed various dietary regimes were examined. Fructose 1,6-bisphosphate aldolase specific activity and the activity per g. liver were greater in females than in males ($p < 0.05$) in all cases (Table VIII). Fructose 1-phosphate aldolase activity showed a similar pattern (Table IX) except that in control animals fed a CDD[R] diet there was no siginificant sex difference in specific activity. When the F 1 P aldolase activities were expressed on a per g liver basis the only sex difference ($p < 0.01$) was found in the sucrose fed animals.

Sex differences in hepatic aldolase activity have not been previously reported in the literature. There is a report that fructose may be metabolized at a faster rate in male baboons than in females, whereas no sex differences in glucose metabolism can be detected (305). However no sex difference in ketohexokinase, triokinase or glycerol phosphate dehydrogenase levels could be detected in experiments similar to those reported in the present study (292). Thus one would expect the rate of utilization of fructose to the triose phosphate level, notably glycerol 3-phosphate, to be faster if anything in the female. This is especially true if aldolase is a rate limiting enzyme in fructose metabolism as the observations that fructose 1-phosphate accumulates both in a liver perfused with 10mM fructose (320) and following intragastric intubation with 70% fructose (81).

It has been proposed that the greater contribution of fructose towards triglyceride formation lies in its additional capacity for glycerol 3-phosphate synthesis (50, 138) which should therefore be greater in females than in males.

The effects of the different dietary regimes on FDP aldolase activity is also shown in table VIII. The sucrose dietary regime results in higher aldolase activities ($p < 0.01$) than those found in the

controls in both male and female animals. Glucose supplementation of the diet of both sexes results in an increase in specific activity ($p < 0.01$) and enzyme activity per g liver ($p < 0.01$) compared to the controls. A comparison of the sucrose and glucose fed animals shows statistically significant differences in enzyme specific activity between the groups ($p < 0.01$). However, enzyme activity per g of liver is only significantly different in the females ($p < 0.01$).

Fructose 1-phosphate aldolase activity is also increased by both glucose and sucrose feeding compared to the control ($p < 0.01$) (Table IX). The specific activity of FIP aldolase in the females fed on sucrose is significantly higher than in females fed on the glucose supplemented diet but no such difference was observed in the males. There was no significant difference in enzyme activity per g liver in the sucrose and glucose fed groups in either sex.

Previous reports (221, 308, 208) have indicated that a high glucose diet will raise aldolase levels to a smaller extent than a corresponding fructose or sucrose diet but the adaptive changes in aldolase are much less than those observed, for example in pyruvate kinase or glucose 6-phosphate dehydrogenase activies. Hepatic aldolase activity has also been reported to fall during fasting (13) and to increase at weaning (165). These diet-induced changes in aldolase activity are surprising in view of the bifunctional nature of the enzyme i.e. it can be involved in either gluconeogenesis or glycolysis depending on the nutritional and hormonal status of the animal. However, it should be noted that the changes observed are smaller than those found for the key lipogenic enzymes.

B. Age-related differences in enzyme levels in male and female rats

Many sex-related differences in enzyme levels were found in the various groups of animals used in the first part of this study and it was of interest, therefore, to determine if these differences persist if the animals are fed a totally different diet. For this study two groups of animals (4 male and 4 female) were fed on Dixon.86 diet for a period of approximately eleven weeks from weaning (Tables X and XI). This is a low fat diet compared to CDD[R] [see IV.A.3]. A similar pattern of enzyme activities was observed in all the enzymes studied, that is, the specific activities and activities per g. liver were greater in the females than in the males ($p < 0.01$) except for 6PGDH which is not so significant ($p < 0.1$) and for GPAT where no sex difference was observed. It is clear therefore that the sex differences observed are not related to the nature of the diet.

It was also of interest to compare the levels of enzyme activity in adult animals with young animals fed on an identical diet (TablesX, XI). During the development of the rat various changes in carbohydrate and lipid metabolising enzymes are observed which are related to the dietary changes occurring during this period. The first change occurs at birth when the high carbohydrate content of food material absorbed through the placenta is replaced by a high fat, milk diet. The second change occurs at weaning when the milk diet is replaced by high carbohydrate laboratory diets.

These changes of diet can be correlated to the efficiency of operation of various pathways, for example, lipogenesis from [^{14}C] acetate by liver slices shows a sharp increase at weaning to a peak at about 35 days followed by a gradual decline with increasing age of the adult rat. There is no apparent sex difference in the young animals (up to 40 days) but lipogenesis declines to a smaller extent in females giving rise to the characteristic sex difference in lipid synthesis in the adult rat. Concomitant changes in G6PDH have also been reported (274). Similarly at weaning there is a rapid increase in FAS synthesis which results in a higher FAS

Table X Sex and age-related differences in the specific activities of some enzymes in animals fed on Dixon .86 diet.

Enzyme	Specific activity (n mole min^{-1} mg^{-1} protein) Young +		Specific activity (n mole min^{-1} mg^{-1} protein) Adult ++	
	Male	Female	Male	Female
ACC	1.8 ± 0.7	1.7 ± 0.3	0.57 ± 0.05	0.82 ± 0.17
FAS	8.2 ± 1.8	7.9 ± 1.1	5.0 ± 0.8	7.3 ± 1.2
G6PDH	8.9 ± 1.8	8.2 ± 2.2	8.3 ± 1.2	13.6 ± 2.3
6PGDH	29.8 ± 8.1	28.1 ± 8.5	28.4 ± 7.4	40.0 ± 8.2
GPAT*	0.43 ± 0.02	0.41 ± 0.02	0.52 ± 0.06	0.49 ± 0.1
FIP Aldolase	24.8 ± 0.7	24.4 ± 0.9	21.0 ± 4.3	33.0 ± 2.0
FDP Aldolase	25.4 ± 0.9	25.1 ± 1.1	24.4 ± 1.9	33.6 ± 1.0

Enzyme activities were determined as described in materials and methods (Section IV.B.)

