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CARBOHYDRATE METABOLISING ENZYMES IN MALE AND FEMALE RATS

A Thesis submitted by

SAID ABDEL FATTAH MAHMOUD

a candidate for the Degree of

Doctor of Philosophy

in

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Department of Biochemistry  
Royal Holloway College  
University of London  
EGHAM HILL  
Egham  
Surrey

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ABSTRACT

It has been reported that the increase in the concentration of triglyceride in fasting serum is more likely to be induced by carbohydrates than by fats in the diet. This fact has focussed the attention of researchers on the possible role of dietary carbohydrates in the aetiology of ischaemic heart disease. Epidemiological studies have also shown that there is a positive correlation between the incidence of atherosclerosis and (1), the amount of sucrose in diet and (2), the level of triglyceride in the fasting serum. Moreover, some epidemiological studies suggest that the increase in the death rate from ischaemic heart disease coincides with the increased consumption of sucrose in western countries.

Feeding sucrose has been reported to produce hypertriglyceridaemia in man and experimental animals and this carbohydrate has a greater hypertriglyceridaemic effect than glucose or starch. Sucrose- or fructose-induced hypertriglyceridaemia is more marked in male animals than females.

These facts when considered together, suggested that an examination of sex differences in the metabolism of sucrose might throw some light on the different incidence of ischaemic heart disease in men and women. Hence the activities of a number of liver enzymes involved in fructose metabolism have been examined using male and female rats on normal and carbohydrate-supplemented diets.

Sex differences were observed in three cases: glycerol kinase ( $\delta > \text{♀}$ ), glycerol 3-phosphate dehydrogenase ( $\text{♀} > \delta$ ) and sorbitol dehydrogenase ( $\text{♀} > \delta$ ) but dietary factors modified or nullified these differences. The conclusion was reached that glycerol 3-phosphate might be produced more readily in female rats than males because aldolase and glycerol 3-phosphate dehydrogenase activities were higher in the females.

The effect of sex hormones on the activity of glycerol kinase was examined using hepatocyte preparations. A rapid activation of this enzyme in cells from both male and female livers was observed when relatively high concentrations of dihydrotestosterone were used. Estradiol-17 $\beta$  had no effect. The activation was most marked with hepatocytes from castrated male rats and the phenomenon could only be demonstrated with intact cells. There was no evidence that the dihydrotestosterone effect was mediated through protein synthesis.

Hormonal control of lipogenesis by estrogens, possibly mediated by insulin and glucagen, has been considered in the Discussion together with the role of lipoprotein lipase in maintaining serum triglyceride levels low in female animals compared with males.

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ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADP	Adenosine 5'-diphosphate
AMP	Adenosine 5'-monophosphate
ATP	Adenosine 5'-triphosphate
Cyclic AMP	Adenosine 3':5'-phosphate
CoA	Coenzyme A
EDTA	Ethylenediaminetetra-acetate
GK	Glycerol kinase
GDH	Glycerol 3-phosphate dehydrogenase
HDL	High density lipoprotein
IMP	Inosine 5'-monophosphate
KHK	Ketohexokinase
KRB	Krebs-Ringer bicarbonate
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
NAD <sup>+</sup>	Oxidized $\beta$ -nicotinamide-adenine dinucleotide
NADH	Reduced $\beta$ -nicotinamide-adenine dinucleotide
NADP <sup>+</sup>	Oxidized $\beta$ -nicotinamide-adenine dinucleotide phosphate
NADPH	Reduced $\beta$ -nicotinamide-adenine dinucleotide phosphate
TPI	Triosephosphate isomerase
TRIS	2-Amino-2-hydroxymethylpropane-1,3-diol
VLDL	Very low density lipoprotein

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

The study described in this thesis was initiated by a number of reports that dietary carbohydrate might be implicated, via blood serum triglycerides, in the development of the disease, atherosclerosis, which is the primary cause of death in western countries.

It was noted that the hypertriglyceridaemic response to some fructose-containing dietary carbohydrates in men and women (and in males and females of other animal species) was different and that, in addition, the incidence of atherosclerosis in men was greater than in women. These points are considered in detail in the text that follows.

The main purpose of the experimental work that is described was to investigate some possible sex differences in the way that fructose is converted to triglycerides. In one case, glycerol kinase, the mode of activation by sex hormones was investigated.

## I. ATHEROSCLEROSIS

### A. THE DISEASE

The most prevalent degenerative change observed in the cardiovascular system is atherosclerosis, which affects the main arteries and leads to ischaemic changes in the myocardium, brain and limbs (6).

The term arteriosclerosis (hardening of the arteries) describes the form of atherosclerosis characterized by accumulation of soft, amorphous, fatty material in the vascular wall.

A study group of the WHO defined atherosclerosis as, "a variable combination of changes of the intima of arteries consisting of focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits and associated with medial changes". (1). This definition emphasizes the pleomorphic character of the arterial lesion, which is often overlooked in studies on atherosclerosis.

Paleopathologists have found evidence of arteriosclerosis (probably atherosclerosis) in mummies from ancient Egypt (27), and medical historians have documented, extensively, reports on atherosclerotic disease over the centuries (3, 8, 28).

Current interest in the study of atherosclerosis is due to the fact that coronary heart disease, one of the complications of the atherosclerotic process, is today the leading cause of death in developed countries. Atherosclerosis of the coronary blood vessels, is a

more frequent cause of death in the United Kingdom than any other disease of the circulatory system (2).

Atherosclerosis begins in childhood, proceeds rapidly in some arteries during adolescence and in others during early adulthood, undergoes a series of complex changes in subsequent decades and then begins to result in clinically manifest disease during early middle age (3).

The first structural abnormality that can be classified as atherosclerosis is the deposition of lipid in the subendothelial space of the arteries and this produces the so-called fatty streaks (4), the appearance of which normally begins in the aorta, in the first year of life, in the coronary arteries in the second decade and in the intracranial arteries in the third decade.

It is well established that fatty streaks chronologically precede fibrous plaques and more advanced lesions. Haemorrhage within the plaque with superimposed thrombosis will result in atheroma formation. The atheroma and complex lesions sometimes show evidence of calcification and/or ulceration (5).

These atheromatous lesions may reduce the size of the arterial lumen and produce partial or complete ischaemia. Ischaemia may then result in angina pectoris, myocardial infarction, cerebral infarction or peripheral gangrene.

Recognition of the importance of atherosclerosis as a cause of morbidity and mortality in industrialized countries has resulted in a great deal of research directed towards an understanding of the causes and the treatment of this disease (2).

#### B. MORPHOLOGY

Atherosclerosis is a disease characterized by the accumulation of lipids in the subendothelial space of the large arteries. From the pathological point of view, the earliest lesions found in children are those classified as fatty streaks, which appear to the naked eye as pale yellow, flat or elevated intimal lesions; microscopically, they consist of macrophages which are apparently filled with lipid droplets (4).

The second type of intimal lesion is the fibrous plaque. These are firm and appear as grey elevated areas of thickening which are composed of collagen and elastic fibres together with smooth muscle cells, but the fat content is less than that found in fatty streaks (4).

The third type of intimal lesion is that which is referred to as atheroma and this consists of soft, fatty, raised lesions in which there may be evidence of intimal haemorrhage with superimposed thrombosis. Calcification and/or ulceration of the atheromatous lesion is a possible complication (5).

In the view of most investigators, the evolution of plaques of the adult type occurs mainly via the fatty streaks. This view is supported by the study of spontaneous and induced atherosclerosis in experimental animals (11). In man, it is difficult, to establish whether the fatty streaks are the precursors of the fibrous plaques. For example, evidence has been presented that the fatty streaks and fibrous plaques appear at different sites in the arterial tree in man (9). A similar conclusion has been drawn from studies on the chemical composition of the different lesions (4). On the whole, however, the pathological evidence, favours the conclusion that atherosclerotic lesions of the adult type commonly evolve from fatty streaks.

#### C. AETIOLOGY

The aetiology of atherosclerosis has been the subject of controversy for over a century. The mechanism of the arterial changes has been explained by a variety of theories (21). The thrombogenic theory is one of the oldest of these theories. It was proposed by Von Rokitansky (12) in the 19th century, when he described a typical atheroma and postulated that it resulted from degeneration of blood proteins (notably fibrin) deposited upon the arterial intima. This view was contested by Virchow (13) in 1856, who introduced the inflammatory theory and regarded the atheroma as an outcome of degenerations that followed inflammatory processes within the intima and the fibrous thickening as a product of proliferation of intimal connective tissue cells in reaction to the pool of degenerative material. In 1941 Leary (14) considered that the atherogenic process was due to a type of fatty infiltration of the vessels, whilst Duguid (15) advanced an alternative explanation for the process of atheromatosis. He revived interest in the theory of Von Rokitansky (12) and presented evidence for the existence of fibrin in arterial lesions. He also said that the fibrous thickenings were not caused by reaction to the process of degeneration, as proposed by Virchow (13), but that they resulted from surface deposits.

In 1951 Wilens (16) drew attention to the slow increase in the thickness of the arterial intima in females relative to males, which proceeds throughout life. He suggested that this might help to explain the difference between the two sexes with regard to the development of atherosclerosis.

The occurrence of appreciable amounts of lipid in atherosclerotic lesions led to the theory of lipid infiltration. This states that the lipid contents of fatty streaks are the result of the deposition of lipids which infiltrate from the blood plasma to the intimal tissues (21). The theory is supported by the observations of Böttcher and Van Gent (10) who have found a similarity between the lipid composition of fatty streaks and that of blood plasma.

The long suspected relationship between the nature of the dietary intake and atherosclerosis was claimed by several investigators, on the basis of dietary studies, in the early years of this century (see also p. 18). In particular, Saltykow (18) emphasized the probable role that dietary cholesterol played in atherogenesis and shortly thereafter Anitschow and Chalатов (19) began to study the effects of excessive cholesterol feeding on rabbits. They showed that aortic, lipid-containing lesions appeared in these animals and they believed that the high level of cholesterol in the plasma initiated atherosclerosis and maintained that the origin of the disease might be traced to a general disorder of lipid metabolism. They further reasoned that increased blood lipoproteins had the tendency to settle in the intima producing the changes typical of atherosclerosis, whereas all other factors (mechanical, chemical and pharmacological) were only of a contributing nature.

In contradiction to this theory, however, the analysis of human arteries for plasma low density lipoprotein (LDL) content has indicated that, proportionally less cholesterol is bound as LDL in the fatty streak than in either normal intima or in advanced atheroma (25). Furthermore, the composition of cholesterol esters in fatty streaks differs considerably from that in adjacent normal intima or in plasma (26). These findings suggest that much of the lipid of the fatty streak is synthesized locally rather than being derived from the plasma.

Developing atheroma leads to the formation of fissures in the endothelial blood vessel tissues overlying the atheromatous mass. These in turn damage blood platelets circulating in the vessel lumen which

accelerates platelet aggregation and thrombus formation. The existence of a thrombus can, of course, threaten blood circulation and thus result in partial or complete blockage of blood vessels often culminating in death if the coronary arteries are affected. The biochemical factors involved in this phenomenon have reviewed (31, 32).

There are numerous other theories of the aetiology of atherosclerosis (21) many of which have not been generally accepted and some which have been subsequently incorporated into broader concepts or have proved to be invalid.

#### D. RISK FACTORS

Great efforts have been made this century to elucidate the pathogenesis of atherosclerosis, and several 'risk factors' for clinical manifestations of the disease have been identified. Risk factors are statistically associated with an increased risk of the development of ischaemic heart disease in an individual (35, 36, 37, 38, 39, 40).

##### 1. Age and Sex

Coronary heart disease is rare in women before the menopause but is an increasing cause of death thereafter. It has been reported that the incidence of coronary occlusion increases with age in both men and women, but throughout all age classes there is a greater incidence for men than for women (7, 36, 43, 48, 80) (See also p.23).

##### 2. Familial Incidence

Attempts have been made to demonstrate a genetic disposition to coronary heart disease and in families of patients with myocardial infarctions, there is commonly a history of atherosclerotic cardiovascular disease and early death (41, 44). As pointed out by Rose (42), however, this cannot be taken as proof of genetic disposition, since family members often resemble each other with respect to dietary habits, physical activity and other factors.

##### 3. Body Weight

In some studies, particularly with men, a greater prevalence of ischaemic heart disease was found in subjects who were over-weight (36, 57, 61), whilst in other studies this was not the case (56).

##### 4. Diabetes Mellitus

Diabetes mellitus is an important risk factor for atherosclerotic diseases - coronary, peripheral and cerebral (7) and there is agreement

in most of the literature that there is a sex difference in the prevalence and incidence of diabetes with more women than men developing the condition (37, 60, 64). It has also been pointed out that atherosclerotic heart disease accompanied by diabetes results in higher mortality rates than atherosclerosis alone. It has been assumed that 'diabetic vascular disease' of the smaller arteries results in a poorer development of collaterals and, therefore, a greater number of fatal cases of arteriosclerotic occlusions (64).

Diabetes is also associated with a marked weight gain (60, 61) which itself predisposes to ischaemic heart disease. In addition, plasma insulin levels show a strong positive relationship with obesity and this is most pronounced in women who are commonly obese. In obesity, the uptake of glucose by the peripheral tissue is reduced, and this produces hyperglycaemia and compensatory hyperinsulinaemia (60, 61). Diabetes mellitus is considered to develop when the compensation is no longer effective.

#### 5. Plasma Lipids

Serum lipids such as cholesterol and triglyceride are always bound to protein to form lipoproteins. In the fasting state, three major lipoproteins can be isolated by ultracentrifugation or electrophoresis: very low (VLDL), low (LDL) and high (HDL) density lipoproteins (72): these have different biochemical, metabolic and pathogenetic properties. Fredrickson et al. (76) have correlated five electrophoretic lipoprotein patterns with clinical syndromes of various types (hyperlipoproteinaemias). Elevated serum LDL and VLDL (type II and type IV hyperlipoproteinaemias) relate to premature ischaemic heart disease and to diabetes and associated vascular disease, respectively. Both of these conditions are influenced by hereditary factors (44, 78). It has also been proposed that reduction in serum HDL (which contains mainly cholesterol, cholesterol esters and phospholipid) may hasten the development of atherosclerosis (29, 75).

A number of epidemiological studies have established a relationship between hypercholesterolaemia and ischaemic heart disease (36, 79, 80, 83) and an association between hypertriglyceridaemia and atherosclerotic heart disease has also been found (51, 84, 85, 86). It is difficult, however, to relate these findings to the associations



claimed for the lipoproteins because the latter all contain mixtures of different lipids and the information concerning hypercholesterolaemia and hypertriglyceridaemia refers to total serum cholesterol and total serum triglyceride, respectively.

Epidemiological studies are scanty in which serum cholesterol and serum triglyceride have been determined in both sexes but there is a good agreement in those studies which have been published. Before the age of about 50 years a lower serum cholesterol level is found in women than in men, whilst the opposite occurs after this age (36, 78). Serum triglycerides are found to increase with age but decrease slowly after the age of 50 years with men having higher values than women in all age groups (76, 78, 89, 90).

#### 6. Smoking

Recent incidence studies have shown that smokers, and particularly cigarette smokers, run the greatest risk of developing myocardial infarction (92, 93, 94, 96), this being so for both men and women (48). However, prevalence studies could not demonstrate with certainty, a relationship between ischaemic heart disease and smoking (97, 98).

The content of carbon monoxide in tobacco smoke has recently been proposed as significant factor in the development of atherosclerosis (101).

#### 7. Hypertension

The cardiovascular consequences of elevated blood pressure have been demonstrated in many incidence studies (36, 38, 80, 102). The risk of atherosclerotic heart disease is higher the higher the blood pressure. There are also strong positive relationships in both sexes between blood pressure and serum triglycerides, obesity and carbohydrate intolerance, and hence maturity onset diabetes (80, 84).

#### 8. Physical Activity

Many investigators assume that the changes which have taken place in the way of life of industrialized countries during this century contribute to the development of atherosclerotic heart disease. Amongst other causes, the decreased demands on physical activity have been blamed for this and regarded as a coronary risk factor (37, 103, 104, 108) and it has been reported that exercise is effective in reducing serum triglycerides (107, 116). In addition, other risk factors such as cigarette smoking, obesity (but not high serum cholesterol levels and hypertension) appear to be positively related to inactivity (105, 106).

9. Serum Uric Acid

In 1951, Gertler et al. (109) published their observations that serum uric acid was significantly elevated in a group of young male patients with myocardial infarctions. Since then, numerous authors have reported a positive relationship between serum uric acid levels and atherosclerotic manifestations (110, 115), as well as with hyperuricaemia and hypertension, obesity and serum triglycerides (7, 66, 115): a relationship of hyperuricaemia with serum cholesterol was denied (7).

All studies on the distribution of serum uric acid in the two sexes have shown that men have higher values than women and in addition the levels show only a slight age-dependence in men (114, 115).

E. DIET AND ISCHAEMIC HEART DISEASE

1. General Considerations

The DHSS report on "Diet and Coronary Heart Disease" (2) considers possible dietary risk factors in ischaemic heart disease (IHD) and in particular: (a), a general over consumption of food; (b), an excess of fat; (c), a low ratio of polyunsaturated to saturated fatty acids in the diet; (d), an excess of cholesterol; (e), an excess of sucrose; (f), a high consumption of common salt; (g), a deficiency of dietary fibre and (h), softness of the water supply.

A consumption of food in excess of energy expenditure results in obesity and there is evidence that this 'disease' is becoming more prevalent in western countries (2). Obesity is associated with low physical activity, a high blood pressure and diabetes mellitus, each of which is considered to be an important risk factor for IHD. Some studies suggest that, in the absence of these associated factors, obesity alone may not contribute greatly to the total risk of IHD in an individual (2, 61).

In the United Kingdom, the amount of protein consumed, expressed as the average weight per person per day, has altered very little since the early years of this century. The consumption of total carbohydrate, on the other hand, during the decade 1950-1960, started to fall and this fall continued into the 1970's. The consumption of sucrose has, however, shown a different trend. This rose from the 1940's until 1958 and then declined slightly, although in 1971 sucrose still provided about 16% of the total available food energy (2, 125).

Over the last decade about 11% of food energy has been available from protein. A comparison of total carbohydrate consumption with that of fat shows that since 1950, the proportion of food energy available from carbohydrate has steadily declined whilst that from fat has risen. In 1971, on average, 46-48% of food energy was supplied by carbohydrate and about 42% by fat (2).

There is some evidence to suggest a positive correlation between the average proportion of food energy which is derived from fat and the death rate from ischaemic heart disease. This relationship is clearest when the proportion of food energy derived from combined saturated fatty acids is considered rather the proportion derived from total amount of fat in the diet (123). The interpretation of the evidence which conforms with the view that a diet rich in fat predisposes to ischaemic heart disease is complicated by the fact that people who consume a high fat diet are often heavy cigarette smokers and tend to have a high sucrose intake (2, 117).

There appear to be strong interrelationships in man between dietary fat and blood cholesterol and ischaemic heart disease (128). In some animal species a diet rich in cholesterol induces the appearance of arterial lesions which have some similarity to those seen in human subjects (11), and comparative studies of different human populations show that those which have a diet rich in cholesterol have a higher death rate from ischaemic heart disease (130). However, a diet rich in cholesterol is usually one which is also rich in saturated fatty acids.

## 2. Sucrose and Ischaemic Heart Disease

It has been claimed (104, 133) that sucrose is an important risk factor in the development of ischaemic heart disease in man. Although this view has been criticized in the scientific literature, it has received considerable public attention.

The hypothesis is based on four main points which can be summarized as follows:

- (a), there is a correlation between the rate of mortality from ischaemic heart disease and sucrose consumption in several countries;
- (b), there was an enormous increase in the incidence of ischaemic heart disease over the last 30 years which was coincident with the marked rise in the sucrose consumption per capita in various countries;

- (c), men with ischaemic heart disease commonly prove to be unusually heavy consumers of sucrose; and
- (d), compared with starch, dietary sucrose leads to elevation of blood serum lipids, mainly triglycerides.

In 1957, Yudkin (104) postulated a relationship between sucrose consumption and the death rate attributed to ischaemic heart disease in 15 countries. The sucrose consumption per capita was calculated from sucrose production, export and import data; death rates were calculated from vital statistics. According to Walker (134) and Keys (132), these two sources of information do not give a true picture of either the actual sucrose consumption or the ischaemic heart disease mortality.

Keys (132) and Grande (129) have pointed to exceptions to Yudkin's original data citing countries with a high sucrose consumption but a low incidence of ischaemic heart disease such as Cuba, Venezuela, Colombia and Costa Rica. Furthermore, in countries with similar sucrose consumptions, such as Sweden and Finland, the incidence of ischaemic heart disease may be different: in the case of the two Scandinavian countries the incidence is higher in the latter than the former (129). The dietary data obtained by Keys (130) in his population studies indicate a correlation between sucrose intake and ischaemic heart disease incidence rate of 0.78 and a correlation between saturated fatty acids and ischaemic heart disease incidence rate of 0.86. However, as noted also by Wretlind (135), there is also a high correlation (0.84) between the amount of sucrose in the diet and the saturated fatty acid content, which is adequate to explain the relationship between sucrose intake and the incidence of ischaemic heart disease in these studies (130, 132).

The view that increases in sucrose consumption and coronary mortality have occurred concomitantly (Yudkin, 104) has been criticized by Keys (132). The data from the U.S. Department of Agriculture reported by Keys, shows that the yearly, per capita average for sugar consumption of 51.7kg in the 1920's and 51.2kg in the 1960's. On the other hand, the U.S. vital statistics show a considerable increase in coronary death rates between these two periods.

Ashton (136) has claimed correlations of 0.64 and 0.55 between death rates from ischaemic heart disease and fat consumption and death rates and sucrose consumption, respectively, in Britain.

Yudkin and his colleagues (137, 138) reported that coronary patients had a higher sucrose intake than controls but Grande (129) and Keys (132), were unable to confirm these findings. It has also been stated by a working party of the Medical Research Council, U.K. (139), that sucrose consumption by men with myocardial infarction is slightly higher than that of normal individuals but the difference was not statistically significant. Furthermore, the Medical Research Council stated that the increase in sucrose consumption amongst coronary patients was likely to relate to an association between cigarette smoking and the intake of sucrose.

The general controversy in this field undoubtedly relates to the multifactorial nature of ischaemic heart disease.

The effect of dietary sucrose on human serum lipids has been the subject of many investigations and is of interest in view of claims that high levels of certain serum lipids can be correlated with a high incidence of ischaemic heart disease (see p. 16).

The term "carbohydrate induced lipaemia" has been coined by Ahrens (140) to signify the type of hypertriglyceridaemia which becomes more apparent following several days of a very high carbohydrate diet. In contrast to alimentary lipaemia, in which the triglyceride concentration is greatest 3-5 hours after a fat meal, in carbohydrate-induced lipaemia the serum triglyceride concentration reaches its highest value after an overnight fast. The elevation of serum triglycerides produced by dietary carbohydrate has been the subject of a review by Kaufmann (141).

Since plasma triglyceride levels are doubled after a few days of carbohydrate-enriched food, persons with initially elevated triglycerides will show a greater increase in triglyceride than will persons with normal triglyceride levels. In other words the effect will be more apparent in hypertriglyceridaemic than normoglyceridaemic individuals.

Many investigations suggest that dietary sucrose (or fructose) is a more marked hypertriglyceridaemic agent than starch or glucose in man. In a review, Grande (129) concluded that the hypertriglyceridaemic effect of sucrose, relative to starch, could not be demonstrated in normolipidaemic men under normal dietary conditions. However, with normolipidaemic subjects on diets with 12% of the calories present as fat (mainly animal fat) and 70% of the calories as carbohydrate, if sucrose

replaces starch then higher serum lipid levels, especially triglyceride, are produced (Macdonald and Braithwaite, 142; cf Antar et al., 148). These latter findings for men and postmenopausal women (but not premenopausal women) were confirmed in later publications (143, 144, 145).

It has also been reported that substitution of sucrose for starch in the diet produces marked elevations of serum lipid levels, particularly the triglycerides, in hyperlipaemic individuals (149, 150, 151). However, not all hyperlipaemic individuals showed this effect. The response to this dietary exchange seems to be related to the degree of hyperlipaemia and to the fatty acid composition of the diet (149, 162). Antar et al. (149) found that sucrose was more hyperlipaemic in patients with hypertriglyceridaemia than in patients with hypercholesterolaemia.

In general, therefore, simple dietary carbohydrate appears to have a greater effect on serum triglycerides than on serum cholesterol or phospholipids. However, with normolipaemic subjects, Keys (147) and Grande and their collaborators (146) found that replacement of 17% of the total calories (present as carbohydrate in fruit, leafy vegetables and legumes) by sucrose or lactose produced significantly higher serum cholesterol values.

It has been clearly shown that sucrose produces hypertriglyceridaemia to a greater extent than glucose or starch not only in humans, but also in experimental animals (153, 154, 155, 156, 157, 158, 159), although this response may be modified by other dietary factors such as the presence or absence of fat (160, 162) and whether the carbohydrate supplement is supplied to fasted or fed animals (154). The hypertriglyceridaemic effect of a sucrose-containing diet has also been found to be dependent on the age of the animal (154, 156), as well as the sex in both man (143) and laboratory animals (154, 155). Dietary studies with the latter are discussed in detail in Section III (p. 54) and the effect of sex in Section IV (p. 68).

It is evident that apart from the hypertriglyceridaemic effect, sucrose can also produce an increase in the adhesiveness of blood platelets, an early stage of blood clotting (161) and an increase in the level of circulating insulin (161). High levels of insulin are associated with peripheral vascular disease, obesity, hypertension and cigarette smoking - conditions that all predispose to ischaemic heart disease (133, 161).

#### F. EXPERIMENTAL ATHEROSCLEROSIS

Until recently the rabbit and the chicken were the only common laboratory animals in which atherosclerosis could be demonstrated (168). It is now recognized, however, that there are a number of other species (rat, mouse, guinea pig, hamsters, monkey and the pigeon) which can be made to develop arterial lesions similar to those found in human blood vessels by dietary manipulations of lipid metabolism (171).

Atherosclerotic lesions have been produced in the rat by adding cholesterol, bile salts and thiouracil to semi-synthetic diets containing the normal nutritional requirements. In addition, large amounts of dietary fat produce lesions and also thrombi (11, 171). The morphological features of the lesions in rat, are said to be more similar to those of man than those found in other experimental animals (171).

More recently, Mahley et al. (50) have succeeded in producing hypercholesterolaemia with a distinctive hyperlipoproteinaemia and subsequent development of atherosclerotic lesion in swine by cholesterol-fat rich diet.

#### G. SEX DIFFERENCES IN THE INCIDENCE OF ISCHAEMIC HEART DISEASE

It has been reported that, coronary heart disease, particularly myocardial infarction, is more common amongst men than women, at least during the middle decades of life (7, 22, 37, 80, 167). The mortality data from the United States reveal that throughout middle age, death rates attributed to ischaemic heart disease are several times higher in white males than white females (37, 165).

Kannel (80), in his Framingham studies, concluded that age, blood pressure, carbohydrate intolerance and serum lipid levels are linked to the rate of arterial atherogenesis in both sexes and with regard to the lipid factor, Hagerup (7) has found that serum cholesterol levels are lower in the men than in the women before the age of about 50 years whilst the opposite is the case after this age. The most common lipid disorder amongst patients with ischaemic heart disease is type IV hyperlipoproteinaemia in which the serum levels of triglyceride-rich lipoprotein VLDL are raised. Salel et al. (74) have found that type IV hyperlipoproteinaemia is 2.7 times more common in men than in women and they suggest that perhaps the type IV disorder is responsible for the sexual differences observed in the incidence

of ischaemic heart disease.

It has been shown that the administration of estrogens decreases the serum cholesterol level and this hormone has been used clinically as preventive treatment for atherosclerosis (23).

Many experiments with laboratory animals have been carried out to examine the effect of sex hormones on atherosclerosis. For example, chickens on atherogenic diets containing significant amounts of cholesterol and fat were treated with estrogens (166). This work showed that the diet itself induced hypercholesterolaemic hyperlipaemia and that estrogen therapy allowed the plasma cholesterol:phospholipid (C/P) ratio to be maintained at or near normal levels (0.35-0.6) via enhancement of the phospholipid concentration. The authors believed that estrogen-induced inhibition of coronary lesions in these animals might be closely interlinked with maintenance of C/P ratios below 0.75-0.80 (166). This tends to support the hypothesis that the human sex differential in susceptibility to ischaemic heart disease may be, at least in part, result from ovarian hormonal secretion (168).

In subsequent studies with chickens it was demonstrated that oral administration of estrogens was effective in producing the decisive triad of hormone effects: feminization, altered plasma lipid patterns and prevention of coronary atherosclerosis (167, 168). These studies reinforced the impression that inhibition of coronary atherogenesis was closely linked to the basic biological actions of the estrogens in Avian species at least.

Studies on estrogen-induced reversal of coronary atherosclerosis with animals on a high cholesterol/fat diet demonstrated that this effect was partially inhibited by administration of either thiouracil or insulin (168, 169). It has been clearly shown that egg-laying hens, in contrast to roosters of the same age, are remarkably resistant to cholesterol-induced atherogenesis (169). This phenomenon of sex difference in chickens shows a parallelism to the human situation regarding susceptibility to coronary atherogenesis.

In addition to studies on estrogens, the effects of androgens and castration have also been explored in animals (167).

Using male and female rats, Uzunova et al. (172) have been able to increase the mortality rate from arterial thrombosis by the administration of testosterone to either sex. On the other hand, estradiol



treatment decreased the mortality rate in both cases. These authors conclude that this may be the first significant experimental evidence for an association between sex hormones and the development of arterial thrombosis.

It should be noted, however, that the protective effect of estrogen against coronary atherosclerosis is apparently not universal with all animal species e.g. the rabbit (168). In addition, it has been claimed that administration of testosterone to cholesterol/fat-fed cockerels retards hypercholesterolaemia: the effect was not observed with castrated animals, however (170).