A statistical analysis of the data is given in Table XII.

+ Rats 4 male and 4 female (50 g 21 days old) were weaned and fed on Dixon .86 for 7 days before sacrificed.

++ Two further groups of rats (4 male and 4 female) were fed on the same diet for 11 weeks before sacrificed.

*Enzyme activity expressed per mg microsomal protein.

Table XI Sex and age-related difference in enzyme activities per g. liver in animals fed on Dixon .86 diet.

Enzyme	Enzyme activity/g liver (μ mol) Young +		Enzyme activity/g liver (μ mol) Adult ++	
	Male	Female	Male	Female
ACC	0.32 ± 0.10	0.33 ± 0.04	0.14 ± 0.01	0.20 ± 0.02
FAS	1.70 ± 0.46	1.68 ± 0.26	0.99 ± 0.17	1.57 ± 0.24
G6PDH	1.91 ± 0.35	1.99 ± 0.08	1.68 ± 0.26	2.83 ± 0.41
6PGDH	6.27 ± 0.60	6.88 ± 2.0	5.75 ± 1.40	8.47 ± 2.41
FIP Aldolase	5.25 ± 0.31	5.10 ± 0.33	4.40 ± 0.60	8.02 ± 0.96
FDP Aldolase	5.36 ± 0.30	5.26 ± 0.30	5.39 ± 0.55	8.23 ± 0.75

Enzyme activities were determined as described in materials and methods (Section IV.B.)

A statistical analysis of the data is given in Table XII.

+ Rats 4 male and 4 female (50 g 21 days old) were weaned and fed on Dixon .86 for 7 days before sacrificed.

++ Two further groups of rats (4 male and 4 female) were fed on the same diet for 11 weeks before sacrificed.

Table XII Statistical analysis of the data from Tables X and XI

Comparison	ACC		FAS		G6PDH		6PGDH		GPAT		FDP Aldolase		FIP Aldolase	
	SA+	TA++	SA	TA	SA	TA	SA	TA	SA	TA	SA	TA	SA	TA
Young animal	NS	NS	NS	NS	NS	NS	NS	NS	NS	ND	NS	NS	NS	NS
Adult animal	$p<0.01$	NS	$p<0.05$	$p<0.02$	$p<0.01$	$p<0.01$	NS	$p<0.05$	NS	ND	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$
Male	$p<0.05$	$p<0.02$	$p<0.05$	$p<0.05$	NS	NS	NS	NS	$p<0.05$	ND	NS	NS	NS	NS
Female	$p<0.01$	$p<0.01$	NS	NS	$p<0.01$	$p<0.01$	NS	NS	NS	ND	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$

+ Enzyme activity per mg protein

++ Enzyme activity per g liver

activity in the liver. However, in the adult the FAS activity is lower than in the weaned animal.

It was of interest therefore in the study to examine age-related changes in the activity of the various enzymes studied. Rats (4 male and 4 female) were obtained from a commercial source at weaning (21 days; 50 g) and fed on Dixon.86 for a further seven days. This allowed time for adaptation to the new diet to take place and the age at which they were killed corresponded to a period when maximum rates of lipogenesis occur (274). A second group of animals (4 male and 4 female) were fed for a further 11-week period on the same diet.

It was of interest to find that no sex differences in the specific activity of any of the enzymes studied were observed in the 28 day old animals but after a period of 11 weeks on the same diet sex-related differences in the levels of various enzymes have become apparent (Tables X and XI). A similar pattern of sex differences in enzyme activities was found in animals fed on Dixon.86. That is the activities of ACC, FAS, G6PDH, 6PGDH and aldolase activities were higher in females than in males whereas no sex difference in GPAT was observed. This is further evidence that the differences in enzyme levels between males and females are not influenced by diet (see Section I.E.).

Acetyl CoA carboxylase levels were lower in the adult animals than in the 28 day old rats ($p < 0.01$ in females, $p < 0.05$ in males). The fall in enzyme activity in the males was more marked than in the female (Tables X and XI). Fatty acid synthetase levels were also lower in the adult males than in the young male ($p < 0.05$) but no age-related difference between the female group was observed. These observations suggest that the lipogenic capacity of the liver

is reduced in the adult animals, especially in the males. This is in agreement with the results of Volpe et al., (194) who found that FAS levels are lower in adult than in young, weaned animals and also with the data observed by Taylor et al., (274) who found no significant sex differences in lipogenesis in animals up to 40 days of age but in mature animals a significant difference was observed. In the latter study the fall in the level of hepatic lipogenesis after the age of 30 days was more pronounced in males than in females.

Glucose 6-phosphate dehydrogenase specific activity and total activity were increased in the adult females compared to the young animals ($p < 0.01$) but no such change occurred in the males (Tables X and XI). This is in agreement with data presented by Taylor et al., (274). 6-phosphogluconate dehydrogenase showed a similar pattern but the difference between the young female and the adult female was not so significant ($p < 0.1$).

Glycerolphosphateacyltransferase specific activity appeared to increase slightly in both sexes in the adult animal but the difference was only significant ($p < 0.05$) in the male. These results are contrary to those of Jamdar et al., (250) who found that GPAT activity decreased in adult rats. Kako et al., (110) however found a small age-related increase in microsomal enzyme activity in male rats.