## II. CARBOHYDRATE AND TRIGLYCERIDE METABOLISM

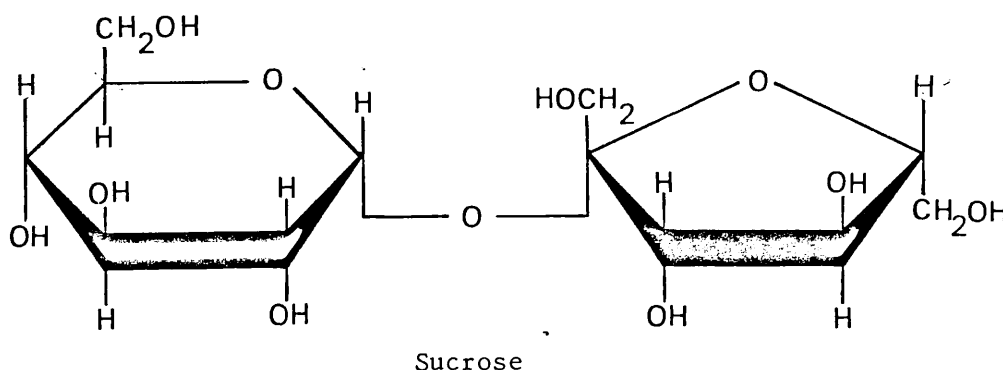
### A. METABOLISM OF CARBOHYDRATES

The disaccharide, sucrose, which is totally derived from plant sources, has been a major and increasing source of dietary calories in western countries for the last 30 years and, hence, any attempt to relate diet to human disease must take this carbohydrate into account.

The evidence linking dietary sucrose with ischaemic heart disease through hypertriglyceridaemia has already been outlined (see p. 19). There is no significant correlation between dietary starch or glucose and ischaemic heart disease.

#### 1. Intestinal metabolism of sucrose

Sucrose is a disaccharide of  $\beta$ -D-fructofuranose and  $\alpha$ -D-glucopyranose with the glycosidic linkage joining the two anomeric carbon atoms. This rather unusual structure imparts chemical properties on the molecule which are not found with most other dietary carbohydrates. It is a non-reducing sugar, which is stable in solutions of alkali but, because of the furanosyl moiety it is more easily hydrolysed by acids than most other dietary oligo- and poly-saccharides.



Dietary sucrose passes through the alimentary tract to the small intestine where, before absorption, it is normally hydrolysed by an invertase (174), present in the intestinal mucosa, to glucose and fructose. Since sucrose is sensitive to acid hydrolysis, it was once suggested that hydrolysis of ingested sugar, catalyzed by the hydrochloric acid of the gastric juice, might start in the stomach (422), but this has recently been refuted by Dahlqvist (174).

Intestinal invertase is an  $\alpha$ -glucosidase which can hydrolyse both sucrose and maltose; it appears in the human mucosa very early in fetal life much earlier than lactase (174). In the rat, the invertase activity varies with age, but not the sex, of the animal (175). Rats 23 days old have approximately two-thirds of the activity found in adult animals.

Dietary sucrose increases the activity of invertase in the jejunum of the rat (175, 176) and, some believe, in man (177, 199). When Blair et al. (175) fed a high-sucrose diet (70%) to rats, the invertase activity increased and there was also enhanced fructose absorption as reported by Deren et al. (176). Rosensweig and Herman (199), have demonstrated the adaptive nature of human disaccharidases; they concluded that the level of disaccharidase activity in human jejunum is controlled by dietary sugars. Specifically, sucrose and fructose are, they claim, capable of regulating invertase activity whilst lactase activity is not changed by these carbohydrates. However, Dahlqvist (174) believes that sucrose has little effect on the invertase activity in humans.

The site of intestinal absorption of the components of sucrose has been studied in man by the use of both oral loading and infusion techniques (178). Both hydrolysis and absorption were found to occur mainly in the jejunum, furthermore, 'sucrose absorption' rates were observed to be considerably more rapid in jejunal segments than in ileal segments. It has been suggested by Gray and Ingelfinger (178) that considerable amounts of glucose and fructose are released into the lumen after the hydrolysis of sucrose by the bound mucosal enzyme and then these monosaccharides are absorbed i.e. the hydrolysis products are not directly absorbed from the mucosal cells. Other workers (179) claim that sucrose is absorbed in both the distal jejunum and in the ileum. In this connection it should be noted that the region of highest invertase activity is in the region of the villi i.e. in the ileum (179).

It has been shown that glucose is absorbed by an active transport system which involves a protein carrier and sodium ions (30, 180, 186). The brush border membrane is the site of action of sodium ions (180). It is assumed that the cell membrane contains a glucose-binding carrier substance which binds glucose at one site and sodium ions at a separate site (see Fig. 1). The sodium ions are assumed to be necessary for the binding of the glucose to the carrier by bringing about a conformational change. It has also been shown that potassium ions can bind at the sodium-binding site and alter the degree of glucose-binding through another conformational change in the carrier (30). Hence, an efflux of potassium ions through the membrane facilitates the release of the glucose by the carrier on the intracellular side of the membrane. The active transport of the sodium ions out from and potassium ions into the cell is believed to be handled by a separate system, via a brush border,  $\text{Na}^+/\text{K}^+$ -dependent membrane ATPase (118), thereby providing the sodium ions necessary for glucose transport.

Work by Honegger (131) very recently reported by Dahlqvist (126), suggests that there are actually two glucose carrier systems in hamster small intestine. They are both  $\text{Na}^+$ -dependent and they can also carry galactose but they differ in sensitivity to phloridzin, the degree of specificity and  $K_m$  values.

Although the bulk of the intraluminal sugars are absorbed into the portal venous blood a portion of these sugars are metabolized by the mucosal epithelial cells of the small intestine to maintain their viability and to enable the absorptive systems to function (30, 191). Absorption of glucose, however, occurs with little conversion of the hexose to other metabolites. Kiyasu *et al.* (182) for example, using rats found that [ $^{14}\text{C}$ ]glucose placed in a loop of intestine, isolated by ligatures, appeared in the blood collected from a vein draining the loop. The total radioactivity recovered in the blood was 97% of which 80-90% was as glucose and the remainder as lactate and alanine.

Fructose is absorbed across and into the small intestinal epithelial cells of man and other animal species at a slower rate than glucose (186). Recently, Guy and Deren (181) have shown that the epithelial cells of the rat small intestine have a special membrane mechanism that allows the rapid entry of fructose. This mechanism is not dependent on sodium ions and is not sensitive to phloridzin unlike the process for glucose transport (121).

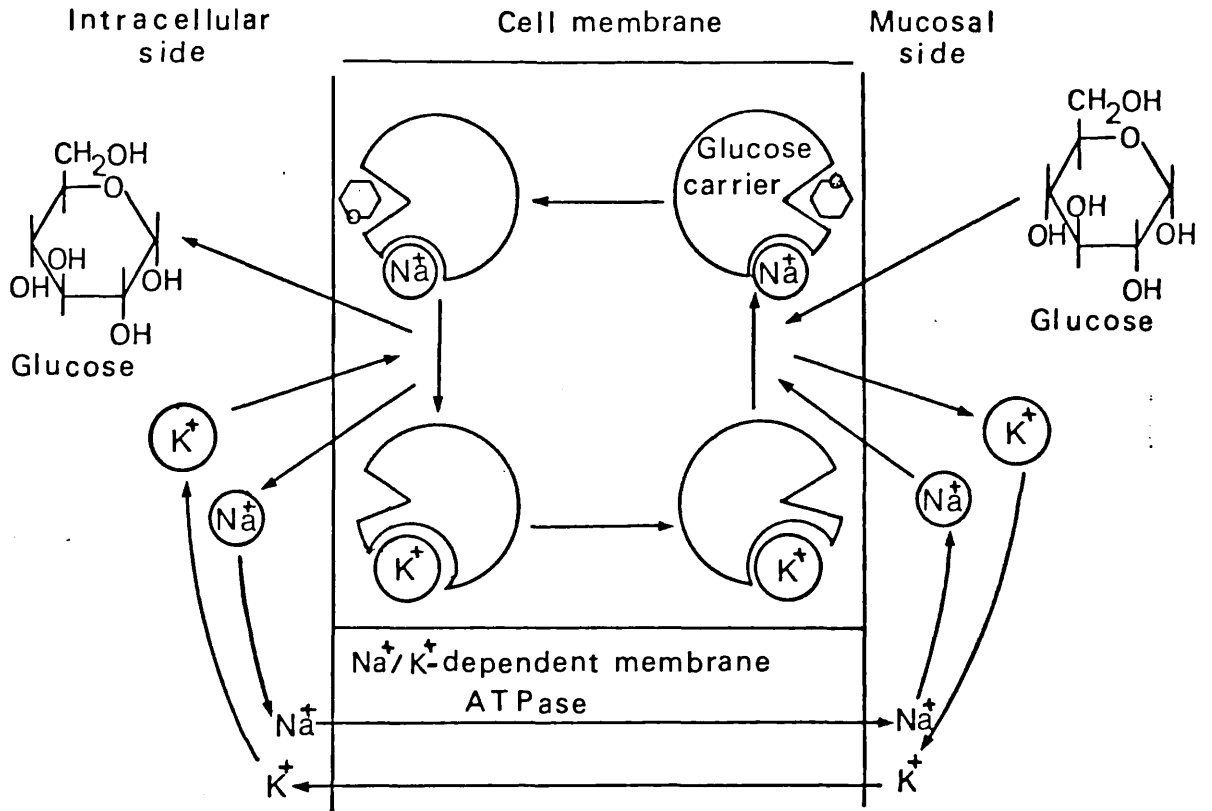


Fig. 1. Hypothetical scheme for active transport of glucose in the small intestine (30)

In the rat small intestine about 10% of ingested fructose is converted to glucose (30, 122, 184), about 60% to lactate (122, 184) and the remaining 30% is absorbed unchanged into the portal venous blood. In the small intestine of guinea pig comparatively more (55-80%) of the fructose is converted to glucose (124, 184), 10% to lactate (124, 184) and the remainder passes into the portal circulation intact.

Man is very different from the other species mentioned in that up to 80-90% of ingested fructose is absorbed from the small intestine as fructose. However, the conversion of some fructose to glucose by the human intestine has been demonstrated (119).

The metabolism of fructose by the small intestine of several animal species has been reviewed by Herman et al. (191). They concluded that in the small intestinal epithelial cells, fructose is transformed into either fructose 6-phosphate (hexokinase-catalyzed) (187) or fructose 1-phosphate (ketoheokinase-catalyzed) (185, 190, 193). According to Herman et al. (191) fructose, per se, can undergo either reaction but fructose which arises from the invertase-catalyzed hydrolysis of sucrose will probably undergo the ketoheokinase reaction preferentially. The reason for this is that the liberated glucose will compete with fructose for hexokinase but not ketoheokinase. Sols (187) found that fructose (6mM) phosphorylation by rat intestinal hexokinase could be inhibited (90%) by the presence of glucose at the same concentration.

Guinea pig small intestine appears to convert fructose to glucose to a greater degree than rat intestine (184, 191) because of the lower levels of ketoheokinase (214) and glucose 6-phosphatase (30, 180, 190) in the latter (see Fig. 2).

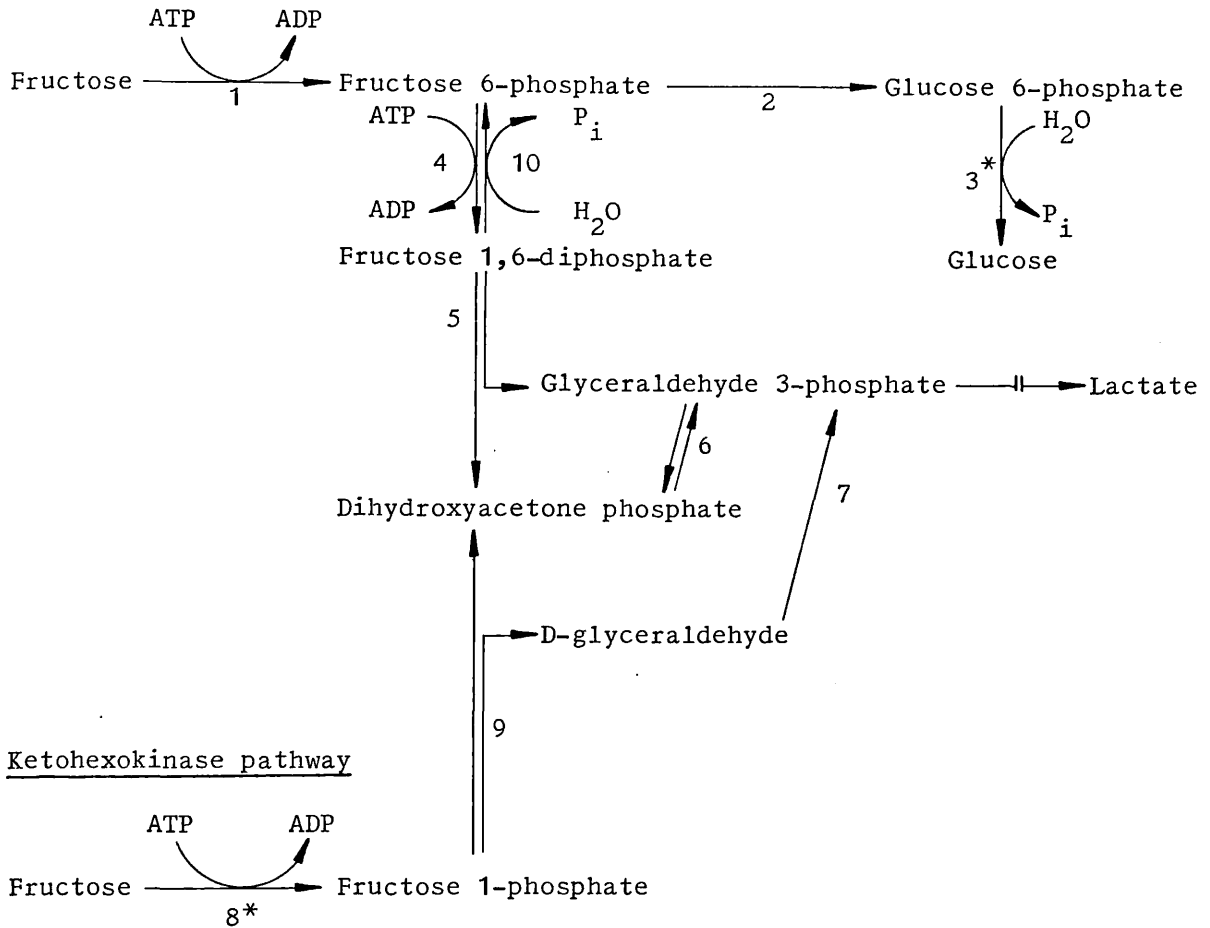
Intestinal ketoheokinase and fructose 1-phosphate aldolase can be regulated by the presence of fructose in the diet. A high fructose diet increases the activity of both enzymes in man (30, 192) and in the rat (30, 193). A glucose diet, on the other hand, lowers the activities of these enzymes (192, 193).

## 2. Metabolism of Glucose and Fructose in the Liver

### (a) General considerations

Glucose and fructose, after being absorbed from the small intestine, pass directly to the liver through the portal circulation. In the liver, however, about two-thirds of the free glucose enters the hepatocytes and is metabolized; the remainder of the glucose passes through the liver to the systemic circulation.

Hexokinase pathway



Ketohexokinase pathway

- 1.Hexokinase
- 2.Hexose phosphate isomerase
- 3.Glucose 6-phosphatase
- 4.Phosphofructokinase
- 5.Fructose 1,6-diphosphate aldolase
- 6.Triose phosphate isomerase
- 7.Triokinase
- 8.Ketohexokinase
- 9.Fructose 1-phosphate aldolase
- 10.Fructose 1,6-diphosphatase.

\* These reactions may be slow in rat tissue; relatively fast in guinea pig tissue.

Fig. 2. Pathways of fructose metabolism by intestinal epithelium

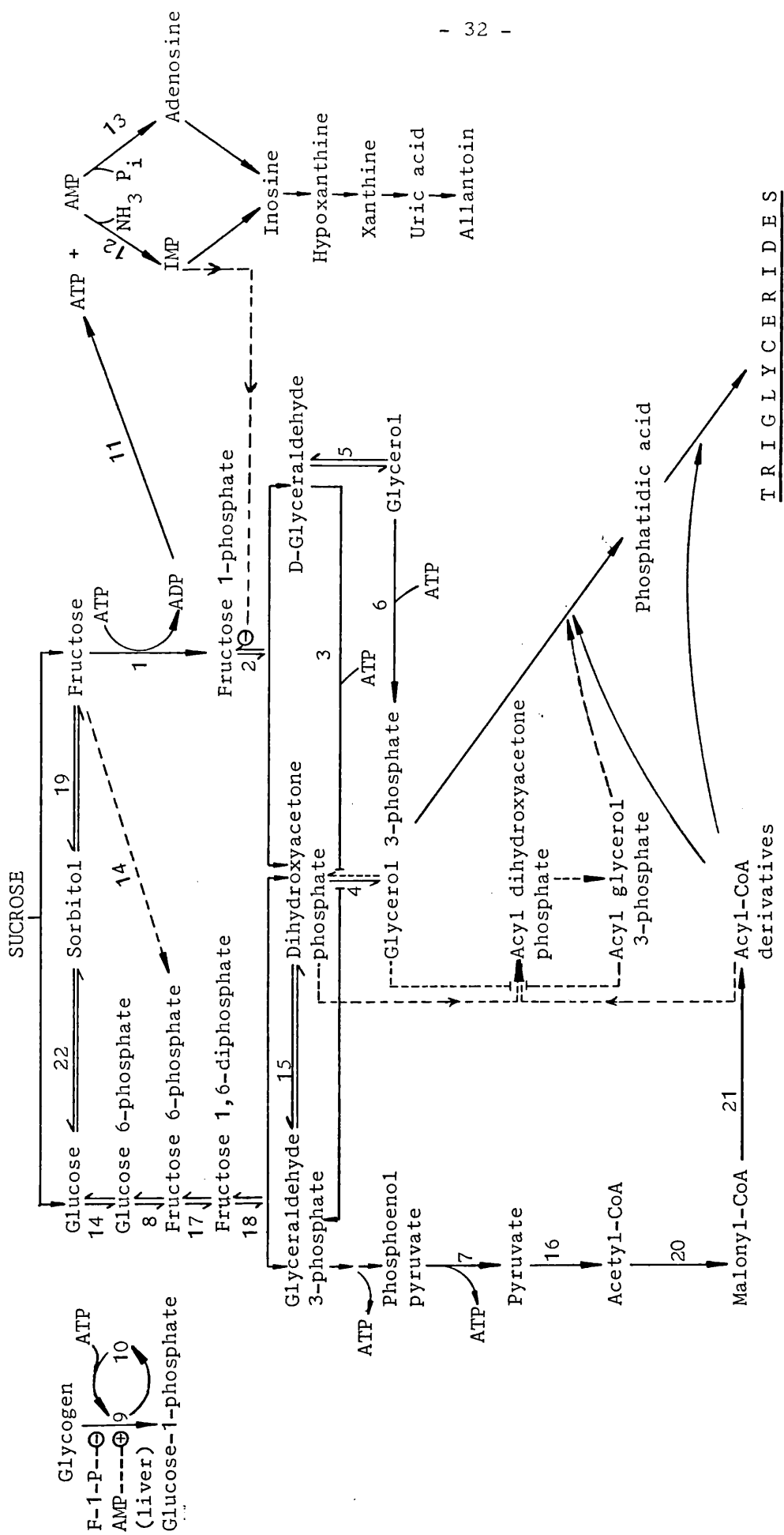
Monosaccharides other than D-glucose, such as D-fructose, D-mannose and D-galactose are all phosphorylated in the liver and can be further transformed into glucose and other products or oxidized to  $\text{CO}_2$ . Systemic blood contains essentially no free monosaccharides other than glucose and minor amounts of fructose.

Cahill and his associates in 1958 (205) believed that hexoses entered the liver cells by free diffusion across the plasma membrane, a view which was widely accepted for 10 years. However, in 1968 Williams *et al.* (33) found that D-glucose was taken up by perfused liver more rapidly than L-glucose and that the uptake was inhibited by phloridzin, a well known inhibitor of glucose transport in various cells. These results were considered as evidence for the existence of a specific transport system for glucose. Baur and Haldt (34) who studied glucose transport using isolated hepatocytes also concluded that there was a glucose carrier system ( $K_m$  30mM and  $V_{max}$   $110\mu\text{mol min}^{-1} \text{g}^{-1}$ ) and that the rate of glucose uptake was much faster than the rate of phosphorylation in the cell. Glucose penetration of liver cells does not appear to be significantly controlled by insulin but high levels of this hormone in the blood do enhance the entry of glucose into extra hepatic tissues such as adipose and muscle (369). Compared to glucose, fructose is taken up more slowly but, again, carrier system ( $K_m$  67 mM and  $V_{max}$   $30\mu\text{mol min}^{-1} \text{g}^{-1}$ ) is involved (222, 232). The results obtained by Sestoft (222) using perfused rat liver, show that the affinity of fructose for its carrier system is lower than the affinity of the ketose for ketohexokinase, the enzyme catalysing the initial phosphorylation stage. Sestoft, therefore, concluded that the rate of carrier-mediated transport of fructose limited its metabolism under physiological conditions.

It has long been known that the routes for glucose and fructose metabolism in the liver are different (see Fig. 3).

In summary the initial step in fructose metabolism is phosphorylation to D-fructose 1-phosphate. This reaction is catalyzed by a specific ATP-dependent enzyme, ketohexokinase. Cleavage of fructose 1-phosphate by liver aldolase yields D-glyceraldehyde and dihydroxyacetone phosphate; the latter may enter the glycolytic pathway and/or be used for triglyceride synthesis or gluconeogenesis.

Four enzymes are present in liver cells for the further metabolism of D-glyceraldehyde. These are: (1), triokinase which converts glyceraldehyde to its 3-phosphate derivative; (2), an NAD-specific aldehyde



1. Ketohexokinase
2. Fructose 1-phosphate aldolase
3. Triokinase
4. Sn-glycerol 3-phosphate dehydrogenase
5. Glycerol dehydrogenases (NAD- or NADP-dependent)
6. Glycerol kinase
7. Pyruvate kinase
8. Hexose phosphate isomerase
9. Phosphorylase a (active)
10. Phosphorylase b (inactive)
11. Adenylate kinase
12. AMP deaminase
13. Nucleotidase
14. Hexokinase
15. Triose phosphate isomerase
16. Pyruvate dehydrogenase
17. Phosphofructokinase
18. Fructose 1,6-diphosphate aldolase
19. Sorbitol dehydrogenase
20. Acetyl-CoA carboxylase
21. Fatty acid synthase
22. Aldose reductase.

Fig. 3. Metabolic pathways for D-glucose and D-fructose in animals



dehydrogenase which produces D-glyceric acid (which can be further phosphorylated to 2-phosphoglycerate by glycerate kinase) and (3), NAD- and NADP-dependent alcohol dehydrogenases which can both reduce glyceraldehyde to glycerol (which in turn can be phosphorylated to sn-glycerol 3-phosphate by glycerol kinase). In different animal species the levels of these enzymes vary (214).

Glucose on the other hand, is phosphorylated to D-glucose 6-phosphate by hexokinase which exists in different isoenzymic forms; this is the first rate-limiting step in hepatic glucose metabolism (214). Glucose 6-phosphate can then be isomerized to D-fructose 6-phosphate which is catalyzed by glucose phosphate isomerase. Further phosphorylation of fructose 6-phosphate at C-1 is catalyzed by the allosteric enzyme phosphofruktokinase. Aldolase-cleavage of the resulting D-fructose 1,6-diphosphate gives rise to dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. After the formation of trioses by aldolase the metabolism of glucose and fructose is similar.

The path of carbon from both fructose and glucose is further regulated at the triose level by the allosteric enzyme, pyruvate kinase, which produces pyruvate for eventual entry into the Krebs cycle. The levels of some of the enzymes involved in both glucose and fructose metabolism are subjected to hormonal and dietary control.

The detailed characteristics of the specific enzymes in the pathways for fructose metabolism and the key regulatory enzymes for glycolysis can now be considered.

(b) Fructose metabolism in the liver

In most animal species the liver is the main organ for fructose metabolism and there are a number of hepatic enzymes which are specific for this purpose (45).

Although fructose can be phosphorylated by hexokinase (EC 2.7.1.1) to fructose 6-phosphate, the low activity of this enzyme in rat and other mammalian livers and the high  $K_m$  (2-5mM) for fructose as compared with the  $K_m$  (0.1mM) for glucose makes it unlikely that the phosphorylation of fructose by this enzyme is at all significant under physiological conditions (214).

D-Fructose is chiefly phosphorylated to fructose 1-phosphate in hepatocytes by an ATP-dependent reaction catalyzed by ketoheksokinase (ATP:D-fructose 1-phosphotransferase; EC 2.7.1.3) (214, 215, 216). This enzyme is found in all mammalian livers (218, 219), in kidney (220)

and in the small intestinal mucosa (214, 221). In the cell, the enzyme is localized in the cytoplasm (68).

Hepatic ketohexokinase has been purified by a number of workers (215, 223, 224, 225, 226) and most recently from rat liver by Sanchez et al. (227). Purified rat liver ketohexokinase has an absolute requirement for  $K^+$  ions (228) and has a  $K_m$  of 0.46mM for fructose and 1.56mM for the ATP-Mg<sup>2+</sup> complex at a  $K^+$  ion concentration of 0.4M (227). ADP, one of its reaction products, is a non-competitive inhibitor towards fructose and competitive with respect to the ATP-Mg<sup>2+</sup> complex (228) and this latter inhibition is partially reversed by  $K^+$  ions. Inhibition of ketohexokinase by fructose 1-phosphate has been reported by Froesch et al. (217) but was not observed by Parks et al. (225) and by Sestoft (222). Ketohexokinase is not specific for D-fructose it also catalyzes the phosphorylation of L-sorbose and D-tagatose. The affinity of the enzyme for these ketoses depends on the concentration of  $K^+$  ions (226, 227).

It has been shown (231) that ketohexokinase is absent from the fetal livers of rats, rabbits and guinea pigs and that the enzyme appears post-partum and reaches adult levels of activity 7-10 days later.

The complete absence of ketohexokinase from the human liver results in a condition called essential fructosuria (233). As a consequence, ingested fructose accumulates in the blood and is excreted as such in the urine.

Ketohexokinase activity varies in different species of animals. In rat liver it is 2.2 U/g and in human liver 1.7 U/g (218). In the rat, the measured enzyme activity equates well with the rate of extraction of fructose from the medium ( $3\mu\text{mol fructose min}^{-1} \text{g}^{-1}$  tissue) during liver perfusion (222). In human liver, an extraction rate of  $1.5\text{-}2.3\mu\text{mol fructose min}^{-1} \text{g}^{-1}$  tissue (or an equivalent rate of 0.41-0.55g/min for a whole liver) has been calculated by Tygstrup et al. (229) and Craig et al. (230). This again correlates well with the level of ketohexokinase in this tissue.

Ketohexokinase activity of rat liver is affected by diet and hormones. Adelman and co-workers (46) have shown that the specific activity of the enzyme, in terms of liver weight, does not change greatly when the diet is manipulated. However, there are marked changes in the total liver activity. This is decreased to about

one-half of the normal level on fasting (48-72 hr) and is restored completely to normal after feeding fructose (70%) for 24 hr. With glucose feeding, the recovery is only partial. Heinz (198) has shown that feeding rats a diet enriched with fructose (65%) for 3 weeks causes an increase in ketohexokinase activity in liver and in kidney. The level of ketohexokinase is unchanged in alloxan-diabetic rats. On the other hand, the enzyme activity of fed, adrenalectomized or hypophysectomized rats is at the fasting level of normal rats and neither fasting nor subsequent feeding of fructose or glucose affects the level (46).

The mammalian liver also possesses an enzyme, sorbitol dehydrogenase (L-iditol dehydrogenase; L-iditol, NAD oxidoreductase; EC 1.1.1.14) which, in vitro, can catalyze the reduction of fructose to sorbitol (glucitol) by NADH. (The EC nomenclature for the enzyme is derived from early work where iditol was used as a substrate (437)).

This dehydrogenase was first partially purified in 1951 by Blakley (437) from rat liver. Since that time it has been shown to occur in various animal tissues (438, 440, 441, 442) where it is mainly located in the cytoplasm and also claimed to be present in the mitochondria of liver, kidney and prostate (439). In the liver the dehydrogenase has a specific activity five times greater than that found in the kidney or prostate (438). In human liver the activity is 25 U/g, and in rat liver 18.9 U/g (218).

The rat liver enzyme has a  $K_m$  of 0.7mM for sorbitol (437) and a  $K_m$  of 100-200mM for fructose (439).

The main fate of fructose 1-phosphate is an aldolase-catalyzed cleavage leading to trioses. The products of the reaction are dihydroxyacetone phosphate and D-glyceraldehyde. The hepatic isoenzyme (aldolase 1B; ketose 1-phosphate aldolase; EC 4.1.2.7) can cleave fructose 1-phosphate at nearly the same rate as fructose 1,6-diphosphate. The isoenzyme is also found in the kidney and the small intestinal mucosa but at lower activities than in the liver (214). A specific fructose 1,6-diphosphate aldolase (aldolase 1A) is present in skeletal muscle, which cleaves fructose 1,6-diphosphate more than 100-fold faster than fructose 1-phosphate (214). In human liver the fructose 1-phosphate aldolase activity is 2.08 U/g and in rat liver, 1.63 U/g (214, 218).

Aldolase 1B of human liver has a  $K_m$  of  $7.5 \times 10^{-3}$  M for fructose

1-phosphate and  $1 \times 10^{-5}$  M for fructose 1,6-diphosphate (214).

It has been shown by Adelman et al. (46) that rat liver aldolase is also regulated by diet and hormones. The activity decreases to about one-third of the normal activity if the animals are fasted for 48-72 hr: the activity is restored completely to normal in 24 hr by fructose feeding. The same authors (46) have shown that with fed, adrenalectomized or hypophysectomized rats, the activity is normal but that it decreases sharply on fasting and does not recover on subsequent glucose or fructose feeding.

Hereditary fructose intolerance is a disorder of fructose metabolism where the activity of liver aldolase is greatly depressed. Hers and Joassin (234) showed that, in patients with this disorder, the ability of liver aldolase to split fructose 1-phosphate is almost completely lacking. It is assumed, therefore, that the toxic effects of ingested fructose are related to the accumulation of fructose 1-phosphate (234).