Both FIP and FDP aldolase activities were increased in adult female rats compared to young animals ($p < 0.01$) but no such change was observed in the males. Lockwood et al., (165) have also found no change in aldolase activity in male rats between 30 day old and adult animals although a rapid increase in enzyme activity was observed at weaning.

It is clear from the data presented in the present study and the work of Taylor et al., (274, 165) that there are sex differences in the changes in enzyme activities which are related to the development of the rats. These differences become

apparent after 30 days of age. For example, increased G6PDH, 6PGDH and aldolase activities were observed only in the females whereas the falls in FAS and ACC levels were much more marked in the males.

C. Discussion

The evidence provided by the results of this study indicates that the activities of some key lipogenic enzymes, notably FAS, ACC, G6PDH, and 6PGDH are significantly greater in adult females than in males although this sex-related difference is not evident in 28 day old animals. There is a considerable body of evidence (see Section I, D), which indicate that these enzymes play a key regulatory role in de novo hepatic fatty acid synthesis and thus may be rate determinant for hepatic VLDL-TG production. Enhanced FAS and ACC activities have frequently been correlated to increased hepatic VLDL production (178, 346).

The sex-related difference in activity of these lipogenic enzymes could be due to two major factors:-
(a) a difference in the catalytic efficiency of the enzyme or
(b) a difference in the quantity of enzyme protein present.

It is assumed that the enzyme levels observed in the present study reflect the total amount of enzyme protein present since optimum conditions were used in all assay procedures. An unequivocal demonstration of this assumption would require specific immunoprecipitation of each enzyme from the soluble protein preparations. The work of Afolabi (347) on the changes in ACC and FAS activity induced by oestradiol treatment indicates that there are no changes in the hepatic properties of the enzymes which is further evidence in support of the view that the activities observed represent changes in the amount of enzyme protein synthesis.

The results presented in this study show a marked age-dependent sex difference in the activities of some key enzymes of hepatic lipogenesis. These sex differences appeared to be independent of the type of diet on which the

animals were fed. Thus following administration of a diet rich in carbohydrate, when high rates of lipogenesis would be expected, then the lipogenic enzyme levels were higher, and in animals fed a relatively high fat diet, the rates of lipogenic enzymes were low. Nevertheless the sex difference occured in all cases. The sex differences found in adult animals were not observed in young, weaned animals. This suggests that the sex differences arise as a result of developmental differences between male and female rats.

The sex-related difference in lipogenic enzymes does not appear to be a general phenomenon for all enzymes since no differences in the levels of ketohexokinase, triokinase, lactate dehydrogenase (299) and glycerol phosphate acyltransferase (this study) occur in adult animals kept under the same conditions. Indeed, glycerol kinase levels appear to be higher in males than in females (292). Thus it can be postulated that some changes occur during the development of either male or female rats (or possibly both sexes) which result in specific changes in lipogenic enzyme levels.

Some obvious factors which may be involved in the regulation of lipogenesis are the levels of circulating sex hormones. There is some evidence for the involvement of the sex steroids in the regulation of lipid metabolism. It is clear from the literature that fatty acid uptake and TG synthesis occur more rapidly in the female liver than in the male (348, 66, 64, 65). In addition TG output is lowered by ovariectomy and subsequently raised by the administration of oestrogen (64). In addition, the higher levels of FAS, ACC and 6PGDH in female rats can be correlated to the changes in enzyme levels which occur when female rats are ovariectomized and subsequently injected with oestrogens (70, 178).

Mandour, Kissebah and Wynn (70) suggest that the oestrogen effect on the lipogenic enzymes and on TG synthesis is mediated via changes in insulin and glucagon levels. These workers showed that oestrogen increases the insulin/glucagon

(I/G) ratio in the portal vein of ovariectomized rats, mainly by lowering the immunoreactive glucagon level. They found that 17 β-oestradiol had a marked suppressive effect on the α cells of the pancreas. The increase in I/G ratio during oestrogen therapy would therefore stimulate hepatic lipogenesis, inhibit gluconeogenesis and slightly lower the blood glucose level. This oestrogen-induced change in the I/G ratio may also explain the sex difference in lipogenic enzymes observed in this study, i.e. the I/G ratio may be higher in normal females than in males. Further evidence for this possibility is that blood glucose levels are known to be slightly but significantly higher in male rats than in females (349). In this connection it has also been reported that female rats have higher insulin levels than males both in the fasted condition and after refeeding a high carbohydrate diet. These observations again are consistent with the explanation for the sex differences in lipogenic enzymes found in adult animals fed either a high fat or a high carbohydrate diet.

The suggested mechanism for these differences is that oestrogen, through its effects on glucagon and insulin can influence both TG and fatty acid synthesis. The synthesis of both TG and fatty acid have been reported to be lowered in diabetic animals and enhanced by insulin (33, 85, 350). Lipogenesis has been reported to be enhanced by insulin in isolated hepatocytes (351, 352) and in perfused livers (353) and the esterification of fatty acid is also increased. Glucagon, on the other hand inhibits lipogenesis by isolated hepatocytes (85, 352, 351) and has also been reported to inhibit TG secretion (33, 105). The activities of GPAT in male and female liver from animals on the same diets were found to be similar (Table VII). Hence if the present hypothesis was correct, it would be expected that the enzyme would not be influenced by insulin. One publication suggests that this is so (265) but a second report claims that microsomal GPAT is raised in the diabetic animal (272).