D-Glyceraldehyde, derived from fructose 1-phosphate, can in theory be acted upon by four different enzymes. Triokinase (ATP: D-glyceraldehyde 3-phosphotransferase; EC 2.7.1.28) can phosphorylate the aldehyde by an ATP-dependent reaction, producing D-glyceraldehyde 3-phosphate. This enzyme can also phosphorylate dihydroxyacetone (214). Triokinase is present in soluble fraction of liver (214), kidney (220) and the small intestinal mucosa (221). The enzyme has been partially purified from guinea pig liver (113), beef liver (112) and, recently, from rat liver (111).

The rat liver enzyme has a  $K_m$  for D-glyceraldehyde of 0.01mM and 0.77mM for ATP-Mg<sup>2+</sup> (111, 236). Free ATP, ADP and ADP-Mg<sup>2+</sup> are competitive inhibitors with respect to ATP-Mg<sup>2+</sup> and non-competitive inhibitors with respect to D-glyceraldehyde (111).

Triokinase activity in human liver is 2.07 U/g, and in rat liver, 1.65 U/g (218). It has been shown (198) that feeding fructose-enriched diets to rats increases the liver and kidney enzyme activities. With normal rats fasting produces a marked decrease in total hepatic activity of the kinase which is completely restored to normal in 24 hr by fructose feeding (46). With fed, adrenalectomized or hypophysectomized rats, the activity is normal but it decreases sharply on fasting and does not recover on subsequent glucose or fructose feeding.

NADP-dependent alcohol dehydrogenase (glycerol dehydrogenase or

aldehyde reductase or alcohol: NADP-oxidoreductase; EC 1.1.1.2) is an enzyme which is highly specific for the reduction of D-glyceraldehyde to glycerol (214). It is found in the liver and in lower activities in the kidney and small intestinal mucosa (214).

Alcohol dehydrogenase has been purified from rat liver (95) and from the skeletal muscles of rabbit (99) and the rat (100). The  $K_m$  is 0.15mM for D-glyceraldehyde and 0.63M for glycerol with the liver enzyme (95, 214). The level of the enzyme is 3.6 U/g in human liver and 0.38 U/g in rat liver (218). A second alcohol dehydrogenase which is NAD-dependent (glycerol dehydrogenase or alcohol: NAD-oxidoreductase; EC 1.1.1.1) can also reduce D-glyceraldehyde to glycerol (214). In human liver the activity of this dehydrogenase is 3.1 U/g and in rat liver four times more active (1.64 U/g) than the NADP-dependent dehydrogenase (218). The rat hepatic enzyme has a  $K_m$  of  $3 \times 10^{-2}$  M for D-glyceraldehyde which is high and for this reason, the physiological role of the dehydrogenase is in doubt (218) (see also p. 63).

Any glycerol produced in vivo from glyceraldehyde (or from lipolysis or dietary sources) can be converted to sn-glycerol 3-phosphate by glycerol kinase (L-triokinase or ATP: glycerol phosphotransferase; EC 2.7.1.30). The tissue distribution of glycerol kinase has been studied in detail in rats. The liver is the chief source of the enzyme and in kidney the activity is mainly concentrated in the cortex (214). Significant activities are also present in the brain (248), adipose tissue (247), heart (247) and the small intestinal mucosa (214).

The activity of the enzyme has also been studied in tissues of guinea pigs (214), hamsters (214), mice (250), rabbits (214) and man (218, 249).

Glycerol kinase has been purified from rat (251, 253, 254), pigeon (252), beef and human (253) livers. The purified enzyme from rat has a  $K_m$  of  $3.2 \times 10^{-6}$  M for glycerol (254). Dihydroxyacetone and L-glyceraldehyde are also phosphorylated by this enzyme (214). Glycerol 3-phosphate, ADP and AMP are inhibitors of the kinase (254, 255) and raising the  $Mg^{2+}$  ion concentration increases inhibition by the latter two compounds (254). Inhibition by AMP is prevented by free ATP (254).

In human liver, the activity of glycerolkinase is 0.62 U/g tissue

and in the rat it was found to be 2.08 U/g (214, 218).

The enzyme level in rats was observed to decrease in response to starvation and in alloxan diabetic animals and after adrenalectomy. The first three days of feeding fat to normal animals also lowered the level. On the other hand, the activity of the kinase was increased after longer ( $\geq 3$  days) periods of feeding fat, after insulin substitution of alloxan diabetic rats and after cortisol replacement of adrenalectomized animals (256). Feeding unsaturated fatty acids to rats also led to an increased activity of glycerol kinase, whilst glycerol had no effect (257).

In adult animals, but not the newborn, glycerol kinase activities show sex differences: males have higher levels in the liver than females (241, 258) and the reverse appears to be true in the case of the kidney (239, 241).

Aldehyde dehydrogenase (D-glyceraldehyde dehydrogenase or aldehyde: NAD-oxidoreductase; EC 1.2.1.3) oxidizes D-glyceraldehyde to D-glyceric acid using NAD as a hydrogen carrier (214).

In rat liver, 60% of the aldehyde dehydrogenase activity is localized in the cytosol and 40% in the mitochondria; the overall activity is 1.4 U/g tissue. In human liver, the enzyme possesses a similar activity of 1.04 U/g (214, 218).

Aldehyde dehydrogenase isolated from rat liver has a  $K_m$  of  $4 \times 10^{-4}$  M for D-glyceraldehyde. Therefore, oxidation of D-glyceraldehyde to D-glyceric acid is a physiological possibility (88, 214).

Aldehyde dehydrogenase in the liver is accompanied by D-glycerate kinase (ATP: D-glycerate 2-phosphotransferase; EC 2.7.1.31) which can phosphorylate glyceric acid to 2-phosphoglycerate, an intermediate in glycolysis.

In human liver, the level of activity of this enzyme is low (0.13 U/g); in rat liver, significantly higher (3.2 U/g) (214, 218).

An enzyme capable of oxidising sn-glycerol 3-phosphate, sn-glycerol 3-phosphate dehydrogenase (sn-glycerol 3-phosphate: NAD-oxidoreductase; EC 1.1.1.8), is widespread in animal tissues. It is NAD-dependent and can readily catalyse the formation of dihydroxyacetone phosphate in vitro. However, at physiological pH values, the equilibrium favours glycerol 3-phosphate formation. In vivo the enzyme is thought to be important for the production of glycerol 3-phosphate from dihydroxyacetone phosphate (a product of the aldolase reaction), the former being an acyl acceptor for triglyceride and phospholipid synthesis (267, 272).

Glycerol 3-phosphate dehydrogenase is present in the cytosol. The tissue distribution of glycerol 3-phosphate dehydrogenase has been studied in rats; significant activities are present in the liver (262, 263), skeletal muscle (260, 262), brain (261), heart and adipose tissue (264), but the highest enzyme level is found in the muscle (239).

The enzyme has been purified from various tissues and several different animals (206, 238, 243, 244, 259, 260, 261, 262, 263, 265). The rabbit liver enzyme has  $K_m$  values of 0.91mM, 0.075mM, 0.083mM and 0.022mM for glycerol 3-phosphate, dihydroxyacetone phosphate, NAD and NADH, respectively (243).

There is some evidence to suggest that the liver enzyme has two allosteric sites, one for glycerol 3-phosphate and the other for NAD. The binding of glycerol 3-phosphate to its allosteric site lowers the  $K_m$  for this substrate but increases the  $K_m$  for dihydroxyacetone phosphate. The case is similar with NAD (265). The effects of sugar phosphates, nucleotides and other phosphorylated compounds on glycerol 3-phosphate dehydrogenase from rabbit liver have also been studied. Lee et al. (210) found that glucose 6-phosphate, fructose 1,6-diphosphate and 3-phosphoglycerate inhibited the oxidation of glycerol 3-phosphate, competitively, with respect to both glycerol 3-phosphate and NAD. On the other hand, glyceraldehyde 3-phosphate was competitive with glycerol 3-phosphate and non-competitive with NAD. Furthermore, nucleotides (AMP, ADP and ATP) and pyrophosphates were found to inhibit both the forward and reverse reactions in a competitive manner with respect to NAD.

It has been shown by Fitch and Chaikoff (266) that feeding glucose- or fructose-enriched diet (60% for 7 days) to rats, resulted in a significant increase in the activity of glycerol 3-phosphate dehydrogenase. There are claims that glycerol 3-phosphate dehydrogenase is under the control of adrenal and pituitary function via glucocorticoids in rat brain but not in muscle (268).

#### (c) Glucose metabolism in the liver

The catabolism of glucose to trioses and eventually acetate, is well documented and differs, in the main, from fructose metabolism in the initial phosphorylation reaction and the subsequent aldolase-catalysed reaction which yields different products. Furthermore, whilst glucose metabolism to acetate is controlled by four rate-limiting enzymes; glucokinase, phosphofructokinase, pyruvate kinase and pyruvate

dehydrogenase, conversion of fructose to acetate would appear to be only controlled by the latter two enzymes.

The first step in glucose metabolism in the liver is phosphorylation which yields glucose 6-phosphate. This is achieved by hexokinases and the process requires ATP and  $Mg^{2+}$  ions (214). In mammalian tissues, two types of hexokinases have been detected the group of, so-called, low  $K_m$  (for glucose) hexokinases (ATP:D-glucose 6-phosphotransferases; EC 2.7.1.1.) which have a low substrate specificity and a single high  $K_m$  form, present only in the liver, which is normally termed glucokinase (ATP:D-glucose 6-phosphotransferase; EC 2.7.1.2) and which is specific for glucose (208).

Using DEAE-cellulose column chromatography and starch gel-electrophoresis Katzen (211) resolved hexokinase activity in rat tissues into different forms. He detected three isoenzymes which were designated hexokinases I, II and III. An additional form, type IV hexokinase, was also found and this is the form which is now commonly known as glucokinase. All four isoenzymes have been detected and purified from livers of several different animal species (163, 194, 211, 213).

In the livers of both man and rat, hexokinases are localized in the epithelial cells of the bile ducts and glucokinase is present in the hepatocytes (209). The hexokinases of rat liver can phosphorylate fructose but it is unlikely, under physiological conditions, that fructose is a substrate for these kinases (see p.33). Glucokinase cannot catalyze the phosphorylation of fructose (207, 208).

The distribution and kinetic data for the multiple forms of rat hexokinases are summarized in Table I (211). Hexokinases are inhibited by their reaction products, glucose 6-phosphate and ADP (208, 214). Glucokinase, on the other hand, is not inhibited by these compounds but acetyl-CoA and phosphoenol pyruvate are inhibitors (200).

In the liver of several mammals and man, the hexokinase activity is approximately the same, i.e. 0.4 U/g tissue. A high glucokinase activity (1.35 U/g) in rat liver compared to human liver (0.08-0.2 U/g) has been reported (214, 218). Rat liver glucokinase is an adaptive enzyme and its activity is increased by a glucose-enriched diet (346). The hexokinases of rat liver, however, are much less subject to dietary modification (346). Sharma et al. (347) found that fasting or alloxan diabetes markedly depressed glucokinase activity without



TABLE I. Kinetic properties and distribution of multiple forms of hexokinase (211).

Type	Tissue	$K_m$ glucose (M)	$K_m$ fructose (M)
I	Brain	$3.0 \times 10^{-5}$	$3.1 \times 10^{-3}$
	Liver	$2.5 \times 10^{-5}$	-
	Muscle	$2.4 \times 10^{-5}$	$5.0 \times 10^{-3}$
II	Muscle	$2.0 \times 10^{-4}$	$2.4 \times 10^{-3}$
	Epididymal fat pad	$1.4 \times 10^{-4}$	$3.0 \times 10^{-3}$
III	Liver	$5.0 \times 10^{-6}$	$2.2 \times 10^{-3}$
IV (Glucokinase)	Liver	$1.6 \times 10^{-2}$	*

\* Fructose is not a substrate at concentrations up to 0.1M.

affecting the activity of hexokinase. Glucokinase activity was restored when glucose was refed to fasted animals or when insulin was administered to diabetic rats. Furthermore, it has been suggested that the responsiveness of liver to dietary glucose in maintaining a high activity of glucokinase requires the action of both insulin and glucose (347).

With regard to the hexokinases, however, there are reports (163, 196) that type II which is predominantly found in insulin-sensitive tissues (heart and skeletal muscles and adipose tissue) but at low levels in liver and other insulin-insensitive organs, is reduced in diabetic or starved rats. Insulin injection and/or refeeding raises the level of type II hexokinase. Glucokinase activity in aged, fasted rats is not restored to normal by glucose feeding. Administration of large amounts of insulin appears to overcome the age-dependent deterioration of the regulatory capacity (201).

Glucose 6-phosphate, produced by the kinases, is isomerized in the glycolytic pathway to fructose 6-phosphate (see Fig. 3) and after this stage the path of carbon from glucose to acetate is further regulated by the allosteric enzyme, phosphofructokinase (ATP:D-fructose 6-phosphate 1-phosphotransferase; EC 2.7.1.11). This enzyme phosphorylates fructose 6-phosphate to fructose 1,6-diphosphate and it is present in most tissues but the levels vary greatly. The liver, for example, possesses a much lower specific activity than muscle (214). The kinase has been purified from tissues from several animal species including muscle (212), brain (242), kidney (245), heart (282, 283) and liver (197, 202, 203). Phosphofructokinase exists in two forms: the M-type is localized in skeletal muscle, heart and brain and the L-type in liver, kidney and adipose tissue (214). These two isoenzymes exhibit different kinetic properties. A comparison of rabbit liver and muscle phosphofructokinases shows similarities in their pH optima and at pH 8.2, cation activation and substrate affinity. On the other hand, differences in the two latter properties were observed when reactions were carried out at pH 7.0. In the presence of 0.75mM ATP, the  $K_m$  values for fructose 6-phosphate for the muscle and liver enzymes were 0.056mM and 0.052mM, respectively, and using 1mM fructose 6-phosphate, the  $K_m$  values for ATP were 0.050 and 0.043mM, respectively (202).

The activity of phosphofructokinase is regulated by a variety of metabolites. It has been shown that ATP and citrate are potent inhibitors of the enzyme (197) and that ADP, AMP, inorganic phosphate and fructose 6-phosphate are activators (197, 246). The liver enzyme is more strongly inhibited by ATP than the muscle-type and it is not sensitive to reversal of inhibition by AMP. Furthermore, the hepatic phosphofructokinase is less inhibited by citrate, phosphoenolpyruvate and other phosphates than muscle enzyme (202, 246). In vitro, inhibition of rat liver phosphofructokinase activity by long chain fatty acids has also been reported (17, 285, 286).

The effect of insulin and glucagon on the activity of hepatic phosphofructokinase has been investigated in rats (20, 349, 350, 351). The enzyme activity has been shown to decrease in the liver of alloxan diabetic rats and during starvation and the activity can be restored after insulin replacement or refeeding normal diet, respectively, in these animals (20). Insulin and glucagon injected intravenously into normal rats produces rapid reciprocal changes in the activity of phosphofructokinase (349, 351): insulin induces an increase whilst glucagon effects a decrease. It is believed that the glucagon-effect is mediated through the action of cyclic AMP on a cyclic AMP-dependent protein kinase as the effect of this peptide hormone on the enzyme activity is preceded by a significant increase in cyclic AMP levels which occur immediately after hormone injection (351). The effect of epinephrine on phosphofructokinase activity has also been investigated in the rat (352). Infusion of the hormone into rat portal vein results in a rapid decrease in the activity of the kinase in liver. Insulin administered 5 min after epinephrine partially reverses the epinephrine effect. The epinephrine effect is also associated with a significant increase in hepatic cyclic AMP concentrations. According to Stifel et al. (352) the epinephrine effect appears to be mediated through the action of cyclic AMP, but on a cyclic AMP-independent protein kinase as was demonstrated by Garrison (373). Pretreatment of rats with actinomycin D or puromycin does not alter the response of the enzyme to insulin, glucagon or epinephrine, indicating that de novo protein synthesis is not responsible for the change in enzyme activity (351).

The enzyme pyruvate kinase (ATP: pyruvate phosphotransferase; EC 2.7.1.40) catalyzes the transfer of the 'energy-rich' phosphate group from phosphoenolpyruvate to ADP producing enolpyruvate and ATP (214).

During gluconeogenesis, phosphoenolpyruvate is produced from pyruvate via oxalacetate in two steps which are catalyzed by pyruvate carboxylase (EC 4.6.1.1) and phosphoenolpyruvate carboxykinase (EC 4.1.1.32), respectively. High levels of these two gluconeogenic enzymes are present in liver and kidney i.e. in tissues capable of catalyzing glucose formation from trioses at a relatively high rate (306).

In the cell, pyruvate kinase is localized in the cytosol (214). At least three different isoenzymic forms of the kinase are present in higher animals (353). The L-type is found as a major component in the liver and a minor constituent in the kidney. The M-type is found in muscle, brain and heart; the third, A-type, occurs in adipose tissue and kidney and to a lesser extent in liver (353, 355). The L- and A-types share a number of regulatory properties; for example, both exhibit sigmoidal kinetics with respect to the concentration of phosphoenolpyruvate and both are allosterically inhibited by citrate and activated by fructose 1,6-diphosphate. They differ, however, with regard to allosteric inhibition by ATP which occurs with the L-type but not the A-type. The M-type pyruvate kinase has none of the above mentioned regulatory properties (353). Pyruvate kinase has been purified from the livers of rats (309, 332, 356) and other animal species (338, 339, 343). More recently human liver pyruvate kinase has been purified (354). The M-type pyruvate kinase (rat muscle) has a  $K_m$  of  $0.7 \times 10^{-4}$  M for phosphoenolpyruvate and  $0.3 \times 10^{-3}$  M for ADP whilst the L-type (rat liver) has  $K_m$  values of  $0.8 \times 10^{-3}$  M and  $0.3 \times 10^{-3}$  M, respectively (214, 344). Rat liver pyruvate kinase is allosterically inhibited by ATP and alanine and inhibition by ATP is reversed by fructose 1,6-diphosphate and high concentration of phosphoenolpyruvate (355). Tanaka et al. (344) have shown that fructose 1,6-diphosphate (0.1mM) can activate purified L-type pyruvate kinase from rat liver at a low concentration of phosphoenolpyruvate and it also reverses inhibition of the enzyme by ATP. Furthermore, in the absence of fructose 1,6-diphosphate the curve of the enzyme activity plotted against the phosphoenolpyruvate concentration is sigmoidal whilst in the presence of fructose 1,6-diphosphate the enzyme follows Michaelis Menten kinetics with a low  $K_m$  for phosphoenolpyruvate (0.08mM) (344, 355). Activation of rat liver pyruvate kinase by fructose 1-phosphate has been reported (277) with most marked activation occurring at low phosphoenolpyruvate

concentrations (0.05 - 0.25mM). At a higher phosphoenolpyruvate concentration (0.8mM), however, no activation by fructose 1-phosphate occurred. Acetyl-CoA has also been shown to inhibit rat liver pyruvate kinase activity (345).

The effects of diet and hormones on hepatic pyruvate kinase have been investigated in rats. Cohen et al. (335) have shown that the activity of liver pyruvate kinase increased 4- to 6-fold, in rats fed a sucrose-enriched diet (72%) for 12 months, as compared to animals fed chow. An increase in the activity of liver enzyme has also been demonstrated by Yudkin and Krauss (197) in rats fed a 60% sucrose diet for 10 days. In alloxan diabetes and during starvation pyruvate kinase activity decreases but it can be restored by insulin treatment or refeeding normal diets (20). Insulin and glucagon injection into normal rats have been shown to raise and lower, respectively, the activity of rat liver pyruvate kinase (349, 351). Glucagon and cyclic AMP have been shown to stimulate gluconeogenesis in the liver and to increase the ratio of phosphoenolpyruvate concentration to that of pyruvate (357, 358). Herman and co-workers (349, 351) found that rapid decrease in rat liver pyruvate kinase activity occurred a few minutes (2-10min) after an intravenous injection of glucagon. As with phosphofructokinase, the glucagon effect is believed to involve a cyclic AMP-dependent protein kinase which phosphorylates pyruvate kinase and inactivates it (355). Rat liver pyruvate kinase has been purified and found to be a substrate for cyclic AMP-stimulated protein kinase (355). At low phosphoenolpyruvate concentrations the activity of pyruvate kinase is inhibited by phosphorylation (because of an increase in the apparent  $K_m$  for phosphoenolpyruvate, from 0.3mM to 0.8mM (in the absence of fructose 1,6-diphosphate) at pH 7.3). Cyclic AMP-stimulated phosphorylation of enzymes and other proteins is reversible, because of the existence of phosphoprotein phosphatases. Thus, the metabolic regulation of liver pyruvate kinase via a phosphorylation-dephosphorylation mechanism is possible. Epinephrine when injected into rats has been shown to produce an effect similar to that of glucagon (352) but in this former case a cyclic AMP-independent protein kinase mediates (373).

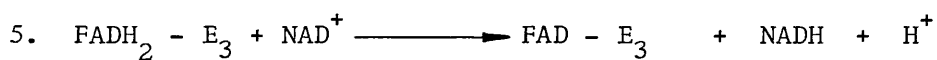
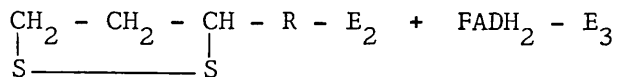
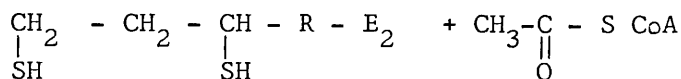
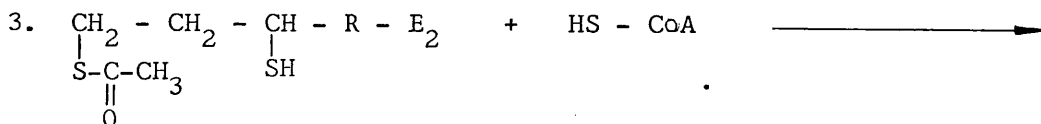
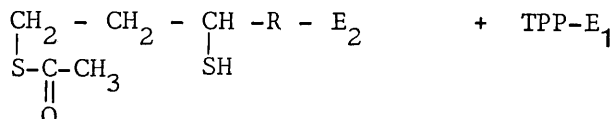
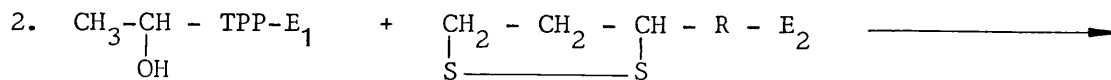
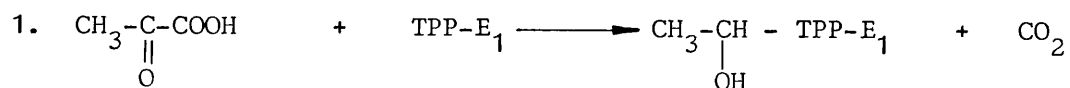
The conversion of pyruvate to acetyl-CoA, a precursor of fatty acids, is catalyzed by the pyruvate dehydrogenase complex (EC 1.2.4.1). This oxidative decarboxylation reaction requires three different enzymes and five different coenzymes organized together into a

multienzyme complex (see Fig. 4): in animal tissues it is localized in the mitochondria. In skeletal muscle, heart, kidney, liver and adipose tissue (214) the pyruvate dehydrogenase complex consists of dihydrolipoyl transacetylase, as a core, to which pyruvate dehydrogenase, dihydrolipoyl dehydrogenase and two regulatory enzymes, pyruvate dehydrogenase kinase and a -phosphatase, are attached (359). The activity of the complex is inhibited competitively by the products of pyruvate oxidation, acetyl-CoA and NADH, and these inhibitions are reversed by coenzyme A and NAD, respectively (360). Furthermore, the transacetylase and flavoprotein components of the complex are the sites of acetyl-CoA and NADH inhibition, respectively.

Pyruvate dehydrogenase is under metabolic and hormonal regulation and a regulatory mechanism, involving phosphorylation and dephosphorylation of the complex has been demonstrated (359, 361, 362). Phosphorylation (inactivation) of the complex is catalyzed by pyruvate dehydrogenase kinase and dephosphorylation (activation) by pyruvate dehydrogenase phosphatase.

This control mechanism has been demonstrated with preparations of pyruvate dehydrogenase from mammalian liver and other tissues (359). The kinetic and regulatory properties of the purified pyruvate dehydrogenase-kinase and -phosphatase from bovine kidney and heart have been described (362). Both ADP and pyruvate are competitive inhibitors of the kinase and they compete with ATP. Recent studies indicate that  $\text{Ca}^{2+}$  is required in addition to  $\text{Mg}^{2+}$  for pyruvate dehydrogenase phosphatase activity (363, 369). Activation of pyruvate dehydrogenase is enhanced by conditions that decrease the ATP/ADP molar ratio or increase the intramitochondrial pyruvate concentration. These observations are consistent with inhibition of pyruvate dehydrogenase kinase by pyruvate and ADP, respectively.

Regulation of the interconversion of the phosphorylated and the non-phosphorylated forms of rat adipose tissue pyruvate dehydrogenase by insulin has been reported (370, 371) and by long chain fatty acids (372) with rat heart, liver and kidney enzymes. Insulin, increases the proportion of the non-phosphorylated form of the dehydrogenase and this effect is antagonized by epinephrine. Metabolic states (diabetes and starvation) associated with increased concentrations of plasma free fatty acids result in an increase in the proportion of the phosphorylated form of pyruvate dehydrogenase (359).



$\text{E}_1$  = Pyruvate dehydrogenase

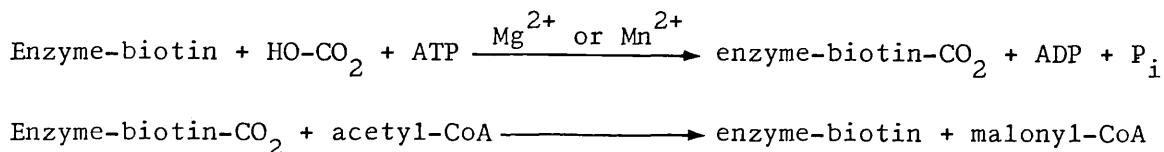
$\text{E}_2$  = Dihydrolipoyl acetyltransferase

$\text{E}_3$  = FAD-containing dihydrolipoyl dehydrogenase

Fig. 4. The reaction steps involved in the oxidative decarboxylation of pyruvate to acetyl-CoA

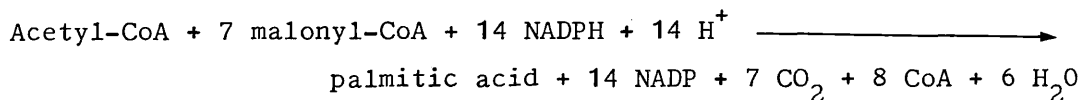
B. TRIGLYCERIDE SYNTHESIS

A major metabolic fate of glucose and fructose in animals in a positive calorie balance is conversion to triglyceride. The mammalian biosynthetic pathways to triglycerides from hexoses mainly involves de novo synthesis of long chain fatty acid esters which are then used to esterify sn-glycerol 3-phosphate. In outline, this occurs as follows. The first step to long chain acyl-CoA derivatives is the addition of CO<sub>2</sub> to acetyl-CoA with the production of malonyl-CoA. This reaction is catalyzed by the biotin-requiring enzyme, acetyl-CoA carboxylase (EC 4.6.1.2) (326):



Allosteric activation of this enzyme by citrate and inhibition by palmityl-CoA (a major product of fatty acid synthesis) can occur (326). Possible control of acetyl-CoA carboxylase by phosphorylation and dephosphorylation of the enzyme has been reported (328, 330). A rat liver protein fraction inactivated the enzyme in vitro with concomitant incorporation of <sup>32</sup>P from [γ-<sup>32</sup>P] ATP and the phosphorylation was reversed by a phosphatase fraction (328). Cyclic AMP has also been shown to inhibit acetyl-CoA carboxylase activity in rat liver slices (331). However, in vivo regulation of the enzyme by phosphorylation-dephosphorylation and the role of cyclic AMP has been questioned (391).

The second step in fatty acid synthesis is the conversion of malonyl-CoA to palmityl-CoA which is catalyzed by a fatty acid synthase multienzyme complex consisting of six enzyme proteins and an acyl carrier protein. In essence the reaction consists of the transfer of C<sub>2</sub> units from malonate to acetyl-CoA and reduced pyridine nucleotide, NADPH, is required (326).



Fatty acid synthase of pigeon liver is stimulated by phosphorylated sugars, especially fructose 1,6-diphosphate (397) and with the rat liver complex inhibition by palmityl-CoA has also been reported (328). Fatty acid synthase activity has been shown to increase when rats are fed a fat-free diet and decrease when fed a high-fat diet or

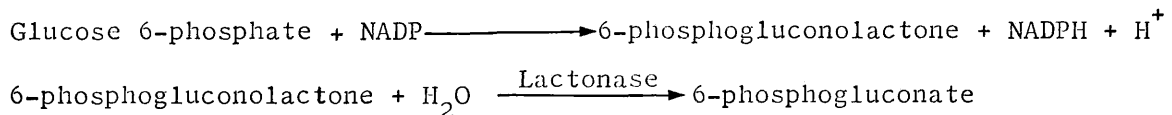


on fasting. It has been demonstrated (397) that the high hepatic fatty acid synthase activity accompanying a fat-free diet returns to normal after gastric intubation of linoleate and linolenate but not after administration of palmitate or oleate.

The adaptive synthesis of fatty acid synthase in the liver of rats fed a fat-free diet following a 48 hr fast has been studied using immunochemical methods (327). The synthesis of the active synthase occurs 3 hr after feeding whereas the synthesis of inactive material which can be precipitated by anti-fatty acid synthase serum, occurs within 1 hr. Furthermore, incorporation of radioactive pantothenate into the synthase during the adaptive synthesis follows the same pattern as the development of enzyme activity, indicating that the inactive but immunochemically identical precursor of fatty acid synthase may represent an apoenzyme which is converted to enzymically active holoenzyme by the incorporation of the 4'-phosphopantetheine prosthetic group.