It is interesting to apply the oestrogen - insulin - glucagon theory to young animals. The levels of the oestradiol are high in both male and female up to 23 days (281). This is consistent with the evidence that high rates of glycerolipid synthesis occur in young animals (250). No sex difference in oestradiol levels can be observed between 25 - 39 days (281) which is in agreement with the evidence from this study that there is no sex difference in lipogenic enzymes in young animals (28 days) and that the sex difference is correlated to the increase in oestradiol levels in the females and the decrease in the males which occur after 39 days of age (281).

It is clear from the evidence from the literature and from this study that female rats have a greater capacity for hepatic TG synthesis and lipogenesis than males. One explanation for this is a higher I/G ratio in the females, but other hormones play an important role in the regulation of lipid metabolism (85, 92, 95, 96, 97) therefore, other hypotheses must be considered.

It is also possible that there is a direct effect of sex hormones on hepatic lipid metabolism. Administration of oestrogen to chick has been shown to result in a marked hyperlipidaemia and arteriosclerosis and that this treatment results in an augmented VLDL and LDL production together with a marked reduction of HDL (354). The increased synthesis of VLDL following oestrogen treatment is blocked by administration of actinomycin-D, suggesting that the synthesis of specific mRNA for VLDL apoprotein is involved (355). However, these authors have not presented evidence for a direct effect of oestrogen on the liver except that there are specific nuclear receptors for oestrogen and that oestrogen treatment leads to an increase in RNA polymerase I and II activities and thus it is postulated to result in the synthesis of specific mRNA. However, so far there is no evidence for

a direct effect of sex steroids on hepatic protein synthesis. It has been suggested that the synthesis of an androgen-induced protein $\alpha_{2\mu}$-globulin by rat liver is a result of a direct effect of the steroid on the liver but recent evidence suggests (356, 357) there is a general enhancement of protein synthesis by androgen. However, specific induction of $\alpha_{2\mu}$-globulin has been observed with a glucocorticoid. It is of interest to note that the protein is not synthesised in either young male or in female rats. The synthesis of $\alpha_{2\mu}$-globulin in the male starts at 35 - 40 days of age (358) and recent evidence points to a complex multi-hormonal regulation, androgen, glucocorticoids, thyroid hormone and growth hormone, all induce the synthesis of the protein and oestrogen appears to act as a repressor in this case (359, 360, 361, 356). Thus we have another example of a sex difference which is not apparent in young animals but which appears in the adult animal.

Another possible explanation for the sex- and age-related differences in enzyme levels found in this study may be found in the levels of thyroid hormones. Some sex differences in thyroid function have been observed in rats. In young animals there is no sex differences in plasma TSH and thyroxine (t4) levels, but in adult rats the levels of TSH are 2.8 fold higher in males than in females (362, 363). However, this does not appear to be reflected in a substantial sex difference in plasma thyroxine levels although there are some reports of a higher level of the latter hormone in male (364, 365, 366) and one report (363) that female T4 levels were higher than those in the male. This may be related to a significant decrease in T3 (Triiodothyronine) generation from T4 by rat liver homogenates from female rats which occurs from abou. 30 days of age. Such a change is not observed in male rats (367).

In hypothyroid animals the fall in glucokinase, pyruvate kinase and pentose phosphate pathway and lipogenic

enzymes are all characteristics of an insulin deficient state (204). Thus from the differences in enzyme levels observed in present study it would be expected that female rats would be slightly hyperthyroid in comparison to the males.

Age-related changes in thyroid function have also been reported. There is a reduced T4 secretion in older animals (368) and there are reports (369, 370) that the responsiveness of the rat to a given dose of thyroid hormone is reduced with increasing age. For example, hepatic glycerol 3-phosphate dehydrogenase and malic enzyme decline with age and the fall is correlated to the nuclear content of T3, T3 nuclear receptor concentration and a decreased response of the enzyme to administered T3. These latter findings may be related to the decrease in both ACC and FAS synthesis observed in the older animals in the study (Tables X and XI).

There is also considerable evidence for the involvement of glucocorticoids in the regulation of lipogenic enzymes and TG synthesis and secretion (106, 107, 95, 96, 97), there is also a report that plasma corticosterone levels are higher in female rats than in males (366). However, Afolabi et al., (178) have found that the effects of oestrogen on hepatic lipogenic enzymes and on plasma TG levels do not occur in the adrenalectomized animal suggesting that the hyper-triglyceridaemia induced by oestrogen may be a consequence of changes in adrenocorticoid function. Thus the higher TG output by female rat livers compared to males may be a consequence of the higher corticosterone levels found in the former (366).

The evidence from the literature and from the present study indicates that the female has a higher potential for hepatic lipogenesis and TG synthesis than the male which may be due to the higher oestrogen levels in the female. However, the effect of oestrogen on the insulin/glucagon ratio (70) and on lipogenesis does not explain why higher levels of serum TG are found in male rats compared to females. It is therefore necessary to invoke differences in triglyceride clearing rates, to explain this phenomenon. Triglyceride

clearance is thought to be mediated by lipoprotein lipase (LPL) in peripheral tissues and this enzyme is known to be subject to hormonal regulation. Insulin has been reported to raise LPL levels (47) and glucagon has been reported to have the opposite effect (371). In the female animal with a relatively high insulin/glucagon ratio a high LPL activity would be expected and, consequently, more rapid TG clearing which could result in a lower serum triglyceride level despite a faster rate of the synthesis of lipid by the female liver. In the case of humans, women have greater amounts of adipose tissue than men and hence the total level of LPL is greater than in men. Women thus have a more efficient TG clearance system than men (119, 342). In normal subjects there is a significant negative correlation in both sexes between the activity of post-heparin LPL and fasting serum TG levels and there is a significantly higher level of post-heparin LPL in females than in males (31). It is of interest to speculate that the same situation occurs in the rat.