Fatty acid synthase in the developing liver has been studied in rat (397): the hepatic activity rises dramatically at the time of weaning when animals change their diet from high fat milk to lower fat chow. Synthase activity is also reduced in livers of alloxan-diabetic rats (397, 401) and administration of insulin to these rats restored the activity.

Two molecules of NADPH are needed for every complete sequence of reactions catalyzed by fatty acid synthase and this reduced coenzyme is derived from glucose 6-phosphate in two stages involving two separate dehydrogenases. In the first stage glucose 6-phosphate is oxidized to 6-phosphogluconate by the action of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) as follows:



Rat liver glucose 6-phosphate dehydrogenase has been crystallized by Matsuda and Yugari (408) who investigated some of the molecular properties of the enzyme (409). The enzyme is inactivated by free fatty acids and this process is facilitated by ATP. Although no effect of ATP itself on the enzyme has been reported by these authors (409), Avigad (410) has described inhibition of the rat liver dehydrogenase by ATP. NADP antagonizes the effect of fatty acids and this relationship

is shown to be of a competitive type (409). Rat liver glucose 6-phosphate dehydrogenase has a  $K_m$  of  $1.3 \times 10^{-5}$  M for both NADP and glucose 6-phosphate. The specific activity of glucose 6-phosphate dehydrogenase in rat liver is  $46 \pm 3$ U/g liver (at pH 7.6) in male rats although the level is higher in females (ca  $104 \pm 12$ U/g liver) (384).

There is some evidence to show that liver glucose 6-phosphate dehydrogenase is under long term dietary regulation. Glock and McLean (98) first reported a marked reduction in the activity of the dehydrogenase in the livers of starved rats. This finding has been confirmed and extended by other workers who observed that re-feeding starved animals led to an increase in the levels of activity (183, 188, 237, 266, 407). Administration of a high-fat diet following starvation does not induce recovery of the enzyme activity (237). A 3-fold increase in activity was observed, however, when a balanced diet was given to fasted rats; the increase was more pronounced (10-12-fold) if the rats were fed with a carbohydrate-enriched diet particularly if the carbohydrate used was fructose (183, 266). There is some evidence that de novo synthesis of glucose 6-phosphate dehydrogenase occurs in response to diets rich in carbohydrate (188, 407). The activity of glucose 6-phosphate dehydrogenase has been shown to decrease in diabetic rats (183) and insulin injection together with a carbohydrate enriched diet raises the activity of the dehydrogenase (416) by increasing the rate of synthesis (80-fold) of the enzyme: the rate of degradation is also increased (5-fold). The rate of synthesis increases in proportion to the caloric consumption of carbohydrate and Rudack and co-workers (416) have concluded that insulin 'induces' glucose 6-phosphate dehydrogenase synthesis, by stimulating the appetite of the animal. Glucagon and cyclic AMP have been shown to prevent the induction of rat liver glucose 6-phosphate dehydrogenase which normally occurs when fasted rats are fed a high carbohydrate diet (418). The hormone decreased the rate of enzyme synthesis without altering the rate of enzyme degradation (417). The effects of sex steroid hormones on this dehydrogenase are considered later (see p. 72).

The second enzyme involved in glucose 6-phosphate catabolism and the regeneration of NADPH is 6-phosphogluconate dehydrogenase (EC 1.1.1.44). In this reaction 6-phosphogluconate undergoes oxidation and decarboxylation to form D-ribulose 5-phosphate.

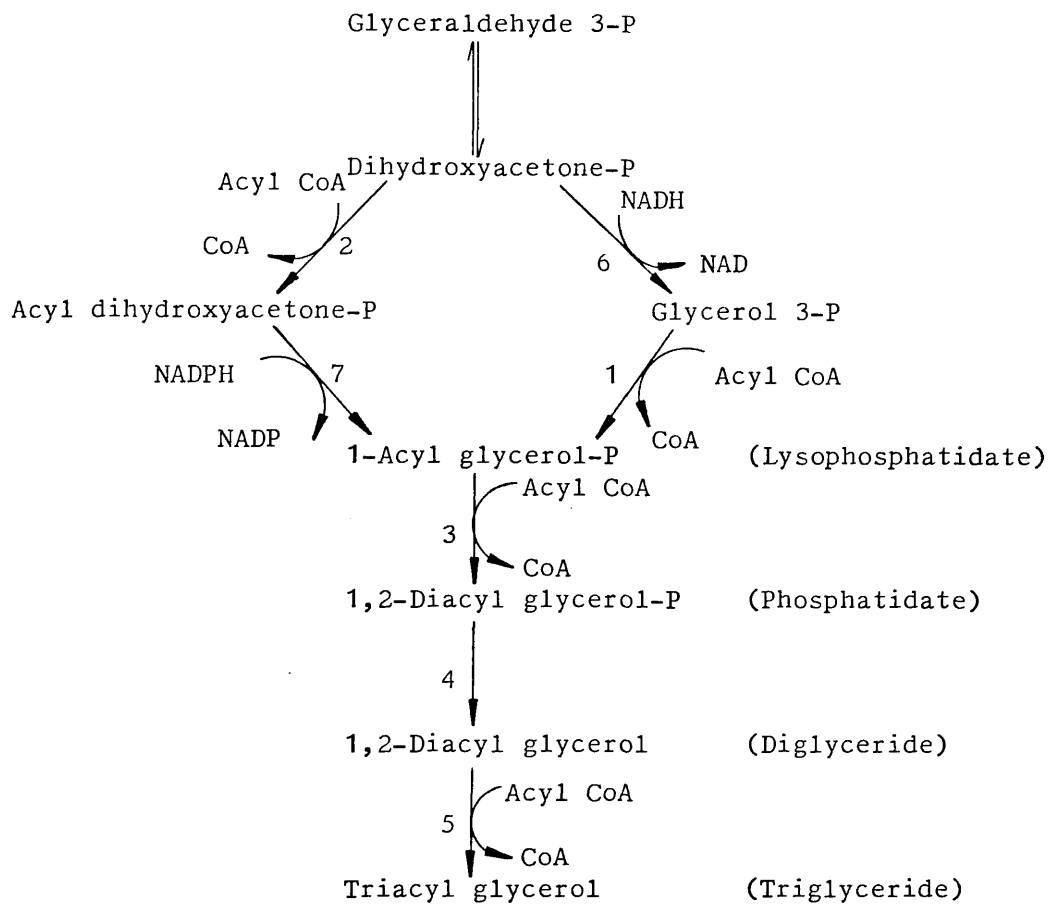


The dehydrogenase from rat liver has been purified (419): the  $K_m$  values for 6-phosphogluconate and NADP are  $7.1 \times 10^{-5} \text{M}$  and  $1.3 \times 10^{-5} \text{M}$ , respectively. The specific activity of 6-phosphogluconate dehydrogenase in male rat liver is  $59 \pm 8 \text{U/g}$  tissue and in female rats this level is, again, higher (ca  $130 \pm 12 \text{U/g}$  tissue) (384). Evidence has been presented that both ATP and NADPH act as competitive inhibitors of the dehydrogenase with respect to NADP (384, 419) and that the NADPH concentration in vivo may be a sensitive regulator of the activity of the enzyme (419). Diet regulates the dehydrogenase in rat liver; a high-fat, carbohydrate-free diet significantly decreases the activity (237). In normal rats, the substitution of glucose or fructose (60% for 7 days) for a stock diet resulted in an increase in the activity of 6-phosphogluconate dehydrogenase (266). When the same dietary substitution was made with diabetic rats, the change in enzyme activity was less than that found with normal rats. The activity of the dehydrogenase was reduced in diabetic rats regardless of the diet fed (183). Glucagon does not appear to be able to regulate the enzyme level (417).

The relative rates of synthesis and degradation of rat liver 6-phosphogluconate dehydrogenase have been studied in animals under different dietary conditions (420). With rats fed a high-carbohydrate, fat-free diet the rates of synthesis were 3.7- and 5.6- times greater than that with animals fed a normal diet or fasted rats, respectively. No significant differences in the rates of degradation of the enzyme in all three nutritional states have been noticed.

The long chain acyl-CoA derivatives synthesized from acetate and malonate are ultimately used as acyl donors for triglyceride formation. The initial acceptor for these acyl groups can be sn-glycerol 3-phosphate or dihydroxyacetone phosphate. The relative importance of the, so-called, dihydroxyacetone phosphate and glycerol 3-phosphate pathways for the synthesis of acyl glycerols has been studied in several different laboratories (272, 273, 297, 304, 305, 307). The two pathways which both produce phosphatidic acids, are illustrated in Fig. 5.

In the first step in the glycerol 3-phosphate pathway a NAD-dependent, reduction of dihydroxyacetone phosphate catalyzed by glycerol 3-phosphate dehydrogenase occurs followed by mono-acylation by glycerol 3-phosphate acyltransferase (EC 2.3.1.15). The highest activities of



E N Z Y M E S

1. Sn-glycerol 3-phosphate acyltransferase (EC 2.3.1.15)
2. Dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42)
3. 1-Acyl sn-glycerol 3-phosphate acyltransferase
4. Phosphatidate phosphohydrolase (EC 3.1.3.4 )
5. Diacyl glycerol acyltransferase
6. Sn-glycerol 3-phosphate dehydrogenase (EC 1.1.1.8 )
7. Acyl didydroxyacetone phosphate reductase (EC 1.1.1.101)

Fig. 5. The de novo synthesis of triglyceride

this transferase are found in the adrenal gland, liver and adipose tissue (267). Studies reported by Lloyd-Davies and Brindley (300) and Lamb and Fallon (301) suggest that glycerol 3-phosphate acyltransferase is a rate-limiting reaction in phosphatidate formation in microsomal fractions of rat liver. After the introduction of a second acyl group by acyl glycerol 3-phosphate acyltransferase which yields phosphatidate, diacyl glycerol is then formed by hydrolysis catalyzed by the enzyme phosphatidate phosphohydrolase (EC 3.1.3.4) (301). Lamb and Fallon (301) believe that this may be a rate controlling reaction for diacyl glycerol and triacyl glycerol formation in liver cells. In the final stage of the process the third acyl group is introduced by diacyl glycerol acyltransferase (326). In this pathway where glycerol 3-phosphate is an intermediate a further contribution to the pool of this phosphate comes from glycerol derived from lipolysis and, possibly, the reduction of glyceraldehyde. Glycerol can be converted directly to glycerol 3-phosphate by glycerol kinase (see p. 37).

In the dihydroxyacetone phosphate pathway, dihydroxyacetone phosphate is converted to acyl derivatives and this can occur in both mitochondria and microsomal fractions (273, 304, 305): acylation is catalysed by the enzyme dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42). NADPH-dependent acyl dihydroxyacetone phosphate reductase (EC 1.1.1.101) can then reduce the carbonyl groups of the acyl derivatives to hydroxyls and the resulting acyl glycerol phosphates are then further acylated to phosphatidates as previously described (326).

The relative contributions of the two pathways for acyl glycerol synthesis have been compared in homogenates of different tissues, Pollock et al. (297) have shown that dihydroxyacetone phosphate enters phosphatidate more rapidly via acyl dihydroxyacetone phosphate pathway than via glycerol 3-phosphate in those tissues which are deficient in glycerol 3-phosphate dehydrogenase. In liver in vivo it was suggested that the glycerol 3-phosphate pathway to acyl glycerols was more active than the acyl dihydroxyacetone phosphate pathway because the activities of glycerol 3-phosphate acyltransferase and glycerol 3-phosphate dehydrogenase were greater than that of dihydroxyacetone phosphate acyltransferase.

Rat liver slices were used to study the relative rates of incorporation of a mixture of [2-<sup>3</sup>H]- or [1,3-<sup>3</sup>H]glycerol with [1-<sup>14</sup>C]glycerol

into lipids. Here, Manning and Brindley (272) showed that 40-50% of the glycerol incorporated into lipid by rat liver slices proceeded via the glycerol 3-phosphate pathway and 50-60% was incorporated via dihydroxyacetone phosphate. This contrasted with studies where isolated hepatocytes were incubated with [U-<sup>14</sup>C, 2-<sup>3</sup>H]glycerol which produced glycerolipids with <sup>3</sup>H/<sup>14</sup>C ratios similar to those found in the intracellular glycerol 3-phosphate and in the original labelled glycerol. This suggested that, in this case, the acyl dihydroxyacetone phosphate pathway did not contribute significantly to the total glyceride synthesis from glycerol since the conversion of glycerol 3-phosphate to dihydroxyacetone phosphate would have resulted in loss of <sup>3</sup>H and a change in the isotopic ratio (307).

### III. EFFECTS OF DIETARY CARBOHYDRATES ON SERUM AND LIVER TRIGLYCERIDES

The role of dietary carbohydrate, particularly sucrose, in producing hypertriglyceridaemia leading to ischaemic heart disease has already been outlined (see p. 19).

In humans, two types of lipaemia are recognized, one of which is induced by fatty meals and the other by carbohydrate in the diet (140, 141). Fructose or sucrose feeding, in comparison with glucose or starch, has been reported by many investigators to increase the serum triglyceride levels in man (143, 144, 145, 149), rat (52, 53, 63) and other animal species (155, 164). It is apparent that it is the fructose moiety of sucrose which is hypertriglyceridaemic (22).

The biochemical mechanisms underlying the hypertriglyceridaemic effect of carbohydrates is poorly understood. It has been postulated that sucrose and fructose cause an increase in plasma triglyceride concentration through: (1), an increased rate of hepatic synthesis of triglyceride (324, 329) and/or (2), a decreased rate of clearance of plasma triglyceride (325).

The male rat has been the main experimental animal used to investigate the effect of dietary carbohydrates on triglyceride levels in blood serum and in the liver from which most of the serum triglyceride is derived. Much of this work is often difficult to compare in detail because of the different concentrations of carbohydrates used in diets and the different times and ways of administration (solid or in solution). In addition, other components accompanying the carbohydrate supplements have often been varied.

These nutritional studies can be divided into short-term feeding (up to 4 weeks) and long-term (from over 4 weeks to 1 year).

Zakim et al. (47), for example, observed that the concentration of triglycerides in serum and liver increased when fasted (48 hr) rats were fed for a short period (48 hr) on glucose- or fructose-enriched diets (70% of calories) in comparison with chow. Fructose produced a higher serum triglyceride concentration than glucose but the liver triglyceride levels were very similar with these two hexoses. Bar-on and Stein (53) carried out a similar investigation and found that with rats, fructose feeding (10% solution for 6-19 days) significantly increased the serum triglyceride levels compared to animals on glucose or chow diets. In comparison to chow, the liver triglyceride levels increased when rats were fed a glucose- or fructose-enriched diet. On the other hand, with guinea pigs in which fructose is known to be converted to glucose in the intestine (184), the serum triglyceride level was not affected by supplements of fructose or glucose to the diet (53). The feeding of a synthetic diet containing 60% sucrose for 8-14 days to rats by Shiff et al. (424) also resulted in a significant increase in serum and liver triglyceride level compared to animals on chow diets and Mukherjee et al. (340) showed that rats ingesting diets containing 12% sucrose or fructose for 30 days, had raised the serum and liver triglyceride levels compared to those animals fed on glucose or starch. Serum triglyceride levels in rats on a glucose regimen were, however, lower than the control animals receiving starch. Nikkila and Ojala (63) reported that both glucose and fructose supplementation of chow diet (10% solution in the drinking water for a period of 2 - 4 weeks) caused an increase of serum triglyceride concentration in rats but the effect of fructose was more marked than that of glucose. Also, the liver triglyceride content was raised by both hexoses although the fructose fed animals did not apparently differ significantly from the control. High (72%) and low (32%) sucrose supplement diets were shown by Lin and Anderson (65) to produce the same raised levels of triglyceride in both the serum and liver of rats. However, the two diets had different fibre and vegetable oil contents.

The age-dependent response of serum triglyceride to dietary fructose has been demonstrated with rats. Hill (154) found that

feeding fructose (10% in the drinking water for 21 days) to rats compared to a chow diet, increased the serum triglyceride and caused an accumulation of liver triglyceride after 24 hr in mature rats. In young animals, however, the serum triglyceride was not affected whilst the liver triglyceride was significantly increased after 24 hr but decreased after 10 days of fructose feeding. Similarly, when Chevalier *et al.* (156) fed young and mature rats on diets (70% carbohydrate diet for 3 weeks) enriched with sucrose, fructose, glucose or starch, the serum triglyceride levels were significantly increased in the mature rats ingesting sucrose or fructose compared to those given glucose or starch diets. The levels of serum triglycerides remained unaffected in young animals, however.

In conclusion, it is apparent that in short-term feeding experiments with rats under a variety of conditions, the serum triglyceride levels are increased by sucrose- or fructose-enriched diets compared to glucose or starch. With the liver triglyceride concentrations, on the other hand, there is not the clear distinction between glucose and sucrose/fructose feeding that is seen with the serum triglycerides.

The effect of long-term feeding of carbohydrates on hepatic and serum triglycerides has again shown an association between sucrose or fructose and elevated serum triglyceride levels in a number of different animal species. Coltart and Macdonald (155), using male baboons, have demonstrated an increase in the fasting serum triglyceride concentration after feeding sucrose (75%) for 17 weeks in comparison with a control chow diet. Palafox (77) has fed white leghorn hens (from 20-76 weeks of age) a diet containing either raw sugar (7.9%) or cornstarch (7.9%) and similarly found that the serum triglyceride levels of the birds ingesting raw sugar were significantly higher than those feeding on starch. When Tuovinen and Bender (55) fed rats with sucrose, fructose or starch (60% for 26 weeks) the hepatic triglycerides were increased significantly in the case of both the sucrose- and fructose-rich diets in comparison with starch. Likewise, Laube *et al.* (157) found that rats fed on a diet supplemented with sucrose (68% for 15 weeks) or an equimolar mixture of glucose and fructose exhibited an increase in total liver lipid and in serum triglyceride and free fatty acids in comparison with rats on a high starch diet. There was no significant difference between the effects of the sucrose and glucose plus fructose diets on these animals. In contrast, studies by Bruckdorfer and Yudkin (333) suggest that carbohydrate induced hypertriglyceridaemia



does not occur with male pigs given a diet in which 50% of the dry weight was either sucrose or starch for one year.

The inclusion of sucrose in the diet not only affects liver triglyceride levels but in the long-term, it also produces changes in the composition of the bound fatty acids. Casal et al. (336) fed young rats (6 weeks) fat-free diets containing either starch, sucrose or glucose (74%) as a carbohydrate source and then the fatty acid composition of the liver triglyceride was analysed. They found that the fatty acids in livers of animals fed on glucose or sucrose containing larger proportions of palmitic, palmitoleic and oleic acids and a smaller proportion of linoleic acid than those fed on starch.

Macdonald (334) has shown that administration of sucrose (18-68% of total calories) to rabbits for 16 weeks increases the hepatic lipid levels compared to animals on starch or glucose diets. However, in this case a study of the fatty acid composition of liver triglyceride revealed that, with dietary sucrose, the proportions of palmitic and palmitoleic acids rose with increasing sucrose intake but with glucose they tended to fall and with starch there seemed to be no change. Dietary sucrose was associated with a more rapid drop in the proportion of combined linoleic acid than was dietary starch or glucose. Recently, Macdonald and his associates (337) have given young rats diets containing 70% of either glucose, sucrose or fructose until they reached 200g body weight. They were then transferred to a chow diet for a further 4 weeks. Other control rats received a chow diet throughout the whole experimental period. The amount of liver triglyceride was found to be significantly greater in the 'fructose group', compared with the other three dietary groups at the end of the period on a high-carbohydrate diet. In the group of animals on the fructose diet there was a significant fall in total liver triglyceride 4 weeks after returning to the control diet: this did not occur with the sucrose- or glucose-fed rats. In these experiments the combined palmitic, palmitoleic and oleic acids in the liver triglycerides after the period of the high carbohydrate diet (glucose, fructose or sucrose) was significantly greater than the levels in the fat from animals ingesting chow. In the case of linoleic acid, however, the reverse was found.

Glycerol (a possible metabolite of fructose) feeding has also been shown to produce hypertriglyceridaemia in rats (82). When male rats

fed on chow diets supplemented by either glycerol or glucose (15% solution in the drinking water for 2 weeks) the serum triglyceride was significantly greater with the glycerol- than with the glucose-supplement. The liver triglyceride content was also increased in both glycerol- and glucose-fed animals as compared to control diet. Macdonald (316) also reported that dietary glycerol was hypertriglyceridaemic in men.

Incorporation of carbon from  $[U-^{14}C]$ glucose or  $[U-^{14}C]$ fructose into lipids has also been investigated with several different animal species and the results tend to confirm the normal dietary studies. Macdonald and Roberts (164) found that with 48 hr fasted baboons, the ingestion of 40g of glucose, sucrose or partially hydrolysed starch all equally labelled with carbon-14, produced serum triglycerides with higher levels of radioactivity in the case of the sucrose-fed animals than with the baboons fed hydrolysed starch or glucose. Gale and Crawford (91) studied the different rates of incorporation of carbon from  $[U-^{14}C]$ fructose and  $[U-^{14}C]$ glucose into lipids in guinea pigs after oral or intraperitoneal administration of the sugars. They found that the contribution of  $[U-^{14}C]$ fructose to the serum triglyceride was 4-6 times higher than that of  $[U-^{14}C]$ glucose. Nikkila and Ojala (63) injected  $[1-^{14}C]$ palmitic acid intravenously into rats previously fed on chow diets supplemented with either glucose or fructose in the drinking water for 2-4 weeks. They found that the radioactivity present in the serum triglyceride of the fructose-fed animals 60 min after injection was 2-4 times greater than that in the serum triglyceride of the control or glucose-fed animals. In the glucose-fed group, however, the radioactivity of serum triglyceride was similar to that in the control animals. Zakim *et al.* (47) compared the conversion of  $[6-^{14}C]$ glucose and  $[6-^{14}C]$ fructose to triglyceride fatty acids and  $CO_2$  in liver slices of rats which had been fed a chow diet then fasted for 48 hr and, finally, fed glucose or fructose diets (70% of calories) for 48 hr. These workers showed that the incorporation of label from  $[6-^{14}C]$ fructose into the combined fatty acids was always greater than the incorporation from  $[6-^{14}C]$ glucose in all dietary groups. Both the glucose- and fructose-fed rats showed higher incorporation into combined fatty acids than the chow-fed animals. Bar-on and Stein (53) have reported that when  $[U-^{14}C]$ glucose or  $[U-^{14}C]$ fructose is injected intravenously into rats, significantly more fructose- than glucose-carbon is recovered in both liver and serum

triglycerides 1 hr after injection. Chevalier and co-workers (348) have studied lipogenesis from [U-<sup>14</sup>C]glucose, [U-<sup>14</sup>C]fructose and [1-<sup>14</sup>C]acetate in liver slices from rats fed fructose- or glucose-enriched (70%) diets for 2-3 weeks. They found that with liver slices from glucose-fed rats more glucose than fructose was converted to combined fatty acids. Dietary fructose, when compared to glucose, enhanced lipogenesis from [U-<sup>14</sup>C]fructose but it had no effect on lipogenesis from [U-<sup>14</sup>C]glucose or [1-<sup>14</sup>C]acetate. Cohen and Teitelbaum (52) fed rats starch-, glucose- or fructose-enriched diets (72% for 65 days), and then measured the rate of incorporation of intraperitoneally injected [1-<sup>14</sup>C]acetate into lipids. Their results demonstrated that animals fed glucose or fructose showed a significantly higher incorporation of labelled acetate into fatty acids and triglycerides in the liver and serum triglycerides compared with rats on a starch diet. There was no significant difference between glucose- and fructose-fed animals in this instance, however. Maruhama and Macdonald (425) using rats on diets low in fat and high in glucose or fructose (11 weeks), demonstrated that after intragastric administration of [U-<sup>14</sup>C]glucose or [U-<sup>14</sup>C]fructose the incorporation of radioactivity into the glycerol moieties of liver and plasma triglycerides was higher than into the fatty acid moieties after both fructose and glucose feeding with fructose contributing more label than glucose.

Using patients who had had a myocardial infarction, Maruhama (341) compared the incorporation of carbon from orally administered [U-<sup>14</sup>C]glucose or [U-<sup>14</sup>C]fructose into serum triglyceride-glycerol and triglyceride-fatty acids over a period of 4 hr. He found that the increase in the specific activity of the triglyceride-glycerol and triglyceride-fatty acids after [U-<sup>14</sup>C]fructose administration was significantly greater in comparison with [U-<sup>14</sup>C]glucose ingestion. The radioactivity of the triglyceride-glycerol was 10 times greater than that of the triglyceride-fatty acids after both glucose and fructose administration. Periera and Jangaard (49) have studied the rate of conversion of [U-<sup>14</sup>C]fructose and [U-<sup>14</sup>C]glucose into various intermediates of carbohydrate and lipid metabolism in rat liver slices. They found that fructose was converted to pyruvic acid and triglyceride-fatty acid 3 times faster than was glucose and 19 times faster than glucose into triglyceride-glycerol.

In conclusion, it appears that the rate of incorporation of carbon from [U-<sup>14</sup>C]fructose into serum triglyceride is much higher than from

[U-<sup>14</sup>C]glucose and this difference is further exaggerated in animals that have received fructose-enriched, rather than glucose-enriched diets, prior to administration of the labelled carbohydrates. In most cases in which glucose and fructose have been compared, the transfer of label from [U-<sup>14</sup>C]glucose to serum triglyceride-glycerol is substantially less than from [U-<sup>14</sup>C]fructose and also the amount of the labelled carbon in triglyceride-glycerol is greater than that found in the fatty acid moieties of the triglycerides no matter which labelled hexose is administered. The incorporation of carbon-14 from [U-<sup>14</sup>C]fructose and [U-<sup>14</sup>C]glucose into liver triglyceride sometimes equates with the serum triglycerides but this may be modified by the dietary regimen preceeding the experiment so that both hexoses behave similarly (cf 348).

The association of dietary fructose, in comparison with glucose, with elevated serum triglyceride levels may relate to different rates of hepatic synthesis and secretion and/or different rates of lipolysis (clearing) of the triglycerides.

The liver is the major source of serum triglycerides and a rapid conversion of fructose to triglyceride, here, could relate directly to high serum triglyceride levels. In the liver, fructose is metabolized as far as aldolase products by a pathway which is distinct from that for glucose but after these trioses the routes for the conversion of glucose and fructose to triglyceride are similar. All of these pathways have already been outlined (see p. 31 and Fig. 3).

The greater capacity for fructose metabolism than glucose metabolism by the liver relates largely to the initial phosphorylation stages. The activity of hepatic ketohexokinase in rat, for example, exceeds the combined activities of hexokinase and glucokinase (and in man the difference is even more marked (214)) no matter what dietary carbohydrate supplements are given to the animals (47). In addition, ketohexokinase is particularly sensitive, in rats, to fructose in the diet which leads to a very significant increase in its activity (46).

The hexokinase (hexokinase I, II and III and glucokinase)-catalyzed phosphorylation of glucose is a rate-limiting step in glycolysis (214). These enzymes are subjected to a number of controls not found with ketohexokinase. The hexokinases which are constitutive enzymes are at very low levels, in rat and human livers (218) and are inhibited by their products, glucose 6-phosphate and ADP. Although this is not true for

glucokinase this enzyme, with a high  $K_m$  for glucose, is under the control of insulin (and dietary glucose-containing carbohydrates) although this is a relatively long term effect.

In rat liver, a phosphorylation rate of 3.0  $\mu\text{mol}$  fructose/g tissue/min and 1.98  $\mu\text{mol}$  glucose/g tissue/min has been calculated based on the levels of ketohexokinase and hexokinase and glucokinase in the tissues under optimal conditions. Thus, fructose could be phosphorylated faster than glucose in rat liver, a fact that has also been established for human liver (218). In vivo, these rates would of course, be influenced by the rates of transport of the two hexoses across cell membranes (cf 173) although differential rates of fructose and glucose phosphorylation are presumably maintained in view of the results obtained by 'feeding' these compounds (see p. 54).

Further apparent evidence for the rapid phosphorylation of fructose in the liver comes from studies where the fructose 1-phosphate levels, following a fructose load, have been assayed. The level of hepatic fructose 1-phosphate has been shown to increase following fructose infusion of rats (271, 274, 279) and perfusion of rat liver (232). However, the biochemical mechanism which results in this increase is reported to involve the rapid decrease in the intracellular concentrations of ATP, as a result of fructose phosphorylation, and subsequent increasing AMP levels and degradation of this nucleotide to IMP (see Fig. 3). IMP inhibits fructose 1-phosphate aldolase activity and, hence, favours the accumulation of fructose 1-phosphate (232). Lamers and Hulsman (270) were unable to demonstrate a marked increase in hepatic fructose 1-phosphate concentration or decrease in ATP 10 min after intraluminal loading of fructose, in contrast to the large effects on the levels of these metabolites observed by the other workers (271, 274, 279) after parenteral administration.

Other enzymes, aldolase 1-B and triokinase, supporting the metabolism of fructose are also at levels high enough to account for the rapid rate of fructose metabolism in rat liver (46). These latter two enzymes also reach maximum levels in response to dietary fructose (46, 198).

A further control of the path of carbon from glucose (but not fructose) to triose occurs at the phosphofructokinase-catalyzed stage of conversion of fructose 6-phosphate to fructose 1,6-diphosphate. This enzyme is subjected to glucagon and epinephrine inhibition and insulin activation and to the influence of a variety of other effectors (see p. 43).

There is no evidence to suggest that the pathways for the metabolism of the trioses produced from fructose and glucose, which are different, play any major role in the more rapid conversion of fructose than glucose to triglyceride. Dihydroxyacetone phosphate is produced from both hexoses but D-glyceraldehyde and D-glyceraldehyde 3-phosphate are the other products from fructose and glucose, respectively. D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are interconvertible via triose phosphate isomerase and, hence, all the glucose carbons and C-1, C-2 and C-3 of fructose pass to pyruvate via the glycolytic pathway and this triose in turn leads to acetate and triglyceride-fatty acid. Likewise the same carbons from glucose and fructose may find their way to triglyceride-glycerol. D-glyceraldehyde, however, derived from C-4, C-5 and C-6 of fructose could conceivably be further metabolized by four different enzyme-catalyzed reactions (see p. 31). The high activity of triokinase in both human and rat livers and the low  $K_m$  of this enzyme for D-glyceraldehyde makes it a strong candidate for the major role in D-glyceraldehyde metabolism via the formation of the 3-phosphate derivative. This view is supported by Hue and Hers (235) who examined the conversion of [4-<sup>3</sup>H, 6-<sup>14</sup>C]fructose to liver glycogen (but not triglyceride) in mice and showed that the glucose was labelled at C-1 and C-6 with <sup>14</sup>C, with the tritium remaining at C-4. On the basis of the kinetic properties of the various enzymes involved in the metabolism of D-glyceraldehyde in the rat, Sillero et al. (236) came to the same conclusion as Hue and Hers (235) that triokinase is the main enzyme involved in the metabolism of D-glyceraldehyde to glucose. Frandsen and Grunnet (111) have speculated that, in the rat, triokinase is mainly involved in the conversion of glyceraldehyde to glucose. These authors support this assumption by two observations: (1), that the amount of fructose converted to glucose in rat liver slices corresponds closely to the activity of triokinase in rat liver (447) and (2), that the enzyme possesses a relatively high  $K_m$  for ATP. The latter could mean that glyceraldehyde phosphorylation is regulated to a significant degree by the ATP level in vivo and that triokinase would, then, only be effective when the ATP concentration was high which would also favour other gluconeogenic processes and inhibit glycolysis and acetate formation. At low ATP levels Frandsen and Grunnet (111) suggest that the glycerate and/or glycerol pathways may operate.

The possibility of D-glyceraldehyde metabolism by oxidation to D-glyceric acid does exist, because the levels of aldehyde dehydrogenase are high in both human and rat livers and because of the low  $K_m$  value for the substrate. However, the hepatic glycerate kinase level in man is very low and it probably plays a minor physiological role in human liver. The situation could be different in the rat: here the glycerate kinase activity is significantly higher than that of aldehyde dehydrogenase which means that the pathway of glyceraldehyde to 2-phosphoglycerate via glycerate could operate but this was not detected by Hue and Hers (235).

The levels of NAD- and NADP-dependent alcohol dehydrogenases in human liver are high but the affinity of the enzymes for D-glyceraldehyde are low which means that the reduction of D-glyceraldehyde to glycerol in human liver may be unimportant. In rat also the evidence does not altogether favour a glycerol pathway. In this animal, NADP-dependent alcohol dehydrogenase activity is low and although the NAD-dependent enzyme appears to be at a similar level to triokinase the  $K_m$  for glyceraldehyde in this case is about 30mM. In addition, isotope studies, have shown that the liver glycogen isolated from rats after administration of [6-<sup>14</sup>C]fructose (58) or [1-<sup>14</sup>C]fructose (189) is labelled only at C-1 and C-6 of the glucose moieties. The reduction of D-glyceraldehyde to glycerol, its conversion to glycerol 3-phosphate by glycerol kinase followed by gluconeogenesis would shift the labelling to positions 3 and 4 of the glucose molecule but this was not observed. However, Landau and Merlevede (58) found that injection of D-[3-<sup>14</sup>C]glyceraldehyde into rats did give rise to the glucose labelling pattern expected for conversion of glyceraldehyde to glycerol. This latter result could be explained by assuming that direct administration of D-glyceraldehyde raises the intracellular concentration of the aldehyde to levels high enough for the NAD-dependent alcohol dehydrogenase to function (236) and raised aldehyde levels are said to occur in response to fructose loading (97).

In summary, the possible fate of D-glyceraldehyde in human liver is its conversion to D-glyceraldehyde 3-phosphate by triokinase whereas the situation in the rat is less clear. There is a possibility of reduction of D-glyceraldehyde to glycerol or oxidation to glycerate in this animal.

As it has been shown with rats that more carbon from [U-<sup>14</sup>C] fructose than [U-<sup>14</sup>C]glucose is incorporated into the glycerol moiety of triglyceride (which in both cases is also higher than the incorporation into triglyceride-fatty acid) (see p. 58) it has been suggested that there is a more rapid synthesis of sn-glycerol 3-phosphate from fructose than from glucose. There is, however, a lack of general agreement: with both in vivo and in vitro studies, there are conflicting results regarding which of the two hexoses is the better precursor of glycerol 3-phosphate (47, 81, 270, 342).

The path of carbon from both glucose and fructose to acetate after the aldolase reaction is further controlled by the two enzymes, pyruvate kinase and pyruvate dehydrogenase, as described earlier (see p. 43). A rapid phosphorylation of fructose in the liver may also indirectly influence the activities of these two enzymes as fructose 1-phosphate, a product of the phosphorylation reaction, has been reported to activate pyruvate kinase (277) and a decreased concentration of ATP would activate pyruvate dehydrogenase (275), hence, acetyl-CoA formation would be favoured on both counts.

A high-fructose diet (47), as well as addition of the ketose to perfused liver (423), both in comparison with glucose, increases the hepatic concentration of acetyl-CoA by 40-60%. This effect could be attributed to: (1), the higher rate of fructolysis as compared to glycolysis which increases the supply of pyruvate; (2), the activation of pyruvate dehydrogenase resulting from a lowering of ATP levels caused by fructolysis (97). As regards (1), however, it is interesting that studies by Tay (431) did not show increased levels of hepatic pyruvate in rats on a sucrose-enriched diet: in comparison with chow the concentration was lowered. The tissue concentration of a metabolite of fructose need not relate directly to the rate of fructolysis and the level of acetyl-CoA, however. In this connection Tay (431) showed that hepatic pyruvate and lactate levels were not necessarily directly related. With reference to (2), a two- to three-fold increase in the proportion of hepatic pyruvate dehydrogenase in the active form has been demonstrated after injection of fructose into rats (275), as well as after administering fructose to perfused livers (276).

The more marked hypertriglyceridaemic effect of fructose than of glucose may then be merely a function of the greater rate of conversion of the ketose to both acetyl-CoA and glycerol 3-phosphate in the liver. The role of insulin in these pathways offers no apparent explanation



for this difference: glucose stimulates insulin production to a much greater degree than fructose (312) and on this basis one might perhaps expect the path of carbon to triglycerides to be greater from glucose as insulin should promote the conversion of glucose to the immediate products of the aldolase-catalysed reaction more rapidly than the conversion of fructose. In reality, it must be assumed that insulin stimulation of glucose metabolism does not compensate for the rapid initial phosphorylation of fructose. A further possible contributory factor which remains to be investigated is that some product of glycolysis or fructolysis may influence the rate of conversion of acetate to long chain fatty acids and/or the esterification reactions. For example a product of fructolysis could conceivably serve as a positive effector of one of the stages in lipogenesis.

Intermediate between hepatic triglyceride synthesis and the appearance of triglyceride mainly as VLDL in the plasma is the process of secretion. Liver perfusion experiments have shown that the release of triglyceride can be directly correlated with the rate of fatty acid synthesis (308, 310). Therefore, secretion in response to a fructose load would be expected to be higher than in the case of glucose. An increase in the rate of secretion in response to fructose in liver perfusion studies has been reported (311) but no comparative studies with glucose appear to have been carried out. If the liver donors are fed sucrose-enriched diets the secretion response to the ketose in the perfusate is even greater (311). Heimberg *et al.* (302) have shown that in alloxan diabetic rats the release of triglyceride from perfused livers is inhibited. When livers from fed rats were perfused with blood containing insulin the secretion of VLDL-triglyceride was enhanced (310). Glucagon has the opposite effect to insulin. It has been suggested that glucagon acts by increasing the concentration of cyclic AMP which activates a hormone-sensitive lipase acting on the small precursor pool of VLDL-triglyceride which, in turn, results in a decrease in the output of triglyceride from the liver (303).

A further important or perhaps most important factor which regulates the level of serum triglyceride is 'clearing' which is achieved by the enzyme lipoprotein lipase (glycerol ester hydrolase; EC 3.1.1.3). This enzyme is a triglyceride lipase present in adipose and many other tissues; it requires lipoprotein-bound triglyceride as a substrate (293).

Lipoprotein lipase is located on the surface of endothelial cells of adipose tissue and skeletal and heart muscles (292). The liver enzyme is predominantly associated with the hepatocyte outer membrane (296). Lipoprotein lipase activity is barely detectable in fasting human plasma, on the other hand, activity can be demonstrated in the plasma compartment a few minutes after the injection of heparin (293, 294).

The mechanism whereby heparin releases lipoprotein lipase from its binding site is unknown. It is believed that the enzyme is present as a membrane bound complex with a glycosaminoglycan and that exogenous heparin (which is also a glycosaminoglycan) either may bring the enzyme into solution by combining with it, or may displace the enzyme from the binding site. In this connection there is some evidence that heparin and related substances have an affinity for an uncharacterized constituent of the blood vessel wall (278). In addition, Malakhova et al. (269) have demonstrated, in vitro, that lipoprotein lipase forms a ternary complex with heparin and chylomicrons and this complex is able to prevent intravascular blood coagulation.

Post-heparin released lipoprotein lipase comes mainly from two sources, adipose tissue and liver (293, 295). The two enzymes differ in their response to inhibition by protamine and concentrated NaCl solutions and activation by specific apoproteins present in chylomicrons and VLDL (281, 284). They also differ in their activity towards their substrates with the liver enzyme only having a limited capacity for hydrolysing lipoprotein-bound triglyceride (294).

Adipose tissue lipoprotein lipase activity has been reported to be markedly enhanced by the presence of HDL in the incubation medium, on the other hand, the liver enzyme is inhibited in the presence of HDL (293). In this connection it is interesting to note that HDL has been implicated as a protective factor in the aetiology of ischaemic heart disease (313).

Little is known about the interaction of triglyceride-rich lipoproteins and the lipoprotein lipase, in situ, i.e. attached to the endothelial lining of the capillary wall. There is evidence to suggest, that the hydrolysis of the triglyceride by the enzyme occurs at, or close to, the luminal surface of the capillary endothelial cells of extrahepatic tissues (where the chylomicrons and VLDL are sequestered) and that the free fatty acids released after the triglyceride hydrolysis then pass across the endothelial cells into the tissues (289).

The activity of lipoprotein lipase is generally considered to be essential for a normal rate of clearance of triglycerides from the plasma, and hence, hypertriglyceridaemia occurs in animals with a low level of this enzyme such as occurs in familial type I hyperlipoproteinaemia (281) and hypertriglyceridaemia of pregnancy in rats (287). In familial type I hyperlipoproteinaemia there is a complete absence of the extrahepatic lipoprotein lipase; the activity of the liver enzyme, however, seems to be at normal levels (281). Types III and IV hyperlipoproteinaemias are also conditions where plasma triglyceride levels are raised and, again, these diseases are believed to be caused mainly by decreased clearing rates and not increased rates of triglyceride synthesis (290). Plasma lipoprotein lipase activity has been studied in 50 patients with ischaemic heart disease within the age group 30 to 50 years. Dutt (291) found that post-heparin lipoprotein lipase activity in the serum was depressed in ischaemic heart disease cases. Here again, it seems possible that the clearance of triglyceride by lipoprotein lipase may be an important factor in the development of ischaemic heart disease.

Epinephrine, norepinephrine, adrenocorticotrophic hormone, glucagon, thyroid and thyroid-stimulating hormones have each been shown to have an inhibitory effect on adipose tissue lipoprotein lipase activity in vitro (292). Moreover, most of these effects have been shown to be inhibited by insulin (292).

Starvation of rats results in a decrease of lipoprotein lipase activity in adipose tissue, whereas insulin or glucose administration has been reported to increase the activity (288, 289, 292). Cryer and his associates (315) fed equicaloric amounts of glucose, sucrose and fructose to starved rats and correlated lipoprotein lipase levels with the resulting insulin levels. Administration of glucose significantly raised both the insulin and enzyme levels beyond the levels obtained with sucrose and fructose. Garfinkel et al. (288) have shown that the stimulation by insulin of epididymal adipose lipoprotein lipase activity in vivo is abolished by cycloheximide treatment. Stimulation of the enzyme activity by insulin has also been demonstrated in the presence and absence of cycloheximide and in this case it was assumed that there was a direct activation of the enzyme by the hormone (280).

Hence, the appearance of more triglyceride in the serum in response to a fructose load than to glucose may well, in part, be explained by the poorer insulinogenic properties of fructose which results in lower

lipoprotein lipase activities and a slower clearing rate. When the relative responses of lipoprotein lipase activity to fructose and glucose loads are considered together with the high rate of conversion of fructose to triglyceride, as compared with glucose, the result is a rational explanation for the hypertriglyceridaemic properties of fructose in comparison to glucose.

#### IV THE ROLE OF SEX HORMONES IN CARBOHYDRATE AND TRIGLYCERIDE METABOLISM

##### A. GENERAL CONSIDERATIONS

The greater hypertriglyceridaemic response to dietary fructose than to dietary glucose is well established in a number of animal species. However, in addition, a sex-dependent hypertriglyceridaemia in response to fructose or sucrose loads has also been reported (143, 154, 155, 392, 396). These two dietary carbohydrates are, in general, associated with higher serum triglycerides in male animals than in female. This association does not apparently hold for glucose- or starch-enriched diets (143, 144, 314).

These observations are of interest because the incidence of ischaemic heart disease in men is greater than in premenopausal women and, in addition, the former have higher fasting serum triglyceride levels (an ischaemic heart disease risk factor (see p. 16)) than the latter. In the case of postmenopausal women fasting serum triglyceride levels are higher than in premenopausal women (143) and the incidence of ischaemic heart disease in the older group is also significantly higher (7).

This raises the question of whether diets rich in sucrose play any part in the aetiology of ischaemic heart disease and in particular, are sucrose and fructose metabolized differently by men and postmenopausal women in comparison with women of a younger age group. If such a difference existed this might be equated with the varying serum triglyceride levels and, hence, the incidence of ischaemic heart disease. The possible role of the sex steroid hormones in the biochemistry of sucrose or fructose and triglycerides is, therefore, of interest.

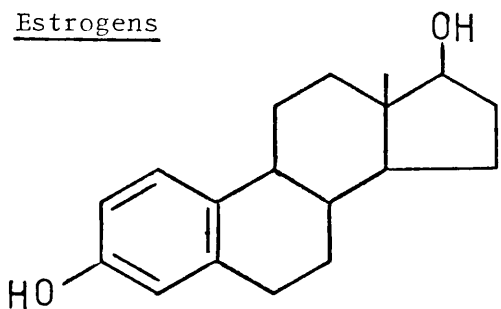
Sex steroid hormone secretion is controlled by releasing factors derived from the hypothalamus which promote the formation of gonadotrophic hormones by the anterior pituitary gland. These hormones then stimulate the gonads, the major sources of sex steroids (Fig. 6). Sex

hormones are produced in a cyclical manner in the female but at a constant rate in the male (323). Once secreted by the gonads the steroid hormones, bound to specific hormone-binding proteins, pass via the blood stream to reach their target cells. The responsive target cells contain specific hormone receptors which are membrane-bound proteins capable of binding the hormone molecule with very high specificity and affinity. In the target cells, the sex hormones, which are lipid-soluble, pass through the cell membrane to the primary receptors which are located within the cell. The binding of the hormone to its specific receptor causes the formation of an intracellular messenger molecule, which stimulates (or depresses) some characteristic biochemical activity of the target tissue and as a consequence the metabolic pathways of the cell are modified. These theories will be discussed further in section V, p. 128.

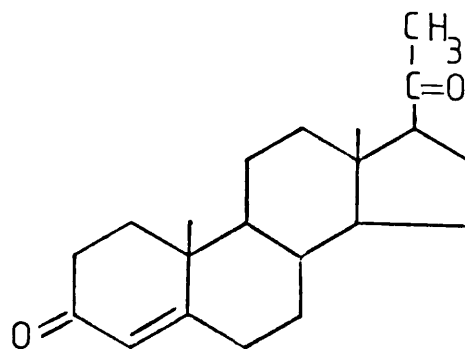
Tissues other than the primary sex organs also appear to be targets for sex steroids. Androgen uptake by peripheral organs has, for example, been investigated by Gustafsson and Pousette (368) after intraperitoneal administration of tritiated testosterone to castrated male rats. They showed that a relatively high accumulation of this steroid occurred in pancreas, adrenals, spleen, muscles, kidneys and liver in addition to the sex organs. A similar treatment of castrated male rats also resulted in appearance of a [<sup>3</sup>H]androstenedione-protein complex in the liver cytosol and this complex is believed to be involved in androgen action in the liver (367).

A cytoplasmic sex hormone receptor has been identified in male rat liver (364) and this receptor binds both androgens (5 $\alpha$ -dihydrotestosterone and testosterone) and estradiol-17 $\beta$ . At a saturating concentration of either dihydrotestosterone or estradiol the receptor binds dihydrotestosterone and estradiol in a molar ratio of 3 : 1. Estradiol inhibits the uptake of dihydrotestosterone by the receptor whereas the presence of dihydrotestosterone only weakly interferes with estradiol binding. The level of the androgen receptor activity in the liver cytosol closely follows the level of the urinary output of  $\alpha_{2u}$ -globulin, an androgen-dependent protein of hepatic origin. Roy et al. (366) have shown that the hepatic synthesis and urinary excretion of  $\alpha_{2u}$ -globulin in male rats is dependent on the production of androgens. Castration of male rats reduces the urinary excretion of  $\alpha_{2u}$ -globulin by 50% and subsequent replacement therapy with testosterone, dihydrotestosterone, androstenedione or dehydroepiandrosterone

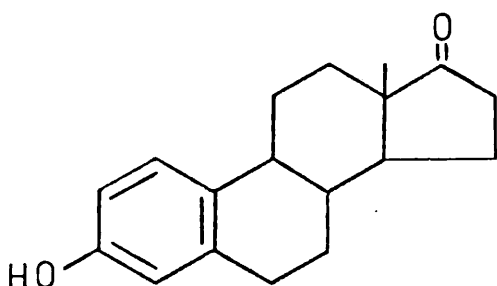
Estrogens



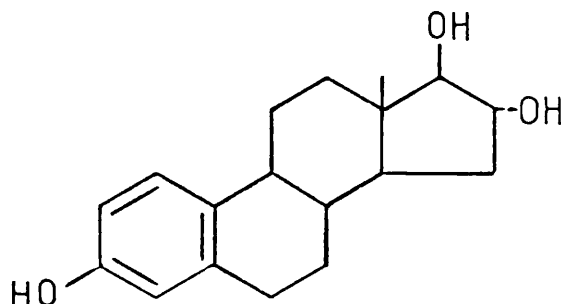
Estradiol-17β



Progesterone

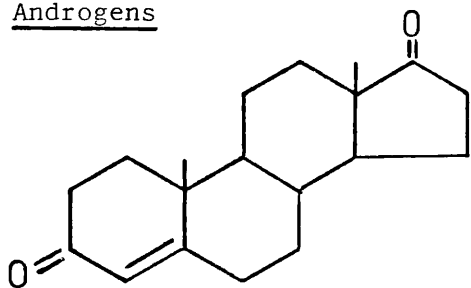


Estrone

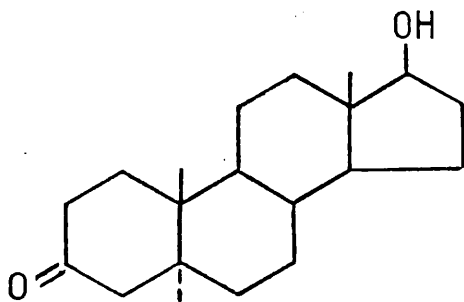


Estriol

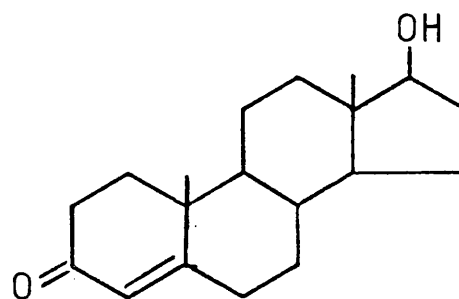
Androgens



Androstenedione



5α-Dihydrotestosterone



Testosterone

Fig. 6. The major naturally occurring mammalian sex hormones.

increases the excretion of  $\alpha_{2u}$ -globulin to normal levels (365). Immature and senile males as well as adult female rats, all of which do not produce urinary  $\alpha_{2u}$ -globulin, lack hepatic androgen receptor activity and androgen treatment of these rats does not induce  $\alpha_{2u}$ -globulin (364). However, administration of androgens to ovariectomized female rats induces the urinary  $\alpha_{2u}$ -globulin.

An estrogen receptor has also been demonstrated in the liver cytosol of adult female rats (319, 320). Similar estrogen-binding sites have been identified in the liver cytosol of adult male rats (318). The presence of these receptors in liver presumably means that sex hormones are involved in the regulation of a number of metabolic pathways in this organ.

Differences in the metabolism of male and female animals must be mediated by differences in enzyme activities and, in general, there have been few studies in this area of biochemistry. However, it is not surprising to find that there are sex differences in the levels of some enzymes which metabolize steroids (379, 394, 395). In addition, male/female differences in the levels of some transaminases (389), glutamate dehydrogenase (389, 390), malate dehydrogenase (390), aryl hydrocarbon hydroxylase (378), acetylcholine esterase (374),  $\beta$ -glucuronidase (375) and tryptophan oxygenase (376, 377) have all been reported. The latter enzyme, which is higher in female rat liver than male has been studied in detail and the effects of exogenous sex hormones both in vivo and in vitro have been investigated. Ovariectomy, adrenalectomy and testosterone propionate injections were all found to reduce the levels of tryptophan oxygenase in female rats. The injection of estradiol benzoate into intact females increased the levels after 14 days whilst similar treatment given to intact male rats raised the level of the enzyme above that of the untreated females. Castration of males had no effect on the level of tryptophan oxygenase but subsequent injections of estradiol to castrated males caused an increase in the activity of this enzyme to that of similarly treated, intact female rats. Braidman and Rose (377), however, found that several estrogenic steroids at relatively high concentrations inhibit the activity of tryptophan oxygenase in vitro by 10-25% but testosterone was without effect. The levels of hormones used were much higher than physiological levels but the authors suggested that some in vivo studies may produce similar levels.

B. EFFECT OF SEX STEROIDS ON ENZYMES OF CARBOHYDRATE AND TRIGLYCERIDE METABOLISM.

The great majority of work on the major pathways of carbohydrate metabolism in animals has been carried out with tissues from male animals and sex differences have not usually been investigated.

As regards fructose metabolism, the specific activity of hepatic ketohexokinase was originally reported to be greater (ca 100%) in adult male rats than in females (380). In addition, testosterone injections were observed to increase the activity of this kinase in livers from both sexes. Estradiol, on the other hand, decreased the level in males but increased it in females. Similar results were obtained when liver slices were incubated with these hormones (380).

C. Pollard in later work in this Department (unpublished results) was unable to confirm these findings. No sex differences in the levels of either glucokinase or hexokinase appeared in results published by Walker and his co-workers (382, 383) who examined rat livers.

Fathipour and Pridham (258) (cf Vernon and Walker (241)) confirmed that the level of glycerol kinase was higher in the livers from male than from female rats and they also showed that in male animals subcutaneous injection of testosterone stimulated the activity and estradiol decreased the level. On the other hand, testosterone administration to castrated male rats increased the activity of the enzyme beyond the level of the control animals. No sex difference was found by these authors (258) in the activities of either NAD- or NADP-dependent alcohol dehydrogenases.

The activity of glycerol 3-phosphate dehydrogenase in the kidney but not liver has also been found to be significantly greater in female rats (241). Sex differences ( $\text{♀} > \text{♂}$ ) have also been found with rat jejunal phosphofructokinase and pyruvate kinase (386) and liver pyruvate kinase (20). Oral administration of either testosterone or estradiol-17 $\beta$  increased the levels of the former two enzymes in both sexes but estradiol was more effective in the female than the male and the reverse was true for testosterone (386). These data may suggest that the response of the jejunal enzymes to sex hormones is determined in part by the sex of the animal. In this work it was noted (387) that the route of hormone administration affected the results; intramuscular injections of sex hormones produced no change in the two kinases. Testosterone and estradiol decreased the levels of jejunal fructose diphosphatase but had no effect on fructose 1,6-diphosphate



aldolase or hexokinase activity in both male and female rats (386). Lufkin et al. (388) have observed that testosterone-deficient adult human males have low activities of certain jejunal glycolytic enzymes (pyruvate kinase, fructose 1,6-diphosphate aldolase and fructose 1-phosphate aldolase). These levels can be raised by the administration of oral or intramuscular testosterone.

Inconsistent sex differences in the levels of lactate dehydrogenase, which were age dependent, were observed by Kerr and Frankel (390) who examined rat livers. These workers did, however, find a significant difference in liver coenzyme levels with female rat liver possessing a higher concentration of NADPH than male. This observation can be related to the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase which are more active in female rat liver than male (384). The activity of glucose 6-phosphate dehydrogenase was reported to be reduced following ovariectomy (385) and subsequent replacement of estradiol restored the activity to the level of the intact animals. The activity of acetyl-CoA carboxylase and fatty acid synthase has also been shown (322) to be significantly higher in the livers of female rats than in those of males. Estrogen injection raises the levels of both enzymes in female animal (403, 421).

#### C. EFFECT OF SEX STEROIDS ON PLASMA AND LIVER TRIGLYCERIDE LEVELS

In the rat, the activities of acetyl-CoA carboxylase, fatty acid synthase and the supply of NADPH should favour a more rapid production of triglyceride from carbohydrate in the female liver than in the male although analysis of liver tissues does not always show this probably because of other factors such as secretion and clearing rates and different dietary regimens.

Takemoto (160) found, for example, that with rats fed on fat-free, fructose-enriched (70%) diet for 30 days the hepatic triglyceride concentration in females was significantly increased compared with male rats. In contrast, however, Touvinen and Bender (55) have reported that feeding rats with fructose (20%) or sucrose (40%) dietary supplements for 26 weeks increases the hepatic triglyceride levels in both sexes to approximately the same degree and Hill (154) stated that liver triglyceride levels were higher in male than in female rats fed on 10% fructose for 10 days.

The effect of exogenous sex hormones on the levels of triglyceride

in liver tissues appears to be complex. Groener and Macdonald (399), for example, have shown that with female rats fed on a high fructose diet (70% for 11 weeks) subcutaneous injections of estradiol decrease the hepatic triglyceride concentration. On the other hand, progesterone administration increases the triglyceride levels in liver. Similarly, Jefferys and White (398) have shown that with male rats fed on sucrose-supplemented diet (75%), subcutaneous injections of estradiol appear to decrease the triglyceride concentration in the liver whilst progesterone has no effect. When these hormones are given together, however, the liver triglyceride levels are raised.

Liver perfusion studies, unlike dietary investigations, clearly indicate that female rat liver can produce more triglyceride than male. Watkins et al. (321) have found that in isolated perfused livers, the output of triglyceride by female rats exceeds that of male rats by a factor of 3. Ovariectomy reduced the secretion of triglyceride from livers of female rats whilst intramuscular administration of estradiol to ovariectomized animals tended to return the output to control levels. Weinstein and co-workers (400) have also demonstrated that livers from normal female rats released more triglyceride into the perfusate than did livers from male animals. Orchidectomy had no effect upon the release of triglyceride from livers of male rats whilst ovariectomy reduced the triglyceride released from the livers of female animals. Livers from female rats also synthesize and secrete more triglyceride than do livers from male animals when both organs are provided with equal quantities of oleate (402). The female rat liver takes up and esterifies fatty acids to triglyceride at a faster rate than male rat liver. There is also a slower rate of oxidation of fatty acids by the female rat liver compared to the male (402).

Further evidence that sex hormones play a role in triglyceride synthesis comes from the work of Hosotani and Yoshida (406) who have shown that the accumulation of fat in the liver, induced by a protein deficient diet, is significantly greater in female rats than in male animals. Farber (404) found that the accumulation of fat due to ethionine treatment occurred only in livers from female rats and administration of testosterone propionate to intact female animals prevented ethionine-induced fatty livers in these animals. Cycloheximide, another protein synthesis inhibitor, also caused a more pronounced accumulation of lipids in the liver of female than in male rats (405).

Sex differences in serum triglyceride levels in humans, baboons and rats associated with dietary sucrose or fructose have also been reported. Hill (154) has demonstrated that the serum triglyceride levels are higher in male than female rats and that feeding 10% fructose for 10 days raises these levels with the differential being maintained. In immature rats, the serum triglyceride levels are the same in both sexes before and after feeding fructose for 21 days. Bruckdorfer et al. (396) have shown that the concentration of serum triglyceride is higher in male than female rats fed on sucrose-enriched (68%) diets for 16 weeks. Kim and Kalkhoff (412) found that with rats fed on chow diet, injections of estrogen alone or in combination with progesterone elevated serum triglyceride levels, whereas progesterone by itself did not alter this parameter.

Coltart and Macdonald (155) showed that dietary sucrose (75%) raised the level of fasting serum triglyceride in male baboons over a 3 week period by 50% in comparison with the initial value. During the next 14 weeks on the disaccharide supplement the triglyceride concentration slowly decreased until it was about 18% higher than the initial value. Estrogen injections prevented this increase. When female baboons were given a similar diet there was no significant change in fasting serum triglycerides and testosterone injection had no effect. The incorporation of label from [U-<sup>14</sup>C]sucrose into serum triglycerides was similar in animals of both sexes prior to administration of the sucrose-enriched diet but following the diet, incorporation into triglycerides was significantly higher in the case of the male rats. Sex hormones appeared to have no significant effects on the incorporation of label, however, Coltart and Macdonald (155) inferred that the different triglyceride response between male and female animals was due to the inhibitory effect of estrogen and was independent of androgen and that the estrogen might regulate triglyceride levels by influencing triglyceride synthesis and/or clearing.

Sex differences in fasting serum triglyceride levels have also been reported in humans with men always having higher levels than women (see p.17). An increase in serum triglyceride levels in men and postmenopausal women, compared with premenopausal women, has also been demonstrated after sucrose or fructose loads (143). Macdonald (143) found that whereas diets containing sucrose or fructose raised the fasting serum triglyceride level in men, the reverse effect seemed

to occur in premenopausal women. Postmenopausal women responded to fructose-containing diets in a similar manner to men. Macdonald (392) has shown that sex differences (human and baboons) in the conversion of fructose to serum triglycerides are not due to differences in the rates of absorption of the hexose.