Oral administration of ethynyl oestradiol to the adult female rat leads to a 39% increase in blood TG levels and a 69% increase in adipose tissue LPL (318) but these authors were unable to show if these effects are the result of direct action of the steroid on the liver and adipose tissues or are due to a secondary metabolic responses.

The effects of dietary carbohydrate on lipogenic enzymes in rat liver have previously been reported by many authors (See Section I). Bruckdorfer et al., (62) have reported that plasma TG levels of male rats fed diets containing sucrose were higher than those of rats fed starch over a 150 day period. These authors suggest that sucrose stimulates hepatic lipogenesis (by modulating FAS, 6GPDH and PK activity) without a parallel change in TG clearance. The reason for the differential response to dietary carbohydrate is not clear. It has been suggested that different dietary carbohydrates may have differential effects on hormone levels and, thus, on

metabolism. For example, Bruckdorfer et al., (55) showed that rats fed for 30 days on fructose and sucrose enriched diets had lower plasma insulin levels than those on glucose diets. However, it has recently been reported (299) that insulin/glucagon ratios are significantly raised by sucrose as compared to starch in a dietary experiment lasting 12 weeks. This effect may be attributed to D-glyceraldehyde, an intermediate in the metabolism of fructose, which is found to be a more potent insulin releasing agent than glucose (77). Such changes in the hormonal levels could result in enhanced rates of lipogenesis and TG output by the liver and may explain the differences in serum TG levels in animals fed on various carbohydrate supplemented diets. If the raised insulin/glucagon ratio is the result of a sucrose enriched diet then an enhancement of all the processes leading to an increased rate of TG secretion should be counter-balanced by an equivalent increase in LPL activity. However, it is possible that the fructose component of the sucrose has an insulin-like effect on hepatic lipid metabolism. For example, Topping and Mayes (56) have reported that both insulin and fructose cause an increased secretion of TG-VLDL in isolated perfused liver. When the two effectors are combined the effects are equal to the sum of the individual effects. Since fructose is rapidly metabolised in the liver (see Section II. A.) it is therefore unlikely that the hexose would have a direct effect on peripheral tissues, e.g. adipose LPL. Thus, the mechanism of the sucrose-induced hypertriglyceridaemia may be a combination of an increased TG secretion and a lower insulin response to the ketose in comparison to glucose.

In a study using similar dietary regimens to those used in the present study Tay (81) found that, sucrose supplementation of the diet results in an increased serum TG in both male and female but that glucose feeding lowered serum TG in the female and raised it in the male. This is an indication that males and females may respond in a different fashion to dietary carbohydrate. However, there is no evidence from the present study for such a differential response of lipogenic enzyme levels. It would be of interest to assay post-heparin LPL in a similar study.

In the present study both sucrose and glucose feeding appeared to raise the specific activity of all the enzymes studied except for GPAT which was raised slightly by sucrose but not by glucose feeding. In general, the sucrose fed animals had higher lipogenic enzyme activities than the glucose fed animals, but the difference was significant only in the case of FAS and G6PDH. The exception was 6PGDH which was raised more by glucose in the female than sucrose. No significant difference was observed in the male.

The effect of dietary carbohydrate on lipid metabolising enzymes has been studied by many workers. Induction of FAS occurs rapidly when fasted rats are fed a fat-free diet (198). Bruckdorfer et al., (55, 80) showed that dietary fructose and sucrose led to higher hepatic FAS activities than glucose and starch.

Zakim et al., (372) have reported that male rats fed on glucose or fructose enriched diets for 48 h showed greater hepatic ACC activities than control, but no significant difference between the effects of the two sugars were observed. However, Cohen et al., (275) in a long-term feeding experiment, showed that sucrose was more effective than starch in raising ACC activity. 6PGDH (275) and G6PDH (275, 330) have been found to be more active in sucrose fed animals in comparison to Chow or starch fed rats. Similarly Romsos and Leveille (45) have found that in vivo hepatic lipogenesis from ^3H_2O is elevated when rats are fed a fructose rather than a glucose diet for 21 days.

Recently Brindley et al., (271) have provided evidence that glucocorticoids may participate in the long-term control of hepatic triacylglycerol synthesis (106) and that feeding rats with fructose significantly increase the serum corticosterone levels in the short term without affecting insulin levels (271). They suggest that the raised TG secretion may be

a result of a fructose induced increase in glucocorticoid levels. Glucose fed animals showed a much reduced glucocorticoid response and a full insulin response. It is of interest to note that prolonged ingestion of fructose can lead to raised serum cortisol levels (271) and to increased PPH and Triacylglycerol synthesis (259). However, the mechanism for the stimulation of corticosterone output by fructose is not clear. Thus a possible mechanism for the sucrose induced hypertriglyceridaemia observed by Tay (81) in a study similar to the present work may be via a combination of the fructose component of the disaccharide on triacylglycerol synthesis and a reduced insulin response.

In conclusion, the results obtained in this study, indicate that the effect of diet on some key hepatic lipogenic enzymes are similar in both sexes. However, the differences between the various diets is more problematical. It is clear that supplementation of the diet with carbohydrate results in increased levels of lipogenic enzymes and it appears that the sucrose diet is more effective than glucose in raising the levels of FAS and G6PDH but that the latter sugar was more effective in raising 6PGDH activity only in the female rats.