Although the studies on factors affecting serum triglycerides so far described all suggest that estrogen is associated with low levels there are reports that are contradictory. For example, Groener and Macdonald (399) claimed that female rats feeding on a fructose supplement had raised serum triglycerides after an estradiol injection (399). In addition, oral contraceptives are associated with raised serum triglycerides in women (24, 393).

In conclusion, it is well established that the perfused female rat liver has a greater capability for triglyceride production than the male organ and that estrogen controls the output in the female liver. However, administration of estrogen to intact female animals lowers rather than raises hepatic triglycerides unless progesterone is present. There is further controversy in the case of serum triglycerides here the level ( $\delta > \text{?}$ ) in three animal species (143, 154, 155, 396) does not reflect the increased output of fat by the female rat liver shown in the perfusion experiments. In addition the work of Coltart and Macdonald (155) shows that injected estrogen lowers serum fats in male baboons. However, there are the two reports (399, 412) that serum triglycerides in female rats are raised by injection of estrogen and that the contraceptive 'pill' has the same effect in women although here estrogen is normally accompanied by progesterone which may modify the action of estrogen (cf 398).

#### D. EFFECT OF SEX STEROIDS ON PLASMA TRIGLYCERIDE CLEARANCE

There are at present, therefore, no clear relationships between hepatic production of triglycerides and the level of these lipids in the sera of male and female animals. Although the rate of synthesis of liver fat must relate to the amount in the serum there is the second process, clearing by lipoprotein lipase, which must be considered.

Higher fasting serum triglyceride levels in men than in women (both on normal and sucrose- or fructose-supplemented diets) can be inversely correlated with reported post-heparin lipolytic specific activities (24, 411, 413). The levels of this enzyme have been shown

to decrease with age but normally the activity in men is always less than in women (411). Sex differences in the levels of lipoprotein lipase have also been reported in rats (415) and again the levels are always higher in females than in males. Nikkila and Kekki (413) studied the kinetics of triglyceride clearing and found that there was a definite difference between normal men and women. The enzyme from women exhibited a lower  $K_m$  than that from men and, hence, it was concluded that the former possessed a more efficient triglyceride clearing system which could be correlated with a lower plasma triglyceride level.

The effects of female sex hormones on the activities of lipoprotein lipase have also been investigated. In the rat, Hamosh and Hamosh (415), for example, found that intraperitoneal injections of estradiol-17 $\beta$  (50 $\mu$ g/week for 8 weeks) into male rats significantly lowered adipose tissue lipoprotein lipase activity. On the other hand, ovariectomy increased the level of the enzyme to almost twice that found in adipose tissue of control animals: subsequent administration of estradiol (25 $\mu$ g daily for 7 days) to ovariectomized females caused a marked decrease in the enzyme activity. Progesterone administration did not affect adipose tissue lipoprotein lipase activity in either male or ovariectomized female rats. Kim and Kalkhoff (412) also found that intramuscular injections of estradiol (5 $\mu$ g/day for 21 days) to female rats significantly decreased the adipose tissue lipoprotein lipase activity and, unlike Hamosh and Hamosh (415), that administration of progesterone (5mg/day for 21 days) increased the level of this enzyme in female animals. Kekki and Nikkila (24) have reported an increased triglyceride-clearing efficiency in normal women using mixed estrogen-progesterone oral contraceptives and they suggested that the progesterone component of the oral contraceptive was responsible for increasing the triglyceride clearance but that this was overbalanced by an estrogen-induced increment in the synthesis of fat resulting in elevated serum VLDL-triglyceride.

In summary, the observation that premenopausal women have a higher lipoprotein lipase activity than men (411) must be an important factor in keeping serum triglyceride levels relatively low in the former. The hormonal basis of this difference is not clear, however, since exogenous estrogen does not enhance the fractional removal rate of plasma VLDL-triglyceride (24, 414) and in experiments with rats, estrogen appears to decrease the adipose tissue lipoprotein lipase activity (415).

RESULTS AND DISCUSSION

The greater hypertriglyceridaemic response of male animals compared with females to dietary sucrose or fructose is a function of the relative rates of hepatic triglyceride synthesis and/or 'clearing'. The present investigation has been concerned mainly with the former in an attempt to establish a clearer picture of triglyceride formation from fructose in male and female rats. Reported sex differences in the activities of some of the enzymes likely to be involved in lipogenesis from fructose have been discussed (see section IV.B, p.72) and that which follows is a description of an investigation of other enzymes involved in the conversion of fructose to trioses which had not been previously investigated or had only been examined superficially with respect to sex differences. In addition, the possibility that different dietary regimens might differentially influence enzyme activities in males and females was investigated. Finally, in the case of glycerol kinase, an attempt was made to establish a link between the activity of this enzyme and sex hormones.

For this investigation male and female wistar rats with approximately the same initial body weight ( $50 \pm 5$ g) were used. In order to investigate possible effects of diet on sex differences in enzyme activity groups of 4 rats of the same sex were caged together and fed on a chow diet together with water or solutions of carbohydrates, all ad lib. (Dixon CDD[R] diet and sometimes Dixon 86 diet) with water to drink and Dixon CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water). The levels of supplementary carbohydrates used were significantly lower than in most previous studies.

The composition of the two commercial chow diets are shown in Tables XVa, b. In general, relative terms, Dixon 86 is a low-fat diet compared with Dixon 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 in the animal house was kept at  $22^{\circ}$  and the light switched on for 12hr daily (08.00 to 20.00hr). The duration of the feeding experiments was 110 days after which the rats were sacrificed by cervical dislocation, between 09.00 and 11.00hr, and the livers excised within 45 sec after death. They were then washed with ice-cold saline and dried between filter paper. Tissues were stored at  $(-70^{\circ})$  until needed for preparations of soluble supernatants for the enzyme assays.

A relatively large number of animals were used for these studies in order to be able to make a 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 a lack of suitable large scale facilities in the animal house. However, the consumption of the carbohydrate supplements by groups of animals was measured and this was shown to be similar for both sexes (7-8g/rat/day) in the case of glucose and sucrose. Unpublished results obtained by C. Pollard in this Department, where a Dixon CDD[R] diet, with water to drink, was fed ad lib. to male and female rat litter-mates for 18 days showed that the male animals consumed approximately 20% more chow than females. This intake of food closely correlates with the difference in final body weights of animals of the two sexes. Studies by Takemoto (160) indicated that for a smaller difference (8%) in consumption of a commercial diet there was a somewhat larger difference (29%) in body weights between males and females after 30 days. It should be noted, however, that Takemoto used a virtually fat-free diet unlike CDD[R].

Although it seems probable that the intake of a chow diet is roughly proportional to the increase in body weight in male and female rats the effects of glucose or sucrose supplements on the consumption of the chow diet are unknown. So although in the present work the supplement consumption was approximately the same for glucose and sucrose with animals of both sexes the two sugars may have differentially influenced the intake of chow. For example, although animals may eat isocaloric amounts of sucrose and glucose the greater sweetness of the former may result in a decreased consumption of chow (cf Allen and Leahy, 452). Mukherjee and his associates (340), however, claimed that addition of starch, glucose, fructose or sucrose supplements to a basic diet had little effect on the total daily food intake of male rats. Mukherjee et al. (340), however, only fed supplements which were 12% of the diet and the animals consumed about 1g of supplement/day (cf 7-8g/day in the present study) and the experiment was only continued for 30 days (cf 110 days in the present work). In addition, there may be a sex difference in the effect of the two carbohydrate supplements on chow consumption. Finally, it should be noted that any biochemical comparison of male and female animals under physiological conditions will always be problematical because of the normal sex differential in food consumption (430).



In view of these feeding problems it has generally not been possible to state unequivocally in this study what the specific effects of the glucose and sucrose supplements on enzyme levels have been. However, it will be clear that carbohydrate-rich diets sometimes affect the male/female sex differentials.

In order to establish whether any gross physiological changes had occurred in response to the diets the body and liver weights of the animals were monitored. This data is also needed in order to understand the full significance of changing hepatic enzyme activities in relation to the whole animal. The capacity for a particular hepatic metabolic function is dependent on the total activities of all of the liver enzymes involved. The relationship between the total activity of a liver enzyme and the body size may change because: (1), the body weight/liver weight ratio changes but not the specific activity or (2), the ratio may remain constant but the specific activity may change.

The effect of different dietary regimens on body and liver weights is shown in Table IIa. Statistical treatment (Student t-test) of the data (Table IIb) indicates that male animals always had significantly greater body and liver weights than female rats when commercial CDD[R] diet or CDD[R] diet supplemented with sucrose or glucose was used. However, these sex differences were not as evident in rats fed on Dixon 86 diet. Male rats on Dixon CDD[R] had a significantly greater body weight than males on Dixon 86. There were no statistically significant differences, between body weights, with male animals on any diet containing CDD[R]. A sucrose-supplement compared with CDD[R] alone did, however, produce significantly heavier livers in the case of males. With female animals body and liver weights were similar using both commercial diets. On the other hand, those rats on CDD[R] diet supplemented with sucrose had significantly greater liver weights than those females on CDD[R] alone or CDD[R] supplemented with glucose. The body weights of female animals on CDD[R] supplemented with glucose were significantly lower than those females on CDD[R] diet alone or CDD[R] supplemented with sucrose.

It is generally believed that body weights and food intake are, at least in part, controlled by steroid hormones. It would seem that estrogens decrease both of these factors in mature female rats (426, 427, 428). Testosterone, on the other hand, appears to have opposite effects (429, 430).

TABLE IIa. The effect of different dietary regimens on total body and liver weights of male and female rats

Diet and Sex	Body weight (g)	Liver weight (g)	$\frac{\text{Body weight}}{\text{Liver weight}}$
Dixon 86			
MALE (n=8)	331 ± 35	10.7 ± 1.8	31
FEMALE (n=8)	305 ± 40	8.4 ± 3.7	36
Dixon CDD[R]			
MALE (n=8)	418 ± 40	11.9 ± 2.4	35
FEMALE (n=8)	287 ± 29	8.8 ± 1.2	33
Dixon CDD[R] + Sucrose			
MALE (n=5)	440 ± 44.1	15.02 ± 2.1	29
FEMALE (n=5)	308 ± 29.5	10.06 ± 0.09	31
Dixon CDD[R] + Glucose			
MALE (n=5)	436 ± 8.22	13.17 ± 1.8	33
FEMALE (n=5)	250 ± 14.1	8.85 ± 0.8	28

Figures in parenthesis represent the number of animals

Values are means ± S.D. The statistical significance of the data is given in Table IIB

Rats (initial body weight 50 ± 5g) were fed for 110 days ad lib. on Dixon 86 or Dixon CDD[R] diets with water to drink and with CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water. Water and carbohydrate solutions were also available ad lib.

TABLE IIb. Statistical analysis (Student t-test) of the data in Table IIa

Group comparison	No. of Animals	Significance	
		Body weight	Liver weight
86 ♂ vs. ♀	16	NS	NS
CDD[R]♂ vs. ♀	16	P<0.01	P<0.01
CDD[R]+S♂ vs. ♀	10	P<0.01	P<0.01
CDD[R]+G♂ vs. ♀	10	P<0.01	P<0.01
86 ♂ vs. CDD[R]♂	16	P<0.01	NS
86 ♀ vs. CDD[R]♀	16	NS	NS
CDD[R]♂ vs. CDD[R]+S♂	13	NS	P<0.05
CDD[R]♂ vs. CDD[R]+G♂	13	NS	NS
CDD[R]+S♂ vs. CDD[R]+G♂	10	NS	NS
CDD[R]♀ vs. CDD[R]+S♀	13	NS	P<0.02
CDD[R]♀ vs. CDD[R]+G♀	13	P<0.02	NS
CDD[R]+S♀ vs. CDD[R]+G♀	10	P<0.01	P<0.01

Dixon 86 = 86; Dixon CDD[R] = CDD[R]; Sucrose = S

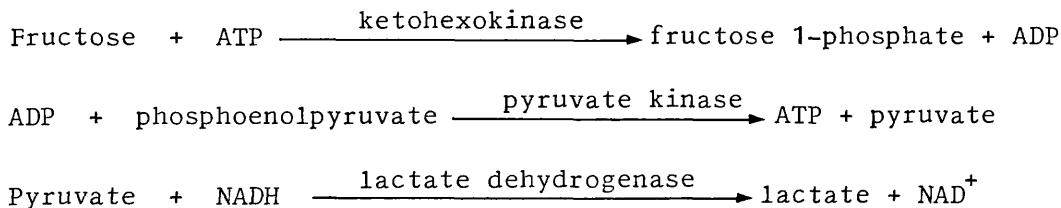
Glucose = G; Not significant = NS

In the present studies although the total intake of food by male rats probably exceeded that consumed by the females (cf 160 and the results of C. Pollard p. 80) the body weight/liver weight ratios were, in general, similar.

In Tables IIIa, b it can also be seen that the protein concentrations in all the 100,000 x g soluble supernatants from liver homogenates are similar. It would appear that CDD[R] with a glucose-supplement gives rise to higher protein contents than the chow with sucrose in both males and females but the differences are not statistically significant. Tay (431) has shown that under similar dietary regimens there is no significant change in the total liver protein content.

Ketohexokinase is the first enzyme in the pathway of fructose metabolism in the liver. Its occurrence and properties have been reviewed (p.33). In the cell, ketohexokinase is localized in the cytosol (214, 380) and 100,000 x g supernatant fractions from liver were used to measure activities in the present study.

Three basic methods have been described for the assay of ketohexokinase. In the spectrophotometric assay (226, 227) the formation of ADP is measured by coupled reactions involving pyruvate kinase and lactate dehydrogenase:



A second method which has been employed by Adelman et al. (226) is based on the conversion of [U-<sup>14</sup>C]fructose to [U-<sup>14</sup>C]fructose 1-phosphate. The product is then separated from the labelled fructose using DEAE-cellulose paper chromatography and its radioactivity measured. A third method for measuring the ketohexokinase activity is based on the estimation of the loss of fructose from reaction mixtures (432): the ketose is estimated using a resorcinol/HCl reagent (434, 435).

In this project it was first decided to try to use the spectrophotometric method of Adelman et al. (226). Several problems with this assay had been reported by these authors and these were examined in an initial study (which is not reported in detail in this thesis). One problem is that the soluble supernatants from liver contain a very

TABLE IIIa. The effect of different dietary regimens on liver protein concentrations from male and female rats

Diet and Sex	Protein concentration mg protein/g liver
Dixon 86	
MALE (n=8)	155.23 ± 25.2
FEMALE (n=8)	163.13 ± 20.67
Dixon CDD[R]	
MALE (n=8)	158.94 ± 16.59
FEMALE (n=8)	160.0 ± 15.95
Dixon CDD[R] + Sucrose	
MALE (n=5)	155.01 ± 30.03
FEMALE (n=5)	155.44 ± 20.20
Dixon CDD[R] + Glucose	
MALE (n=5)	168.47 ± 24.79
FEMALE (n=5)	166.93 ± 29.96

Figures in parenthesis represent the number of animals

Values are means ± S.D. The statistical significance of the data is given in Table IIIb

Rats (initial body weight 50 ± 5g) were fed for 110 days ad lib. on Dixon 86 or Dixon CDD[R] diets with water to drink and with CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

Water and carbohydrate solutions were also available ad lib.

TABLE IIIb. Statistical analysis (Student t-test) of the data in Table IIIa

Group Comparison	No. of Animals	Significance
86 ♂ vs. ♀	16	NS
CDD[R] ♂ vs. ♀	16	NS
CDD[R]+S ♂ vs. ♀	10	NS
CDD[R]+G ♂ vs. ♀	10	NS
86 ♂ vs. CDD[R] ♂	16	NS
86 ♀ vs. CDD[R] ♀	16	NS
CDD[R] ♂ vs. CDD[R]+S ♂	13	NS
CDD[R] ♂ vs. CDD[R]+G ♂	13	NS
CDD[R]+S ♂ vs. CDD[R]+G ♂	10	NS
CDD[R] ♀ vs. CDD[R]+S ♀	13	NS
CDD[R] ♀ vs. CDD[R]+G ♀	13	NS
CDD[R]+S ♀ vs. CDD[R]+G ♀	10	NS

Dixon 86 = 86; Dixon CDD[R] = CDD[R]; Sucrose = S

Glucose = G; Not significant = NS

active sorbitol (glucitol) dehydrogenase which reduces fructose in the presence of NADH, to sorbitol. Secondly, the addition of ATP alone to the liver preparation results in rapid ADP formation presumably due to the activity of ATPase.

It had been suggested by Adelman et al. (226) that both acid and heat treatments of the soluble supernatants could be used to completely destroy sorbitol dehydrogenase and ATPase. These workers also suggested that the effect of the dehydrogenase would be minimized if the concentration of the fructose was kept at about 1mM. The explanation for this is that the  $K_m$  of ketohexokinase is ca 0.5mM whereas for sorbitol dehydrogenase it is 100-200mM. A low fructose concentration would also prevent interference from hexokinase which also has a high  $K_m$  (2-5mM) for fructose. However, in this study, after preliminary investigations, it was felt that it would be wise to avoid the acid and heat treatments where comparisons of ketohexokinase activities in a number of samples were required. Hence, the method of Hers (432) was eventually adopted where the loss of fructose from the incubation mixtures is measured. In this assay procedure N-acetyl D-glucosamine was added to the reaction mixture: this inhibits hexokinase activity without affecting ketohexokinase (436).

The specific activities of ketohexokinase from livers of adult male and female rats were examined after feeding the various dietary regimens already described. The results are expressed in terms of mg protein and it should be noted that, for comparative purposes, this is satisfactory as there were no indications of changes in the protein contents of the livers as a result of the different diets. (Specific activities of other enzymes in this study are expressed in the same way although in Appendix I all results are given in an alternative form in terms of liver weight). The specific activity of the enzyme obtained from rats on the unsupplemented chow diet (Table IVa) are in good agreement with those obtained by Heinz (214), Heinz et al. (218), Heinz and Weiner (219), Walker (231) and Stifel et al. (193). It is also clear that supplementation of chow with carbohydrate significantly raises the specific activity of ketohexokinase. This finding is supported by the short term studies of Stifel et al. (193) and of Adelman et al. (46). Both of these groups also report that ketohexokinase activity rises more sharply in response to dietary fructose than other sugars. The results in Tables IVa, b also show clearly

TABLE IVa. The specific activities of ketohexokinase in male and female rat livers

Sex	Specific activity nmol min <sup>-1</sup> mg <sup>-1</sup> protein		
	DIXON CDD[R] DIET		
	No Supplement	Sucrose	Glucose
Males	18.0 ± 3.1 (n=8)	44.6 ± 4.0 (n=4)	41.5 ± 4.0 (n=4)
Females	18.6 ± 2.2 (n=8)	44.6 ± 1.1 (n=4)	41.8 ± 2.0 (n=4)

Values are means ± S.D. A statistical analysis of the data is given in Table IVb

Figures in parenthesis represent the number of animals examined

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



TABLE IVb. Statistical analysis (Student t-test) of the data in Table IVa

Group comparison	No. of Animals	Significance
CDD[R] ♂ vs. ♀	16	NS
CDD[R]+S ♂ vs. ♀	8	NS
CDD[R]+G ♂ vs. ♀	8	NS
CDD[R] ♂ vs. CDD[R]+S ♂	12	P < 0.01
CDD[R] ♂ vs. CDD[R]+G ♂	12	P < 0.01
CDD[R]+S ♂ vs. CDD[R]+G ♂	8	NS
CDD[R] ♀ vs. CDD[R]+S ♀	12	P < 0.01
CDD[R] ♀ vs. CDD[R]+G ♀	12	P < 0.01
CDD[R]+S ♀ vs. CDD[R]+G ♀	8	P < 0.05

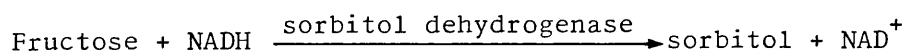
Sucrose = S; Glucose = G; Dixon CDD[R] = CDD[R]

Not significant = NS

that there is no sex difference in hepatic ketohexokinase specific activities no matter which diet is considered. This result differs from that reported by Hay and Pridham (380) who claimed that the activity was at a higher (ca 100%) level in males than in females. However, the latter workers only used animals which were maintained on Dixon 86, the high-carbohydrate, low-fat diet but C. Pollard (unpublished) who also fed rats on Dixon 86 diet (for 100 days) was unable to confirm the report by Hay and Pridham (380). This anomaly remains unexplained. As regards hepatic metabolism it is of interest to note that Tay (431) could find no difference in the concentrations of fructose 1-phosphate in male and female rat livers. This might be expected if the ketohexokinase activities were similar although the level of the ketose phosphate would also be determined by the rates of further metabolism.

The specific activity of sorbitol dehydrogenase is, interestingly, one of the highest (i.e. 18.9 U/g) of all the carbohydrate-metabolizing enzymes in rat liver. The enzyme catalyzes the interconversion of fructose and sorbitol and, in theory, under conditions of high fructose concentration, it could be involved in metabolism of the ketose in vivo. A literature search revealed no information on sex differences in the levels of the enzyme and, hence, this was investigated, again using animals on different dietary regimens.

For this project, the spectrophotometric method adopted by Gerlach and Hiby (439) for sorbitol dehydrogenase was employed where the reduction of fructose by NADH is followed spectrophotometrically at 340nm:



The results (Table Va) firstly show that the specific activity of the enzyme in male animals fed on chow is 1.4 times higher than that reported by Heinz, Lamprecht and Kirsch (218). Secondly, in the case of the unsupplemented diet the specific activity of the dehydrogenase is significantly higher in the livers of the female rats than of males. Supplementation by either glucose or sucrose appears to lower the activity in both sexes but the effect is more marked with the glucose-supplement. In addition, both carbohydrate-supplements nullify the sex differences which were observed in the case of the chow diet (see also Table Vb).

TABLE Va. The specific activities of sorbitol dehydrogenase in male and female rat livers

Sex	Specific activity nmol min <sup>-1</sup> mg <sup>-1</sup> protein		
	DIXON CDD[R] DIET		
	No Supplement	Sucrose	Glucose
Males	216 ± 25 (n=8)	184.8 ± 26 (n=5)	139 ± 14 (n=5)
Females	294 ± 34 (n=8)	215.6 ± 19 (n=5)	140 ± 13 (n=5)

Values are means ± S.D. A statistical analysis of the data is given in Table Vb.

Figures in parenthesis represent the number of animals examined.

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

TABLE Vb. Statistical analysis (Student t-test) of the data in Table Va

Group comparison	No. of Animals	Significance
CDD[R] ♂ vs. ♀	16	P < 0.01
CDD[R]+S ♂ vs. ♀	10	NS
CDD[R]+G ♂ vs. ♀	10	NS
CDD[R] ♂ vs. CDD[R]+S ♂	13	NS
CDD[R] ♂ vs. CDD[R]+G ♂	13	P < 0.01
CDD[R]+S ♂ vs. CDD[R]+G ♂	10	P < 0.01
CDD[R] ♀ vs. CDD[R]+S ♀	13	P < 0.01
CDD[R] ♀ vs. CDD[R]+G ♀	13	P < 0.01
CDD[R]+S ♀ vs. CDD[R]+G ♀	10	P < 0.01

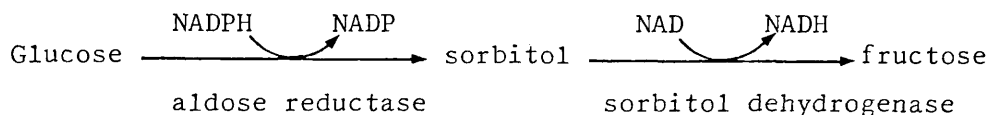
Dixon CDD[R] = CDD[R];

Sucrose = S;

Glucose = G;

Not significant = NS

The physiological significance of the presence of very high levels of sorbitol dehydrogenase in liver has not been explained. In other tissues, e.g. seminal vesicles, this enzyme appears to function together with aldose reductase (EC 1.1.1.21) to produce fructose from glucose (443).

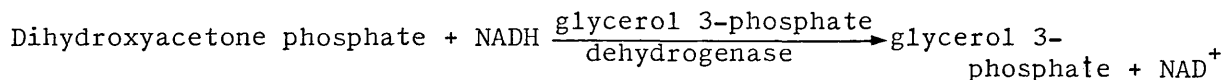
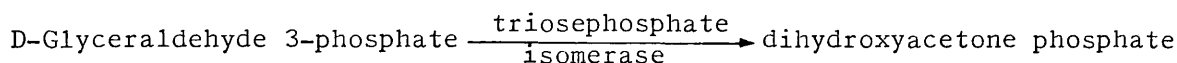
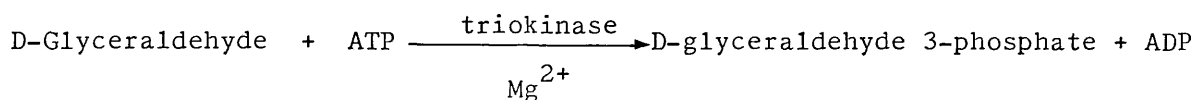


In the liver, however, there is little evidence to suggest that this complete pathway is operative in either direction and the activity of the aldose reductase in this tissue appears to be low (457). The metabolism of sorbitol by rat liver slices has been examined (437, 444) and the hexitol was shown to be converted mainly to glucose and to a lesser extent fructose. This was explained (437) by the assumption that sorbitol was first oxidized to fructose by NAD and sorbitol dehydrogenase and that the ketose product was then converted to glucose via fructose 6-phosphate and glucose 6-phosphate. (This has also been assumed to occur, in human liver (445)). This study did not, however, rule out the direct conversion of sorbitol to glucose via aldose reductase and NADP and the explanation by Blakley (437) also required fructose in the slices to have reached a relatively high concentration in order that hexokinase might react. Blakley's investigation (437) was also made prior to the discovery of ketohexokinase (433) which with a low  $K_m$  for fructose is more likely in liver slices to catalyze fructose phosphorylation (to fructose 1-phosphate) than hexokinase (p. 33).

There is little doubt, however, that sorbitol can be oxidized in the liver by sorbitol dehydrogenase to fructose (437, 444, 446) and that this is the first step in the metabolism of oral loads of the hexitol, which are given to diabetics, and of small amounts of sorbitol of endogenous origin or of dietary origin. As regards the reverse reaction, i.e. fructose to sorbitol, sorbitol dehydrogenase possesses a high  $K_m$  (100-200 mM) for fructose and, hence, it is difficult to envisage any role for this enzyme in the metabolism of fructose under normal tissue concentration of this ketose. The reasons for the very high levels of sorbitol dehydrogenase in liver and the significant sex difference in the levels therefore remain obscure.

The metabolism of D-glyceraldehyde, an intermediate (produced by aldolase) in the metabolism of fructose, may proceed via at least three pathways: one of these is the phosphorylation of the aldehyde to glyceraldehyde 3-phosphate catalyzed by the enzyme triokinase. The problem of the D-glyceraldehyde 'cross roads' has been reviewed (see p. 62).

The spectrophotometric method adopted by Heinz and Lamprecht (112) has been extensively used for measuring the specific activity of triokinase and in this present study the same method has been employed. The principle of the assay is to follow the phosphorylation of glyceraldehyde by coupled reactions with triosephosphate isomerase and glycerol 3-phosphate dehydrogenase, measuring the loss of NADH at 340nm:



The specific activities of triokinase in livers from male and female rats on different diets has been investigated and the results are shown in Table VIa. They demonstrate that sex differences in the levels of this enzyme are only evident with those animals on the high-carbohydrate, low-fat, Dixon 86 diet with males having statistically significant (Table VIb) higher levels than females.

As regards male rats on Dixon CDD[R] diet alone, the results are in a good agreement with the specific activities published by Adelman et al. (46) based on liver weight, although they appear to be somewhat lower than those quoted by Heinz et al. (218).

Comparisons of the specific activities of triokinase in rats fed on the two commercial diets, Dixon 86 and CDD[R], reveal that the levels of the enzyme are always higher in those animals on Dixon 86 and this is almost certainly due to the fact that Dixon 86 contains appreciable amounts of molasses (sucrose). This is further supported by the fact, that Dixon CDD[R] diet supplemented with

TABLE VIa. The specific activities of triokinase in male and female rat livers

Sex	Specific activity $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein			
	DIXON 86 DIET		DIXON CDD[R] DIET	
	No supplement	No supplement	Sucrose	Glucose
Males	9.69 $\pm$ 1.04 (n=10)	6.44 $\pm$ 0.6 (n=8)	11.38 $\pm$ 1.3 (n=5)	6.07 $\pm$ 1.7 (n=5)
Females	6.91 $\pm$ 0.72 (n= 9)	6.34 $\pm$ 0.3 (n=8)	10.1 $\pm$ 0.8 (n=5)	5.30 $\pm$ 1.4 (n=5)

Values are means  $\pm$  S.D. A statistical analysis of the data is given in Table VIb

Figures in parenthesis represent the number of animals examined

Rats (initial weight 50  $\pm$  5g) were fed for 110 days ad lib. on DIXON 86 or DIXON CDD[R] diets alone or CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water

TABLE VIb. Statistical analysis (Student t-test) of the data in Table VIa.

Group Comparison	No. of Animals	Significance
86 ♂ vs. ♀	19	P < 0.01
CDD[R] ♂ vs. ♀	16	NS
CDD[R]+S ♂ vs. ♀	10	NS
CDD[R]+G ♂ vs. ♀	10	NS
86 ♂ vs. CDD[R] ♂	18	P < 0.01
86 ♀ vs. CDD[R] ♀	17	P < 0.05
CDD[R] ♂ vs. CDD[R]+S ♂	13	P < 0.01
CDD[R] ♂ vs. CDD[R]+G ♂	13	NS
CDD[R]+S ♂ vs. CDD[R]+G ♂	10	P < 0.01
CDD[R] ♀ vs. CDD[R]+S ♀	13	P < 0.01
CDD[R] ♀ vs. CDD[R]+G ♀	13	NS
CDD[R]+S ♀ vs. CDD[R]+G ♀	10	P < 0.01

Dixon 86 = 86; Dixon CDD[R] = CDD[R];

Sucrose = S; Glucose = G; Not significant = NS



sucrose produces higher specific activities in both sexes than an unsupplemented diet or a diet supplemented with glucose. Adelman et al. (46) also showed that with rats initially on a normal commercial diet, the level of triokinase was decreased by fasting (48 hr) and that it could be restored back to normal levels by refeeding a fructose-, but not a glucose-rich diet for a short period (70% for 24 hr). It is interesting to note that triokinase levels are raised by dietary supplements of sucrose or fructose but not glucose and this presumably reflects the importance of the enzyme for fructose metabolism (see also Heinz, 198).