IV. Materials and methods

A. Materials

1. Chemicals: all chemicals used were of Analar grade obtained from BDH, Chemical Ltd., Poole, England except for enzyme substrates and enzymes which were obtained from Sigma Chemical Company, P.O. Box 14508, St. Louis, Mo. 63178, U.S.A. Glass distilled deionized water was used for preparation of all solutions.

2. Rats: all rats used in this study were of the Wistar strain and were either bred in this laboratory or obtained from a commercial source (A. Tucks and Son, Rayleigh, Essex).

3. Diets: the two types of commercial diet used in feeding experiments were obtained from E. Dixon and Sons (Ware) Ltd., Crane Mead Mills, Ware, Herts. The compositions of these diets are shown in Tables XIII and the calculated percentage compositions are shown in Table XIV.

Table XIII Composition of Dixon CDD [R] and Dixon .86 diets*

Dixon CDD R	cwt/ton	Dixon .86	cwt/ton
Wheat	10.8	Wheat	10
Maize	1.5	Barley	5
Soya meal	2.5	Concentrated meat meal	1.5
White fish meal	3	White fish meal	1.4
Milk powder	0.5	Gross	1
Yeast	0.39	Yeast	1
Shredded suet fat	1.2	Molasses	1
Pirkbent mineral salt	0.25	Salt	0.2
Salt	0.16	Vitamin 706	0.022
Vitamin 17	0.223		

*Figures are approximate, obtained from manufacturer (E. Dixon and Sons (Ware) Ltd., Crane, Mead, Mills, Ware, Herts).

Table XIV Calculated percentage 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
Metablizable energy	3,548	3,538

* Values obtained from manufacturer (E. Dixon and Sons, (Ware) Ltd. Crane, Mead, Mills, Ware, Herts).

B. Methods:

1. Feeding experiment

Young Wistar rats aged 21 days (50-60 g) were obtained from a commercial source or bred in the laboratory animal house. Five male and five female rats were fed on Dixon.86 diet for seven days. Further groups of five animals of each sex were fed on Dixon CDD[R], Dixon CDD[R] supplemented with 5% sucrose in the drinking water or Dixon CDD[R] supplmented with 5% glucose in the drinking water. These latter groups were fed ad lib. for 110 days. Each group was housed in a separate cage under similar conditions. The temperature of the animal house was kept constant at $22^{\circ} \pm 2^{\circ}$ and the animals subjected to a 12 h light/12 h dark regime with the light on between 08.00 and 20.00 h. The intake of glucose and sucrose depended on the age of the animal but there was no marked sex difference in the amount of the sugar consumed. Approximately 7.5 g/day of each sugar was taken in by the mature animals.

A further group of stock animals was used in part of the study. These animals were fed on Dixon.86 from weaning at 21 days until they were 14 weeks of age.

All animals were killed by cervical dislocation between 09.00 h and 10.00 h. to minimise differences in enzyme levels arising from diurnal variations. The liver was rapidly removed, weighed, and freeze clamped in liquid nitrogen within 60 s of death or, in some instances, the fresh liver was homogenised immediately in the appropriate buffer. The freeze clamped liver was stored at -70° until required. The liver was used for the enzyme preparations as described in the following sections.

2. Enzyme assays

a-Acetyl-CoA carboxylase

(i) Enzyme preparation: the frozen liver sample was washed in ice cold 0.05 M Tris-HCl buffer pH 7.5 containing 20 mM sodium citrate, 0.5 mM EDTA and 5 mM 2-mercaptoethanol. The tissue was blotted using filter paper and then weighed. A portion of liver from each lobe was minced with scissors into a glass homogenizer containing the above buffer (1:3 w/v) and homogenized using a close-fitting (clearance 0.4 mm) teflon pestle rotating at 2000 r.p.m. The homogenate was centrifuged at 1,000 g for 30 min. and then the supernatant was recentrifuged at 100,000 g. in an MSE Superspeed 65 centrifuge for 1 h. The supernatant was carefully separated to avoid any contamination with either the floating lipid particles or the pellet at the bottom and used as the source of enzyme. All enzyme preparation steps were carried out at 0-4°C.

(ii) Enzyme assay: the radioisotopic method of Inoue and Lowenstein (151) was followed. It is based on the incorporation from [^{14}C] bicarbonate into the carboxyl group of malonyl-CoA (148). The reaction involves the conversion of an acid-volatile compound into an acid-stable compound. Addition of acid (HCl) will stop the reaction, unreacted [^{14}C] bicarbonate escapes as $^{14}CO_2$ during the drying of the mixture and the labelled malonyl-CoA will remain. Citrate and Mg^{++} can activate the enzyme (see section I.D.3) particularly when the crude extract is used as the source of enzyme. The enzyme was activated by adding 0.5 ml of the enzyme preparation to 0.5 ml of an activation mixture containing 20 mM sodium citrate, 20 mM $MgCl_2$, 1 mM dithiottreitol (DTT) and 0.5 mg/ml BSA (fatty acid poor) in 50 mM Tris-HCl buffer pH 7.5. This mixture was incubated for 30 min. at 37°C. The assay mixture contained 100 mM Tris-HCl

buffer pH 7.5, 1 mM DTT, 0.2 mM acetyl-CoA, 20 mM NaH $^{14}CO_3$ (0.25 μCi/μ mole), 5 mM ATP, 20 mM sodium citrate, 20 mM $MgCl_2$ and 0.5 mg/ml BSA. The reaction was started by adding the activated enzyme mixture and the reaction was allowed to proceed at 37°C for 5 min. The final volume of assay mixture was 0.4 ml and the reaction was stopped by the addition of 0.1 ml of 4M HCl. The mixture was dried in a gentle stream of nitrogen in a fume cupboard to remove volatile $^{14}CO_2$ and the residue was dissolved in 1.0 ml of deionized water and then 9 ml of the scintillant added. The scintillant consisted of a mixture of PPO in toluene (5 g/l) and Triton X-100 (1:3 v/v). The radioactivity was assayed in a Packard liquid scintillation counter with the appropriate correction for chemical quenching. The blank consisted of either the full reaction mixture with 4 M HCl added at zero time, or, with the complete reaction mixture except for acetyl-CoA.

Specific activity was expressed as n moles of malonyl-CoA formed at 37°/min/mg of protein.

b. Fatty acid synthetase

(i) Enzyme preparation: rats were killed by cervical fracture, livers were removed, washed with ice cold potassium phosphate buffer pH 8, blotted with filter paper, weighed and used for assay on the same day. The liver was homogenized and centrifuged according to the method described by Hsu et al.(337). The liver was minced, using scissors, into ice cold phosphate-carbonate buffer (7 mM $KHCO_3$, 85 mM $K_2H\ PO_4$, 9 mM KH_2PO_4, 3 mM EDTA, 1 mM 2-mercaptoethanol) pH 8.0 (1.5:1 w/v) and homogenized in a glass homogenizer with a teflon pestle (0.4 mm clearance) rotating at 2,000 rpm for 30 s. The homogenate then centrifuged at 1,000 g for 30 min. The supernatant decanted

and then recentrifuged at 100,000 g for 1 h. The clear supernatant was carefully separated using a Pasteur pipette to avoid contamination of the soluble protein fraction, this was used as the source of enzyme. All procedures were carried out at 0-4°C.

(ii) Assay of enzyme activity: the enzyme activity was determined according to Lynen (150) by a spectrophotometric method. Changes in absorbance at 340 nm were followed using a Pye Unicam SP1800 spectrophotometer equipped with a constant temperature cell housing. The assay was based on the oxidation NADPH during the reaction and was followed by measuring the decrease in absorbance at 340 nm and 25°C. The assay mixture (total vol. 2.0 ml) was set up in a quartz cuvette and contained 200 μ moles; potassium phosphate buffer, pH 6.5, EDTA, 5 μ moles; cysteine, 20 μ moles; BSA, 0.6 mg; acetyl-CoA, 0.12 μ mole; NADPH, 0.3 μ moles and the enzyme preparation (300 μl). The mixture was diluted to a volume of 1.98 ml using deionized water. The absorbance change was followed for 3-4 min.as a blank (the change in asorbance due to endogenous reaction was subtracted from the absorbance change observed afterthe addition of malonyl-CoA). The reaction was started by addition of 20 μl of 7 mM malonyl-CoA and the decrease in absorbance followed for up to 4 min. The change in absorbance with time was linear and was proportional to the protein content of the assay mixture. The enzyme specific activity was expressed as the amount of enzyme which, under these conditions, consumed one n mole of malonyl-CoA per min. per mg protein at 25°C.

c. 6-Phosphogluconate dehydrogenase

(i) Enzyme preparation: a supernatant fraction was prepared from the liver samples which had been stored at -70°C. Liver samples were washed in 0.15 M KCl and then homogenzied in the same medium (1:5 w/v) using a glass homogenizer with a teflon pestle as described in B(i).

The homogenate was centrifuged at 1,000 g for 30 min. and the supernatant obtained was recentrifuged at 100,000 g for 1 h and the particle-free supernatant used as a source of enzyme.

(ii) Enzyme assay: enzyme activity was measured spectrophotometrically by following the increase in O.D. at 340 nm due to the reduction of NADP. The assay was a modification of the method of Rudack et al., (222). The assay mixture (total volume 1 ml) contained 120 mM Tris-HCl buffer pH 8.0, 10.4 mM $MgCl_2$, 0.9 mM NADP, 1.2 mM 6-phosphogluconate and the reaction was started by addition of the enzyme preparation. The assay was carried out at 30°C. The specific activity was expressed as n moles NADP reduced per min. per mg protein.

d. Glucose 6-phosphate dehydrogenase

(i) Enzyme preparation: see section C.1.

(ii) Enzyme assay: The method of Rudack et al., (222) with minor modifications, was used to assay G6PDH in this study. The change in absorbance due to the reduction of NADP was followed spectrophotometrically at 340 nm, in the presence of both 6-phosphogluconate and glucose 6-phosphate. This assay gives the combined glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity. In order to obtain glucose 6-phosphate dehydrogenase activity, the 6-phosphogluconate dehydrogenase activity was subtracted from the combined activity. The assay mixture contained 120 mM Tris-HCl buffer pH 8.0, 10.4 mM $MgCl_2$, 0.9 mM NADP, 0.5 mM glucose 6-phosphate, 1.2 mM 6-phosphogluconate and enzyme in a total volume of 1 ml. The activity of 6-phosphogluconate dehydrogenase was determined by eliminating glucose 6-phosphate from the assay. The assays were carried out at 30°C and the enzyme specific activity expressed as n moles NADP reduced per min. per mg protein.