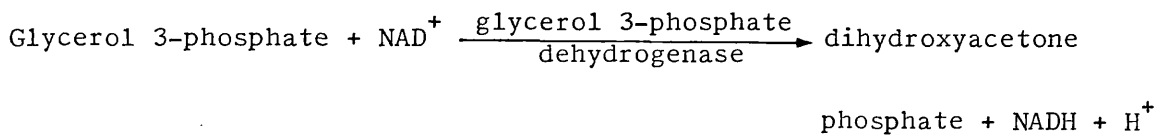
In addition to glyceraldehyde, dihydroxyacetone phosphate is also produced in the liver by the joint action of ketohexokinase and aldolase on fructose. This triose phosphate may: (1), be converted to glyceraldehyde 3-phosphate and enter the glycolytic pathway which eventually leads to acetate and (2), serve as an important source of triglyceride-glycerol. As regards the latter, dihydroxyacetone phosphate carbon can enter triglyceride either via the dihydroxyacetone phosphate pathway or via the glycerol 3-phosphate pathway: the glycerol 3-phosphate pathway is believed to be the more important in liver (p. 53).

The reduction of dihydroxyacetone phosphate to glycerol 3-phosphate can, in vitro, be catalyzed by two different enzymes. One is the cytosolic NAD-linked glycerol 3-phosphate dehydrogenase, and the other the mitochondrial enzyme which utilizes FAD as a hydrogen carrier. The two enzymes, acting in concert, are thought to provide one of the mechanisms in the cell for the reoxidation of extramitochondrially-generated NADH via the respiratory chain with the cytosolic dehydrogenase reducing dihydroxyacetone phosphate to glycerol 3-phosphate and the mitochondrial enzyme catalyzing the reverse reaction (239).

At physiological pH values the equilibrium for the cytosolic enzyme-catalyzed reaction greatly favours glycerol 3-phosphate formation and it is difficult to demonstrate the reverse reaction in vitro without raising the pH to a strongly alkaline condition and 'trapping out' the dihydroxyacetone phosphate product with hydrazine.

As glycerol 3-phosphate dehydrogenase is important for the provision of glycerol for the synthesis of hepatic triglycerides, it was decided to examine rat liver for sex differences in the levels of the enzyme.

Glycerol 3-phosphate dehydrogenase activity has been assayed spectrophotometrically by measuring the rate of formation or disappearance of NADH at 340nm which accompanies the oxidation of glycerol 3-phosphate at pH 10 or the reduction of dihydroxyacetone phosphate at pH 7.5, respectively (265). In the present study the spectrophotometric method reported by Lee and Craine (265) was adopted using glycerol 3-phosphate as substrate:



The specific activity (see Table VIIa) of the hepatic enzyme from male animals on chow (CDD[R]) diet agrees with the results of Baquer et al. (448). It was stressed earlier (p. 81) that it would be difficult to define exactly the effects of the different diets on enzyme activities. However, it would appear that the two basic chow diets had affected the dehydrogenase activities in both male and female rats quite differently with Dixon CDD[R] being associated with a significantly higher specific activity (Table VIIb). With the exception of male animals ingesting CDD[R] with a glucose supplement, this was also apparent with all the CDD[R]-containing diets. This result may be a function of the higher fat content of the CDD[R] diet as compared with the 86. In this connection Harding et al. (449), using chicken, have demonstrated that the specific activity of hepatic glycerol 3-phosphate dehydrogenase is decreased by a high-carbohydrate, low-fat diet and increased with a high-fat, low-carbohydrate regimen. The low specific activities obtained with the Dixon 86 diet were probably not a function of either the relatively high total carbohydrate content of this diet or the high sucrose content otherwise low activities would have been obtained with the CDD[R] supplemented diets. In other studies (266, 450) it has been claimed that in the short term, both glucose- and fructose-enriched diets increase hepatic glycerol 3-phosphate dehydrogenase in male rats (but not female; see De Gomez et al. (451)) and Mukherjee and co-workers (340) state that dietary sucrose or fructose is more effective than glucose in this respect.

TABLE VIIa. The specific activities of sn-glycerol 3-phosphate dehydrogenase in male and female rat livers

Sex	Specific activity nmol min <sup>-1</sup> mg <sup>-1</sup> protein			
	DIXON 86 DIET		DIXON CDD[R] DIET	
	No supplement	No supplement	Sucrose	Glucose
Males	82.4 ± 10.3 (n=8)	143.2 ± 19.7 (n=8)	170.0 ± 24.8 (n=5)	89.9 ± 16.7 (n=5)
Females	91.1 ± 10.4 (n=8)	170.8 ± 24.8 (n=8)	175.5 ± 20.8 (n=5)	127.4 ± 22.1 (n=5)

Values are means ± S.D. A statistical analysis of the data is given in Table VIIb

Figures in parenthesis represent the number of animals examined

Rats (initial weight 50 ± 5g) were fed for 110 days ad lib. on DIXON 86 or DIXON CDD[R] diets alone or CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water

TABLE VIIb. Statistical analysis (Student t-test) of the data in Table VIIa

Group Comparison	No. of Animals	Significance
86 ♂ vs. ♀	16	NS
CDD[R] ♂ vs. ♀	16	P < 0.05
CDD[R]+S ♂ vs. ♀	10	NS
CDD[R]+G ♂ vs. ♀	10	P < 0.02
86 ♂ vs. CDD[R] ♂	16	P < 0.01
86 ♀ vs. CDD[R] ♀	16	P < 0.01
CDD[R] ♂ vs. CDD[R]+S ♂	13	NS
CDD[R] ♂ vs. CDD[R]+G ♂	13	P < 0.01
CDD[R]+S ♂ vs. CDD[R]+G ♂	10	P < 0.01
CDD[R] ♀ vs. CDD[R]+S ♀	13	NS
CDD[R] ♀ vs. CDD[R]+G ♀	13	P < 0.01
CDD[R]+S ♀ vs. CDD[R]+G ♀	10	P < 0.01

Dixon 86 = 86;

Dixon CDD[R] = CDD[R];

Sucrose = S;

Glucose = G;

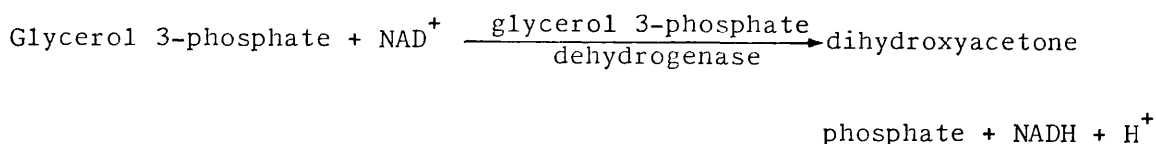
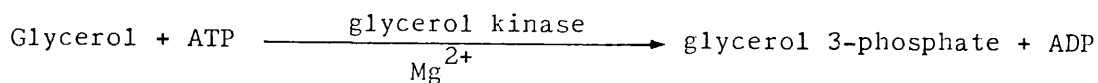
Not significant = NS

In the present long term study, it is also clear that the relationship between sucrose and glucose in both male and female rats is similar to that reported by Mukherjee et al. (340) i.e. dietary sucrose raises the dehydrogenase activity more than dietary glucose. It is also of interest that a sex difference in the levels of glycerol 3-phosphate dehydrogenase exists in the case of animals ingesting CDD[R] and CDD[R] and glucose supplement ( $\text{♀} > \text{♂}$ ) but that this does not occur with rats taking a sucrose-enriched diet. It should also be noted that there is no sex difference with the rats on Dixon 86 which is rich in molasses. Vernon and Walker (241) observed no sex differences in dehydrogenase activities with rats feeding on a commercial pelleted diet but in female kidney tissue the activity was significantly higher than in the corresponding male tissue.

Although there is some controversy over the further metabolism of glyceraldehyde (pp. 49, 50) the consensus of opinion is that it is first phosphorylated by triokinase. It should be noted, however, that this theory is derived from metabolic studies on the conversion of the aldehyde to glucose and the possibility still exists that it is, instead, reduced to glycerol or oxidized to glycerate (111). There appears to be no clear evidence that one or both of these reactions is not involved to some degree in triglyceride synthesis. In the former case the resulting glycerol could readily be converted to glycerol 3-phosphate by glycerol kinase and this metabolite is the major precursor of triglyceride-glycerol. Glycerol of dietary origin or produced by lipolysis, is also metabolized in the same way.

Two main methods have been used for measuring glycerol kinase activity in soluble fractions from rat liver. The first procedure, is the radiochemical method described by Newsholme et al. (458), which is based on the conversion of [ $^{14}\text{C}$ ]glycerol to [ $^{14}\text{C}$ ]glycerol 3-phosphate and adsorption of the latter on DEAE-cellulose paper discs. The radioactivity adhering to the paper is then measured in a liquid scintillation counter. A second method for measuring glycerol kinase activity was published by Bublitz and Wieland (459) and in this assay the glycerol kinase reaction is coupled with the glycerol 3-phosphate dehydrogenase reaction in the presence of hydrazine to 'trap out' dihydroxyacetone phosphate. The formation of NADH during the oxidation of glycerol 3-phosphate is then measured

spectrophotometrically at 340nm.



In the present investigation, glycerol kinase was assayed by the latter method (459) which is more rapid and convenient than the procedure of Newsholme and co-workers (458).

The specific activities of glycerol kinase in livers from male and female rats on different diets are shown in Table VIIIa. As regards male rats on Dixon CDD[R] diet alone, the results are in a good agreement with the specific activities published by Heinz (214) and Heinz, Lamprecht and Kirsch (218) but lower by a factor of three than those published by Vernon and Walker (241). The results also demonstrate clearly that male livers have a higher activity than female livers with rats on Dixon 86 or Dixon CDD[R] diets alone or CDD[R] diet supplemented with sucrose but, interestingly, this sex difference is not evident in the case of animals ingesting the glucose supplement. This latter phenomenon was also observed with glycerol 3-phosphate dehydrogenase (Table VIIa.) and sorbitol dehydrogenase (Table Va). Similar sex differences in the specific activities of hepatic glycerol kinase with rats on normal chow diets have also been reported by Fathipour and Pridham (258) and Vernon and Walker (241) but no attempt to examine the effect of diet was made by these workers. Unlike the liver, the level of glycerol kinase activity in female rat kidney is higher than in the male (241).

Comparisons of the specific activities of hepatic glycerol kinase (Table VIIIb) in rats fed on the two commercial diets, Dixon 86 and CDD[R], reveal that the levels of the enzyme, in both sexes, are always higher in those animals on Dixon CDD[R] (the high-fat, low-carbohydrate diet) with or without carbohydrate supplements, and, as with glycerol 3-phosphate dehydrogenase, this is probably due to the fact that Dixon CDD[R] contains a higher fat content than 86. This result is supported by observations made by Kida and his associates (257), using rats, and Harding *et al.* (449),

TABLE VIIIa. The specific activities of glycerol kinase in male and female rat livers

Sex	Specific activity nmol min <sup>-1</sup> mg <sup>-1</sup> protein			
	DIXON 86 DIET		DIXON CDD[R] DIET	
	No supplement	No supplement	Sucrose	Glucose
Males	5.56 ± 2.2 (n=8)	16.2 ± 1.4 (n=8)	17.04 ± 2.4 (n=5)	14.6 ± 2.6 (n=5)
Females	2.71 ± 1.1 (n=8)	11.4 ± 1.5 (n=8)	9.4 ± 4.5 (n=5)	13.6 ± 1.1 (n=5)

Values are means ± S.D. A statistical analysis of the data is given in Table VIIIb

Figures in parenthesis represent the number of animals examined

Rats (initial weight 50 ± 5g) were fed for 110 days ad lib. on DIXON 86 or DIXON CDD[R] diets alone or CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water

TABLE VIIIb. Statistical analysis (Student t-test) of the data in Table VIIa

Group Comparison	No. of Animals	Significance
86 ♂ vs. ♀	16	P < 0.01
CDD[R] ♂ vs. ♀	16	P < 0.01
CDD[R]+S ♂ vs. ♀	10	P < 0.01
CDD[R]+G ♂ vs. ♀	10	NS
86 ♂ vs. CDD[R] ♂	16	P < 0.01
86 ♀ vs. CDD[R] ♀	16	P < 0.01
CDD[R] ♂ vs. CDD[R]+S ♂	13	NS
CDD[R] ♂ vs. CDD[R]+G ♂	13	NS
CDD[R]+S ♂ vs. CDD[R]+G ♂	10	NS
CDD[R] ♀ vs. CDD[R]+S ♀	13	NS
CDD[R] ♀ vs. CDD[R]+G ♀	13	P < 0.02
CDD[R]+S ♀ vs. CDD[R]+G ♀	10	NS

Dixon 86 = 86;      Dixon CDD[R];      Sucrose = S;

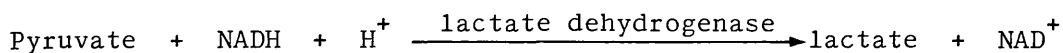
Glucose = G;      Not significant = NS



with chickens. Both of these groups found that hepatic glycerol kinase activities were increased by feeding high-fat, low-carbohydrate diets and decreased by low-fat, high-carbohydrate diets. Again, as with glycerol 3-phosphate dehydrogenase, the low specific activities obtained with the Dixon 86 diet were probably not a function of either the relatively high total carbohydrate content of this diet or the high sucrose content otherwise low activities should have been obtained with the CDD[R] supplemented diets.

With a view to using hepatocytes to investigate the effects, in vitro, of sex hormones on glycerol kinase activity, lactate dehydrogenase was examined as a marker enzyme for hepatocyte viability. This enzyme was of further interest as a possible 'control enzyme' without male/female differences in activity, which could be assayed easily, and which could be studied in parallel to glycerol kinase. Initially, a difference in the level of the dehydrogenase was considered to be a possibility because of reports by Kerr and Frankel (see p. 73; (390)) and Tay (431) that the concentration of lactate in male rat liver was approximately 60% higher than that present in female tissues when the animals were ingesting a sucrose-enriched diet. There was no difference in the case of rats on an unsupplemented chow (Dixon CDD[R]) diet, however.

In this present study lactate dehydrogenase was assayed spectrophotometrically by the method of Bergmeyer and Bernt (460) which is based on the reduction of pyruvate by NADH which is monitored at 340nm:



The results in Tables IXa, b, show that there was no significant difference in the specific activities of the dehydrogenase from male or female rat livers no matter which diet the animals were ingesting. This result does not help to explain the sex difference in hepatic lactate concentration reported by Kerr and Frankel (390) and Tay (431): the answer to this problem may perhaps lie in differences in the rate of lactate excretion and/or the rate of pyruvate production.

The enzyme was, therefore, considered to be suitable for use as a 'control' in an in vitro investigation of the effects of steroid hormones on glycerol kinase activity.

TABLE IXa. The specific activities of lactate dehydrogenase in male and female rat livers

Sex	Specific activity nmol min <sup>-1</sup> mg <sup>-1</sup> protein		
	DIXON CDD[R] DIET		
	No Supplement	Sucrose	Glucose
Males	3206 ± 367 (n=8)	3205 ± 347 (n=5)	3281 ± 298 (n=5)
Females	3461 ± 993 (n=8)	2957 ± 124 (n=5)	3001 ± 207 (n=5)

Values are means ± S.D. A statistical analysis of the data is given in Table IXb

Figures in parenthesis represent the number of animals examined

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

TABLE IXb. Statistical analysis (Student t-test) of the data in Table IXa

Group Comparison	No. of Animals	Significance
CDD[R] ♂ vs. ♀	16	NS
CDD[R]+S ♂ vs. ♀	10	NS
CDD[R]+G ♂ vs. ♀	10	NS
CDD[R] ♂ vs. CDD[R]+S ♂	13	NS
CDD[R] ♂ vs. CDD[R]+G ♂	13	NS
CDD[R]+S ♂ vs. CDD[R]+G ♂	10	NS
CDD[R] ♀ vs. CDD[R]+S ♀	13	NS
CDD[R] ♀ vs. CDD[R]+G ♀	13	NS
CDD[R]+S ♀ vs. CDD[R]+G ♀	10	NS

Dixon CDD[R] = CDD[R];

Sucrose = S;

Glucose = G;

Not significant = NS

It had previously been reported by Vernon and Walker (241) and Fathipour and Pridham (258) that the specific activity of hepatic glycerol kinase was higher in male than in female rats: Vernon and Walker (241) also found no differences between the sexes in the case of immature rats. Fathipour and Pridham (258) examined the effect of sex hormones on the activity of glycerol kinase in rat liver both in vivo and in vitro. They found that testosterone injection had little effect on the activity of the enzyme in normal male animals whilst a similar treatment of castrated male rats resulted in a significant increase in the kinase activity. The hormone was administered in relatively high concentrations (0.5 - 5.0 mg/kg body weight) in corn oil although injections were subcutaneous and, therefore, the plasma level of the steroid may not have been far beyond the physiological range. In, in vitro, studies using liver slices Fathipour and Pridham (258) also found that testosterone had an activating effect on the enzyme in slices from female rats and from both normal and castrated male animals.

In the present study an attempt was made to elucidate the mechanism of androgen activation of hepatic glycerol kinase using isolated hepatocytes from male and female rats as an in vitro system. The rats used for this work were fed on unsupplemented Dixon CDD[R] diet with water to drink ad lib.

Over the past few years, suspensions of isolated hepatocytes have become generally accepted as an important system for studying hepatic metabolism and its control and hepatocyte preparations are now normally used instead of liver slices because experimental condition can be carefully controlled and ischaemia prevented. Liver slices consist mainly of, apparently, intact cells but they serve as poor material for in vitro studies because essential elements of liver metabolism become greatly impaired during the slicing procedure. Thus the capacity for gluconeogenesis from lactate and amino acids, for oxidizing fatty acids to ketone bodies and for maintaining normal concentrations of the adenine nucleotides is largely lost. Hepatocytes, however, are comparable to perfused liver in a variety of biochemical parameters including gluconeogenesis and protein synthesis (461).

There were many attempts during the past 40 years to produce viable, isolated liver cells (462). Early methods depended on the

use of mechanical force to dissociate the cells from the liver (463). Later, various chelating agents (citrate, tetraphenyl boron and EDTA) in conjunction with mechanical disruption were used to separate the cells (464), inevitably resulting in damage to cell membranes.

Shortly after 1964 when Rodbell (465) had demonstrated that adipocytes could be isolated by incubating fat tissue with collagenase, Howard et al. (466) described a similar method for isolating liver cells by incubating liver slices with collagenase and hyaluronidase. These enzymically prepared cells appeared to be morphologically intact and metabolically active. In 1969, Berry and Friend (467) reported that Howard's method could be modified by perfusing the liver with collagenase and hyaluronidase in situ in a recirculating liver perfusion apparatus. This modification greatly increased cell yields and improved the integrity of the isolated liver cells. In 1973, Krebs and co-workers (468) described a number of modifications of the original procedure of Berry and Friend (467). The most recent procedure, that of Wagle and Ingebretsen (469), exposes the liver to low concentrations of collagenase (10-20 mg/100ml) and for only a short period of time (10-15 min).

In the present study, the method adopted for the isolation of hepatocytes was that which was originally devised by Berry and Friend (467) and later modified by Krebs et al. (468). In this procedure the perfusate used is Krebs-Ringer bicarbonate solution initially without  $\text{Ca}^{2+}$  ions. In the later stage of perfusion  $\text{Ca}^{2+}$  ions have to be added to activate the collagenase. No hyaluronidase is used with this technique.

The biochemical properties of hepatocytes do vary with different batches of cells and it is necessary to be able to use preparations with similar viabilities in order to compare the results of experiments carried out with different preparations.

During the isolation of hepatocytes, the cells can be damaged by removal of  $\text{Ca}^{2+}$ , by the action of lytic enzymes and by mechanical stress. Thus suitable criteria for estimating their viability have to be considered: for example, fine structure, permeability of the plasma membrane, permeability to substances of high molecular weight, ability to respire, the adenine nucleotide content and the ability to synthesize glucose from lactate have all been used.

The trypan blue exclusion test is widely used as an index of cell viability. Some workers claim viabilities of 90-100% for hepatocyte preparations in the presence of albumin using this dye as the only measure of cell viability (469). Baur et al. (54) have demonstrated clearly that reversible, pH-dependent staining of cells by the dye can occur with maximum penetration of the hepatocytes at low pH values.

Bovine serum albumin is commonly added to the medium to aid cell suspension and to prevent clumping which results in the loss of metabolic activity (468) and it has been shown by Tay (431) that the viability of hepatocytes, as tested by the ability to exclude trypan blue, can be changed by the presence of this protein. Albumin decreases the number of cells stained by the dye probably because trypan blue binds to albumin preferentially (467). In addition, some investigators believe that cells with stained nuclei but not cytosol are still viable and consider the cells to be 'dead' only when both nuclei and cytosol are stained (9). Some controversy, therefore, exists about the definition of the term 'viability' with respect to hepatocytes and the use of the trypan blue exclusion test. In spite of this, most authors have confined themselves to the use of the test as a measure of viability.

In this present study it was of importance to be able to define the viability of cell preparations because of both the need to compare results obtained with hepatocytes from animals of both sexes and to determine whether sex hormones affected cell viability. Hence, initially, a number of methods for determining hepatocyte viability were compared using preparations with similar cell concentrations (ca  $8-10 \times 10^6$  cells/ml) and containing bovine serum albumin and  $Ca^{2+}$  ions. In all cases cells were gassed continuously with an  $O_2/CO_2$  mixture and incubated in a shaking water bath at  $37^\circ$ .

The leakage of  $K^+$  from hepatocytes into the medium can be used as a measure of the integrity of the cells (467, 468, 469) and likewise the leakage of lactate dehydrogenase (9, 54, 467, 468, 469).

The results of a comparison of these methods together with the trypan blue exclusion test is given in Fig. 7. Cells from both male and female rats were suspended in Krebs-Ringer bicarbonate

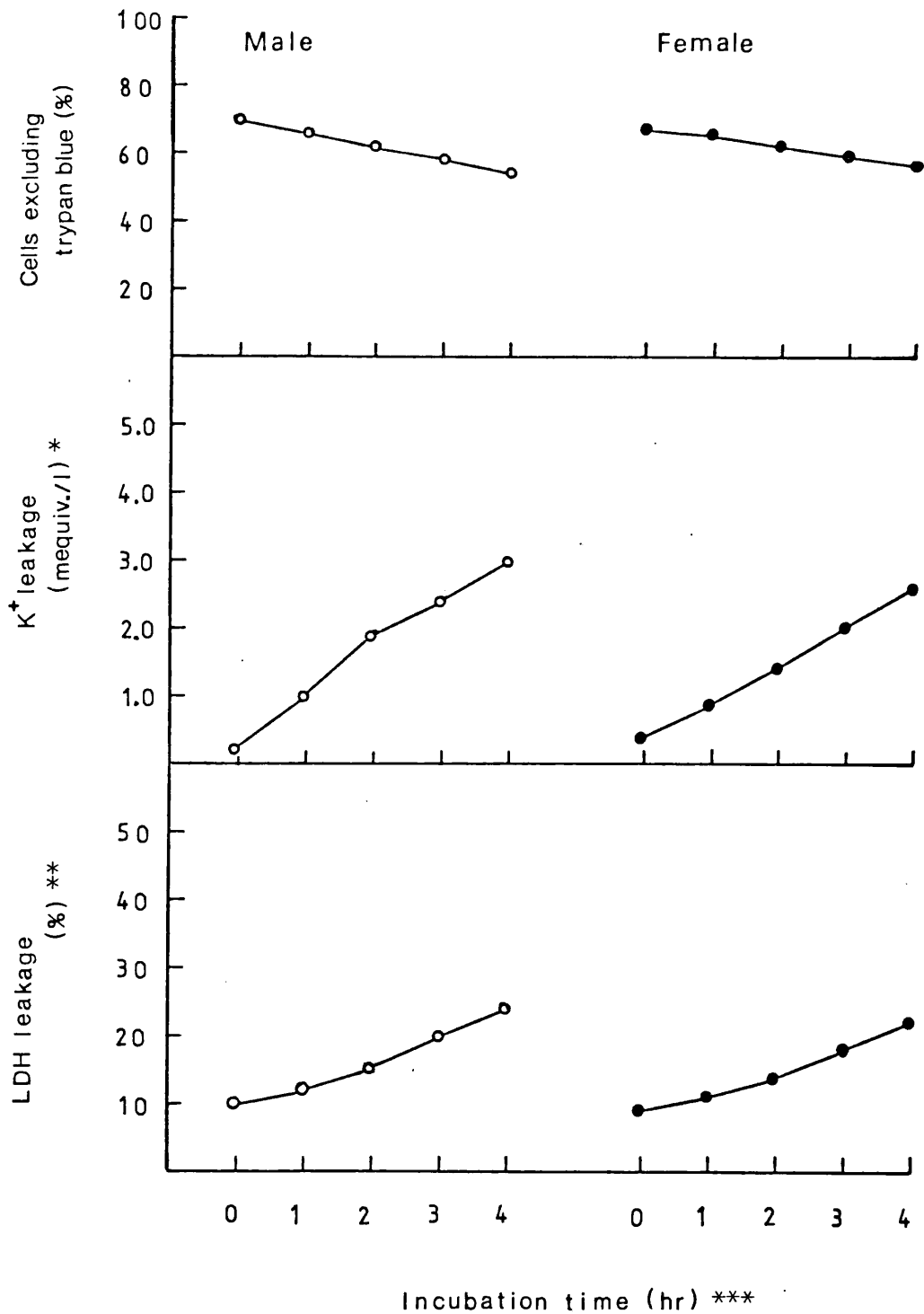


Fig. 7.

A comparison of different viability tests using hepatocytes from male and female rats.

\* K<sup>+</sup> leakage is corrected for K<sup>+</sup> ions initially present in the incubation medium.

\*\* LDH leakage is given as the activity in the medium expressed as a % of the total activity in the medium plus the cells.

\*\*\* Cells were preincubated for 1hr at 37<sup>o</sup> prior to the tests.

medium, incubated for varying times and then examined. The graphs show that cells from male and female rat livers can be prepared with similar viabilities and loss of viability in both cases occurs at the same rate when examined by any one of the three procedures. The decrease in the unstained 'viable' cells (from ca 70% to 55%) over a 4hr incubation period is accompanied by leakage of both  $K^+$  ions and lactate dehydrogenase into the medium. The rate of loss of enzyme from the cells is relatively low which suggests that in both preparations cell membranes are not grossly damaged. The total  $K^+$  ions lost from the hepatocytes during 4hr was not measured in this study but an approximate value of only 4% is probable if the data on the  $K^+$  content of hepatocyte preparations given by Krebs and his associates (468) is used as a basis for the calculation.

As a further test of the integrity of the hepatocyte preparations used in this study the rate of gluconeogenesis from L-[U- $^{14}C$ ] lactate was also examined using the procedure described by Claus et al. (69) and isolating the products by the method of Exton and Park (70).

The results shown in Fig. 8 demonstrate that the rate of gluconeogenesis from lactate by the isolated liver cells from male rats is linear with time for up to 60 min. The rate of 10 $\mu$ mol glucose/g hepatocyte/hr obtained with 2mM lactate in this study, can be equated with the values reported by several other groups of workers (69, 431, 468, 469), bearing in mind that the rate has been shown to be linear with lactate concentrations up to 10mM (431, 469). It can also be seen in Fig. 8 that glucagon (10nM) stimulates the gluconeogenic rate by approximately 30% over a period of 1 hr (cf 69, 71, 73, 87).

It is clear, therefore, from all the data that the technique used for hepatocyte isolation produced high quality cells as judged by the apparent low degree of membrane disorganisation and the existence of an intact gluconeogenesis pathway under hormonal control.