e. Glycerol 3-phosphate acyltransferase

(i) Enzyme preparation: the microsomal fraction was prepared according to Mookerjea and Marai (278). Samples of frozen liver which were stored at -70°C were used for the preparation. The tissue was washed in ice cold 0.35 M sucrose-TMK buffer (TMK buffer: 0.035 M Tris-HCl, pH 7.8; $MgCl_2$, 0.01 M; and KCl, 0.025M) and blotted with filter paper, weighed and was then cut into small pieces which were immersed in 2.5 vol. of ice cold 0.35 M sucrose-TMK buffer. The liver was homogenzied in a glass homogenzier using a teflon pestle. The homogenate was filtered through four layers of cheese-cloth and then centrifuged at 17,000 g for 10 min. The post-mitochondrial supernatant was carefully aspirated by Pasteur pipette and diluted with 2 vol. of 0.9 M sucrose, containing 0.01 M $MgCl_2$ and 0.025 M KCl, and then recentrifuged at 100,000 g for 1 h. The supernatant was decanted, the microsomal pellet was mixed with TMK buffer and resuspended (1 ml/g of liver) using a teflon pestle.

(ii) Enzyme assay: enzyme activity was measured by the radio-isotopic method of Husband and Lands (241), using [^{14}C] glycerol 3-phosphate as substrate. The labelled lipid formed was extracted by chloroform-methanol and the remainder contained the total lipid which was assayed by liquid scintillation counting. The assay mixture (total volume of 0.35 ml) contained 57 mM Tris-HCl buffer pH 8.0, 0.28 mM (Sn-[^{14}C] glycerol 3-phosphate, 0.29 mM stearoyl-CoA and approximately 1 mg of microsomal protein. The reaction was allowed to proceed for 2 min. and then 7 ml of chloroform-methanol (2:1 v/v) was added followed by 1.4 ml of 0.03 M HCl containing 2% acetic acid. The mixture was shaken and then two phases allowed to separate and the upper phase was decanted together with the denatured protein at the interface. The lower phase was washed twice with the upper phase of a mixture of chloroform methanol and 0.03 M HCl containing

2% acetic acid (20:10:7.5). The lower phase which contained labelled lipid was gently evaporated in a stream of N_2 and the lipid obtained was dissolved in Triton X-100: 8 g PPO in 1 litre toluene (1:2v/v) and counted by liquid scintillation counting. The appropriate quench correction was applied. The specific activity of glycerol phosphate acyltransferase was expressed as the amount of glycerol 3-phosphate incorporated into total lipid per min per mg protein.

f. Fructose 1,6-bisphosphate aldolase

(i) Enzyme preparation: the soluble cytoplasmic fraction was prepared according to Penhoet and Rutter (306). A sample of frozen liver was washed in ice-cold Tris-HCl buffer (10 mM Tris-HCl, 1 mM EDTA) pH 7.5, blotted with filter paper and then weighed. The tissue was minced into small pieces, using scissors, into 2 vol. (v/w) of the same buffer and homogenzied in a glass homogenizer with ten strokes of a teflon pestle (0.4 mm clearance). The homogenate was centrifuged at 100,000 g for 1 h. All extraction steps were carried out at 0-4°C. The clear supernatant was separated carefully using a Pasteur pipette to avoid any contamination by membrane-bound particles. The supernatant was used as a source of enzyme.

(ii) Enzyme assay: enzyme activity was measured spectrophotometrically according to Rajkumar and Rutter (343). The principle of the assay (see section IV.B.2.f) was based on the cleavage of FDP to DHAP and G3P, the latter was converted to DHAP by triose phosphate isomerase (TPI). The total DHAP formed was converted to G3P by glycerol 3-phosphate dehydrogenase (GDH) in the presence of NADH. The change in absorbance due to the oxidation of the NADH was followed at 340 nm. Two μ moles of NADH were oxidized following the cleavage of one μ mole FDP. The assay mixture (total volume 1 ml) contained 49 mM glycylglycine buffer

pH 7.5, 0.1 mM NADH, 2 mM FDP, 59 μ g GDH-TPI mixture and enzyme solution (20 μl). The assay was carried out at 28°C. The reaction was linear withtime and the initial rate was proportional to the protein concentration under these assay conditions. The specific activity was expressed as n moles NADH oxidized per min per mg of soluble protein.

g. Fructose 1-phosphate aldolase

(i) Enzyme preparation: see f(i).

(ii) Enzyme assay: the enzyme assay procedure was identical to that of fructose 1,6-bis-phosphate aldolase except that fructose 1,6-bis-phosphate was replaced by fructose-1-phosphate (10 mM) in the assay mixture. In this case the products of the reaction are DHAP and glyceraldehyde. Since the latter does not undergo further reaction under these conditions then one μ mole of fructose 1-phosphate yields 1 μ moles of NAD^+. The enzyme activity was expressed as n moles FIP utilized per min per mg soluble protein.

3. Protein assay

Protein was estimated by the Biuret method in all cases (344). Bovine serum albumin was used to prepare a standard curve for the protein assays.

4. Statistical analysis

The statistical analysis of the data from the present study involved the application of the t test to determine whether or not differences observed were statistically significant (Zivin and Bartko (345).

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

Where $\bar{X}$ = mean of arithmetic average $\xi \bar{X} / N$

SD = standard deviation

N = number of observation

("degree of freedom" = n-2)

Standard deviation was calculated from the formula

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

Where ξ = "sum of"

$\bar{X}$ = mean of arithmetic average $\xi X / N$

X = observed values

N = number of observation.

The t-test is a method of statistical analysis which 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 (i.e. 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 fundamentally important about a 5% level. It is only by convention that difference occur with $P < 0.05$ are called "statistically significant".

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