The viability tests were extended to examine whether the addition of sex steroid hormones (5 $\alpha$ -dihydrotestosterone and estradiol-17 $\beta$ ) to isolated male and female cells would affect cell viability after an incubation period of 3hr at 37 $^{\circ}$ . From the results in Fig. 9 and 10 where trypan blue exclusion and  $K^+$  and lactate dehydrogenase leakage tests have been carried out, it appears that



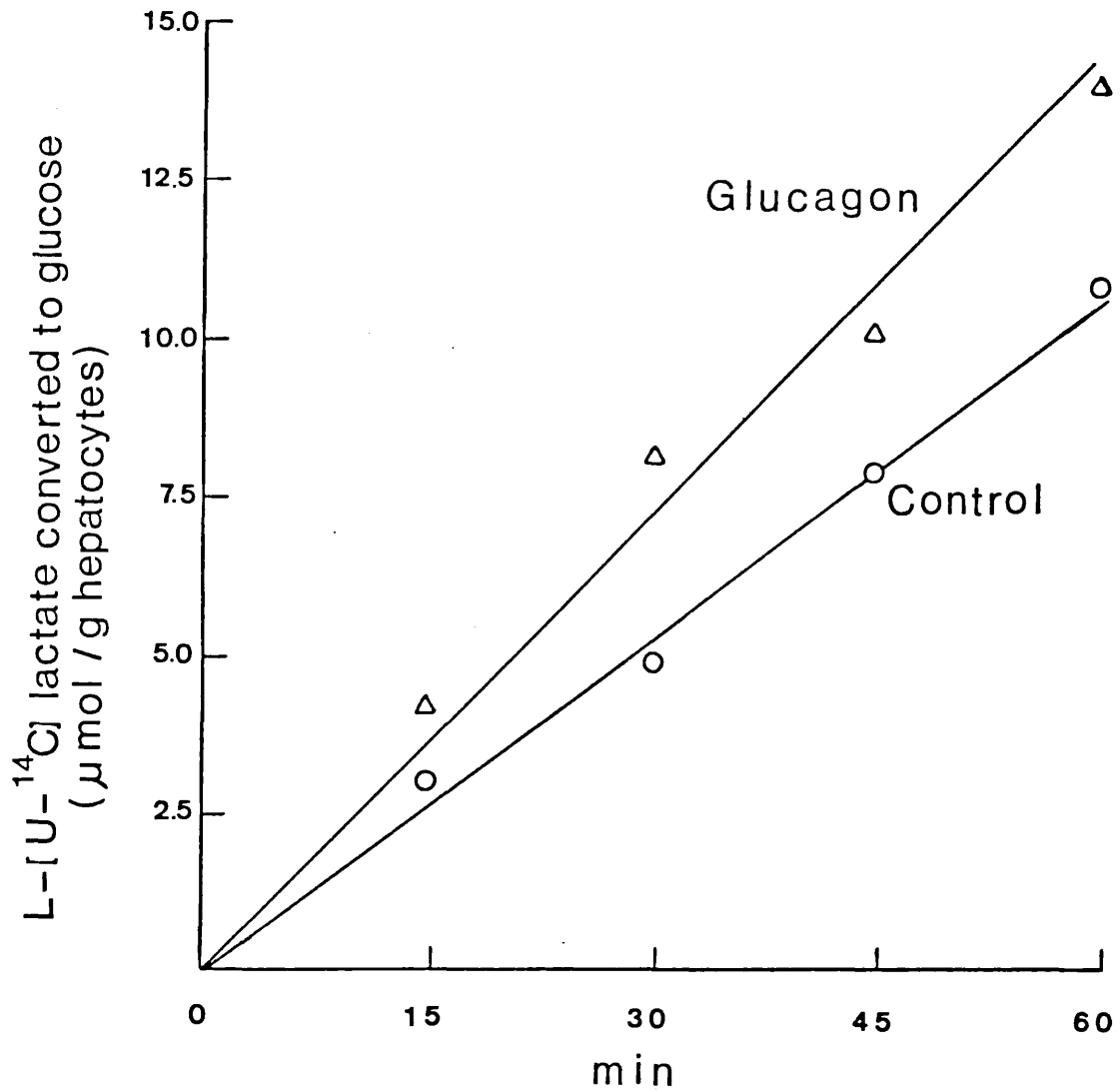


Fig. 8. The rate of gluconeogenesis from L-[U-<sup>14</sup>C]lactate

Isolated hepatocytes ( $3.2 \times 10^6$  cells/ml) from fed male rats were preincubated for 1hr without substrate and then with L-[U-<sup>14</sup>C]lactate (2mM) for various times. Glucagon (10nM) was added at zero time. Experimental details are described in Section VI.B.5.b. (p. 147)

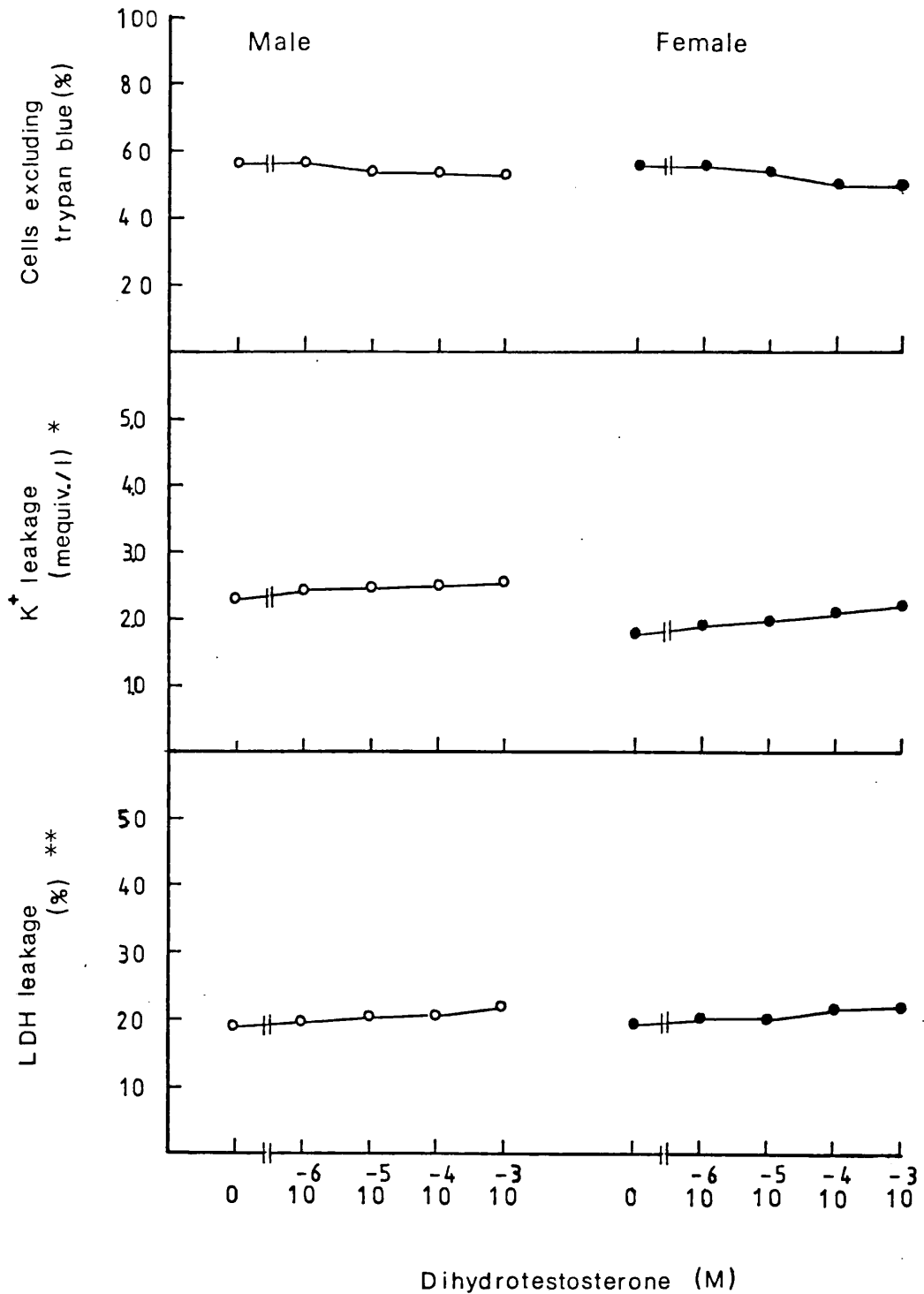


Fig. 9. The effect on viability of incubating hepatocytes from male and female rats with dihydrotestosterone for 3hr at 37<sup>o</sup>.

Cells were preincubated for 1hr at 37<sup>o</sup> prior to the tests.

\* K<sup>+</sup> leakage is corrected for K<sup>+</sup> ions initially present in the incubation medium.

\*\* LDH leakage is given as the activity in the medium expressed as a % of the total activity in the medium plus the cells.

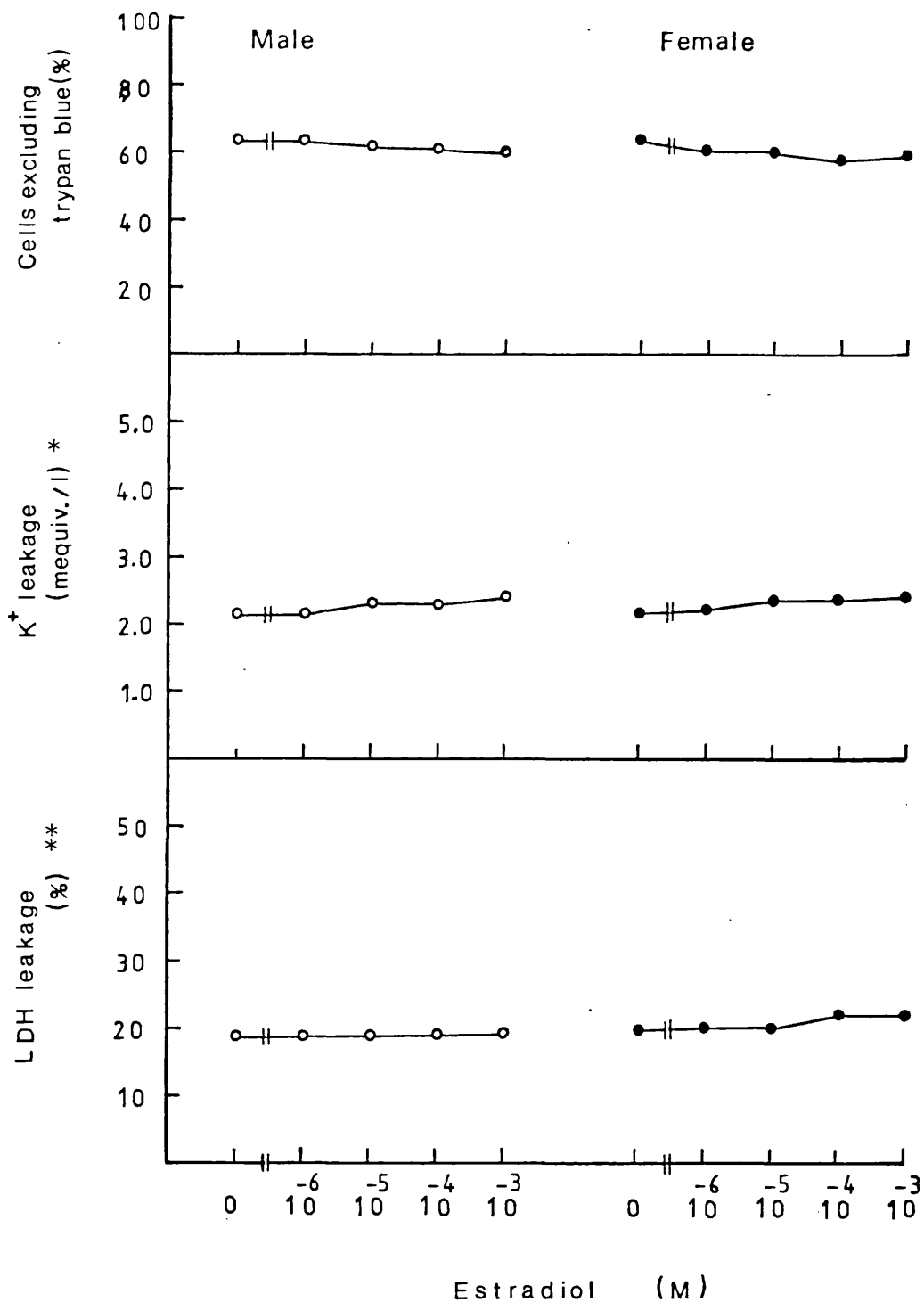


Fig. 10.

The effect on viability of incubating hepatocytes from male and female rats with estradiol for 3hr at 37°.

Cells were preincubated for 1hr at 37° prior to the tests.

\* K<sup>+</sup> leakage is corrected for K<sup>+</sup> ions initially present in the incubation medium.

\*\* LDH leakage is given as the activity in the medium expressed as a % of the total activity in the medium plus the cells.

addition of either dihydrotestosterone or estradiol at concentrations up to  $10^{-3}$  M and incubation for 3hr, has no apparent effect on cell viability according to the three measured parameters.

The specific activities of glycerol kinase were examined in hepatocytes prepared from adult male and female rats to ensure that the sex difference found with soluble preparations from livers was reflected by the cell preparations. The levels of lactate dehydrogenase in hepatocytes from both sexes were also assayed. The specific activity values for both enzymes were expressed as  $\mu\text{mol min}^{-1} \text{g}^{-1}$  liver assuming that approximately  $1.38 \times 10^8$  cells were equivalent to 1g wet liver (468): the values represent the total activity in the cells plus the medium.

In Table X it can be seen that the specific activity of glycerol kinase was significantly higher (22%) in hepatocyte preparations from male than from female rats and that castration produced a marked decrease in activity (30%), compared with intact animals, which exceeded that reported by Fathipour and Pridham (13%) (258) who measured glycerol kinase activity in soluble supernatants from livers of intact and orchidectomized rats. The observed difference in activities in Table X are probably not due to differences in viabilities of the cells as in all preparations the viability range was 80-90% as shown by the trypan blue exclusion test.

The results in Table XI also show (as in the case of the soluble liver preparations (Table IXa)) that there is no significant sex difference in lactate dehydrogenase activity in the hepatocytes.

The effects of sex hormones on the activity of glycerol kinase in hepatocyte preparations from male and female rats were next investigated in an attempt to demonstrate hormonal control of glycerol kinase at the cellular level. For this study, the hormones were dispersed in the hepatocyte bathing medium by following a method used with testosterone and estradiol- $17\beta$  by Schweppe and Jungman (120). In this method, the hormones are dissolved in propan 2-ol to give the required concentrations (for full description see Section VI.B.5.c., p.147). The final concentrations of the hormone in the incubation flasks ranged from  $10^{-6}$  to  $10^{-3}$  M. Hepatocyte preparations were pre-incubated for 1hr at  $37^{\circ}$  before incubation with the hormone which was

TABLE X. The specific activity of glycerol kinase in male and female rat hepatocytes

Sex	No. of Animals	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes	Significance
Males (intact)	8	2.24 $\pm$ 0.17	male (intact) vs. male (castrated) P $<$ 0.01
Males (castrated)	3	1.56 $\pm$ 0.22	male (intact vs. female (intact) P $<$ 0.01
Females (intact)	8	1.83 $\pm$ 0.11	male (castrated) vs. female (intact) NS

Values are means  $\pm$  S.D. of the total specific activity of glycerol kinase in the cells and the incubating medium

Rats were fed on Dixon CDD[R] diet with water to drink ad lib.

TABLE XI. The specific activity of lactate dehydrogenase in male and female rat hepatocytes

Sex	No. of Animals	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes	Significance
Males	8	$354.91 \pm 61.3$	male vs. female  NS
Females	8	$302.59 \pm 45.7$	

Values are means  $\pm$  S.D. of the total specific activity of lactate dehydrogenase in the cells and the incubating medium

Rats were fed on Dixon CDD[R] diet with water to drink ad lib.

for either 3hr or 'zero hr' (hormone was added to the cells and the cells were separated from the incubation medium by centrifugation for 10 min). In the case of both incubation times a total period of about 70 min was required after incubation to obtain a soluble supernatant preparation for the enzyme assay.

It was reported by Bruchovsky and Wilson (298, 299) that 5 $\alpha$ -dihydrotestosterone was the main metabolite of testosterone in rat prostate, both in vivo and in vitro, and that 5 $\alpha$ -dihydrotestosterone was more firmly bound than testosterone to nuclear chromatin material in androgen target tissues such as prostate. It was also shown by Hansson et al. (317) that after intravenous administration of [<sup>3</sup>H]5 $\alpha$ -dihydrotestosterone to adult castrated male rats, the highest levels of the hormone were found in the prostate and in the liver. These authors believe that 5 $\alpha$ -dihydrotestosterone is the active intracellular androgen and that testosterone is the main transport form of the androgen in blood. In view of these reports it was decided to use 5 $\alpha$ -dihydrotestosterone instead of testosterone in the present experiments.

The effect of incubating hepatocytes from male rats with different concentrations of 5 $\alpha$ -dihydrotestosterone on the activities of glycerol kinase and lactate dehydrogenase are presented in Fig. 11. The results demonstrate clearly that dihydrotestosterone, at concentrations of 10<sup>-6</sup> and 10<sup>-5</sup>M, has no apparent effect on glycerol kinase activity but at higher hormone concentrations, 10<sup>-4</sup> and 10<sup>-3</sup>M, there is a statistically significant increase in the activity of the kinase (ca 65%, after 3hr incubation) with 10<sup>-3</sup>M hormone compared with the controls containing no steroid. This 'activation' appears to be instantaneous as there are no significant differences between the zero and 3hr incubations in either case.

No statistically significant differences in the levels of lactate dehydrogenase are apparent when hepatocytes are incubated with or without dihydrotestosterone.

The effect of 5 $\alpha$ -dihydrotestosterone on glycerol kinase activity in hepatocytes from castrated male rats was also examined because it was believed that in the absence or at a low level of endogenous hormone, stimulation of the enzyme by exogenous steroid would be greater. The results of this experiment (Fig. 12) show that, as with intact male rats (Fig. 11), relatively high concentrations of

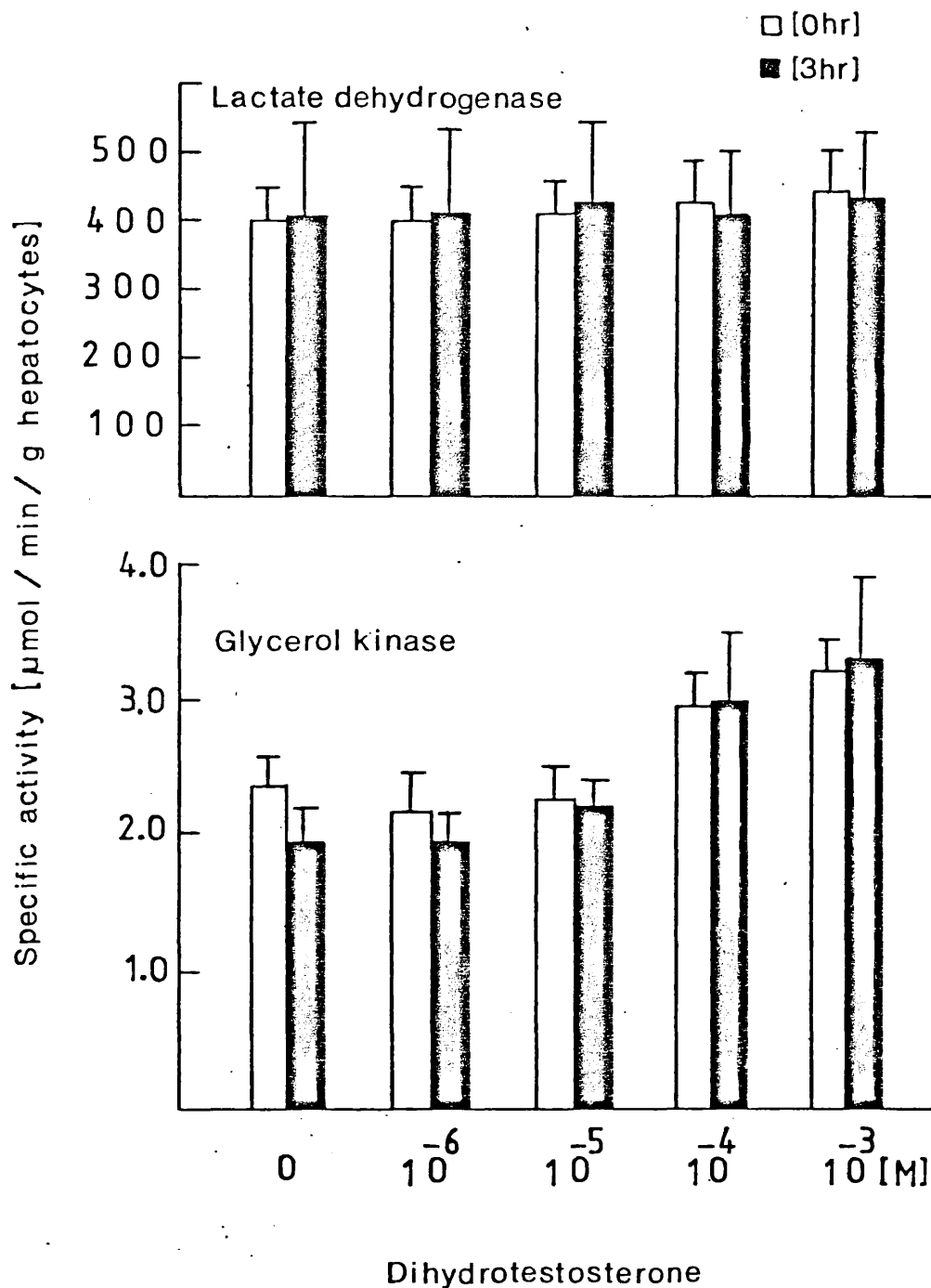


Fig. 11.

The effect of incubating hepatocytes from male rats with different concentrations of dihydrotestosterone on the activities of glycerol kinase and lactate dehydrogenase. For experimental details see Section VI.B.5. (p. 145).  
(see Appendix 2 for numerical data).



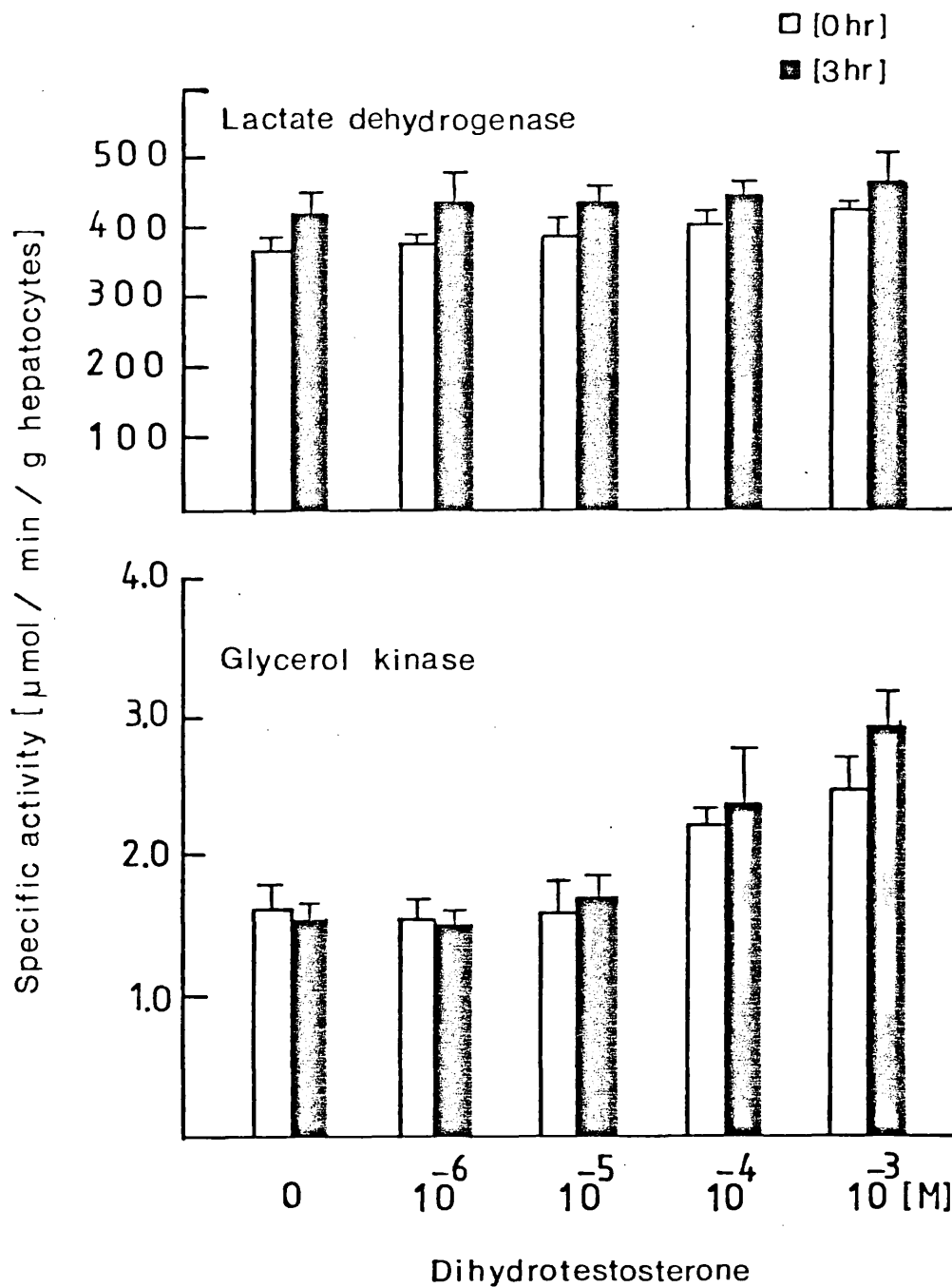


Fig. 12.

The effect of incubating hepatocytes from castrated male rats with different concentrations of dihydrotestosterone on the activities of glycerol kinase and lactate dehydrogenase. For experimental details see Section VI.B.5. (p. 145).

(see Appendix 3 for numerical data).

5 $\alpha$ -dihydrotestosterone raise the level of the kinase activity and the difference (90%) between the activities in the absence and presence of the hormone ( $10^{-3}$  M) is somewhat higher than in the experiment with cells from intact animals (Fig. 11). Again, zero time and 3hr incubations gave very similar values for each hormone concentration indicating that the activation was rapid.

With lactate dehydrogenase activities (Fig. 12) in hepatocytes from castrated animals there was, again, no evidence of hormone effects.

In further studies the effect of estrogen was investigated by incubating hepatocytes from intact male rats with different concentrations of estradiol-17 $\beta$  (Fig. 13). Unlike the results obtained with 5 $\alpha$ -dihydrotestosterone, increasing concentrations of estradiol have no significant effects on glycerol kinase activity if either the 'zero time' or the 3hr incubations are compared with the control (no hormone). However, with the 3hr incubations there does appear to be a trend towards decreasing activities with increasing estrogen concentration. This may be correlated with an increasing difference in activity between the zero and 3hr incubations with increasing hormone levels.

Estradiol had no significant effect on lactate dehydrogenase activity at any of the steroid concentrations used.

The experiments described using hepatocytes from male rat livers were repeated using cells from the livers of intact female animals. The results which are given in Fig. 14, show no significant changes in lactate dehydrogenase activity with increasing 5 $\alpha$ -dihydrotestosterone levels but, with glycerol kinase, the results are very similar to those obtained with male hepatocytes (Fig. 11). That is, the specific activity increased with increasing steroid concentrations and, again, the activation was rapid and was apparent in the 'zero hour' incubation mixtures.

With estradiol and female hepatocytes (Fig. 15) there were no significant changes with either of the enzymes and, in the case of glycerol kinase, there was no trend of increasing inactivation with increasing hormone levels using a 3hr incubation period as was found with male cells (Fig. 13).

In view of the apparent activation of glycerol kinase produced by incubating either male or female hepatocytes with 5 $\alpha$ -dihydrotestosterone, it was decided to examine the direct effect of the hormone on glycerol kinase which had been released from the cells.

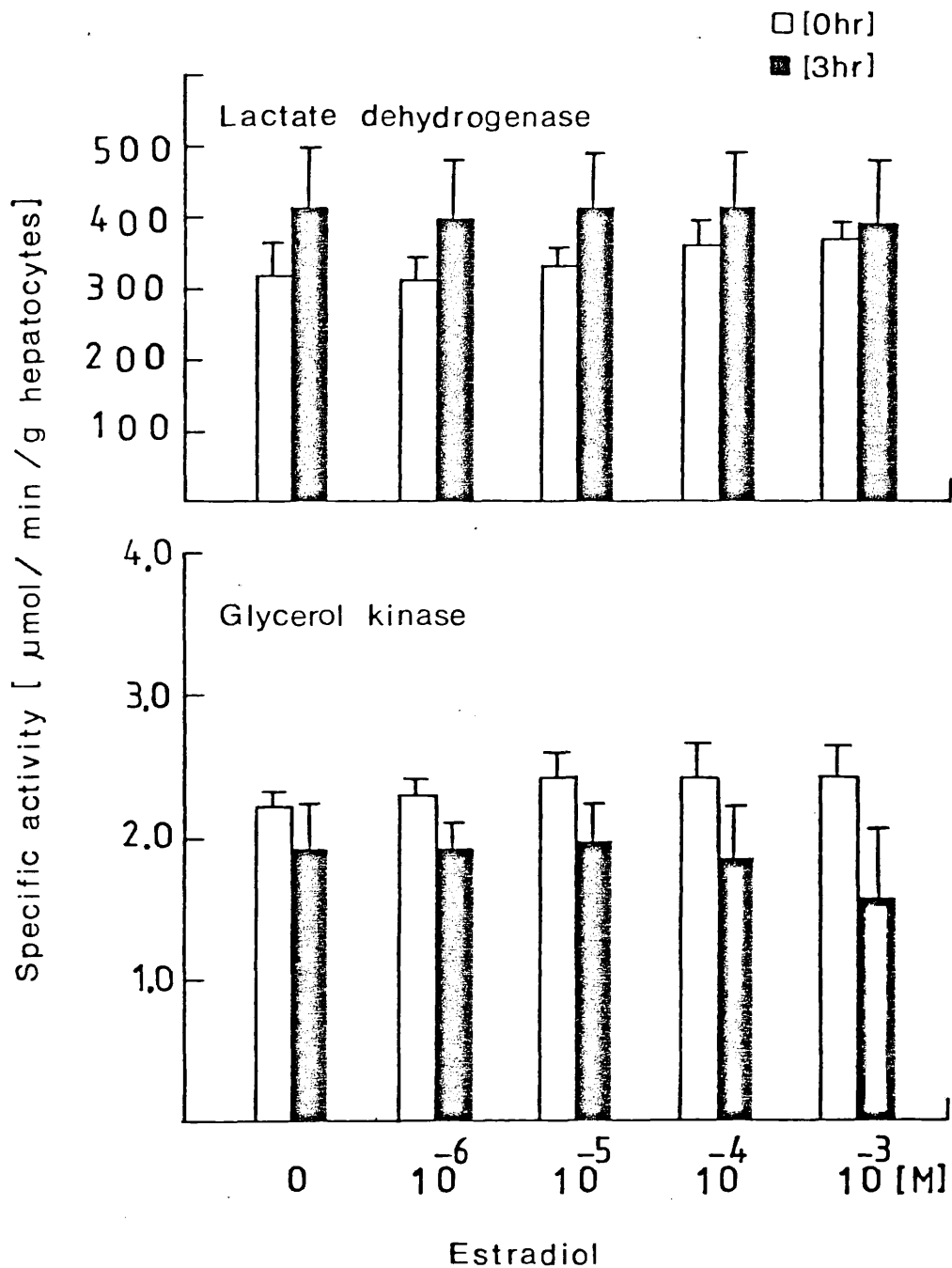


Fig. 13. The effect of incubating hepatocytes from male rats with different concentrations of estradiol on the activities of glycerol kinase and lactate dehydrogenase. For experimental details see Section VI.B.5. (p. 145).  
(see Appendix 4 for numerical data)

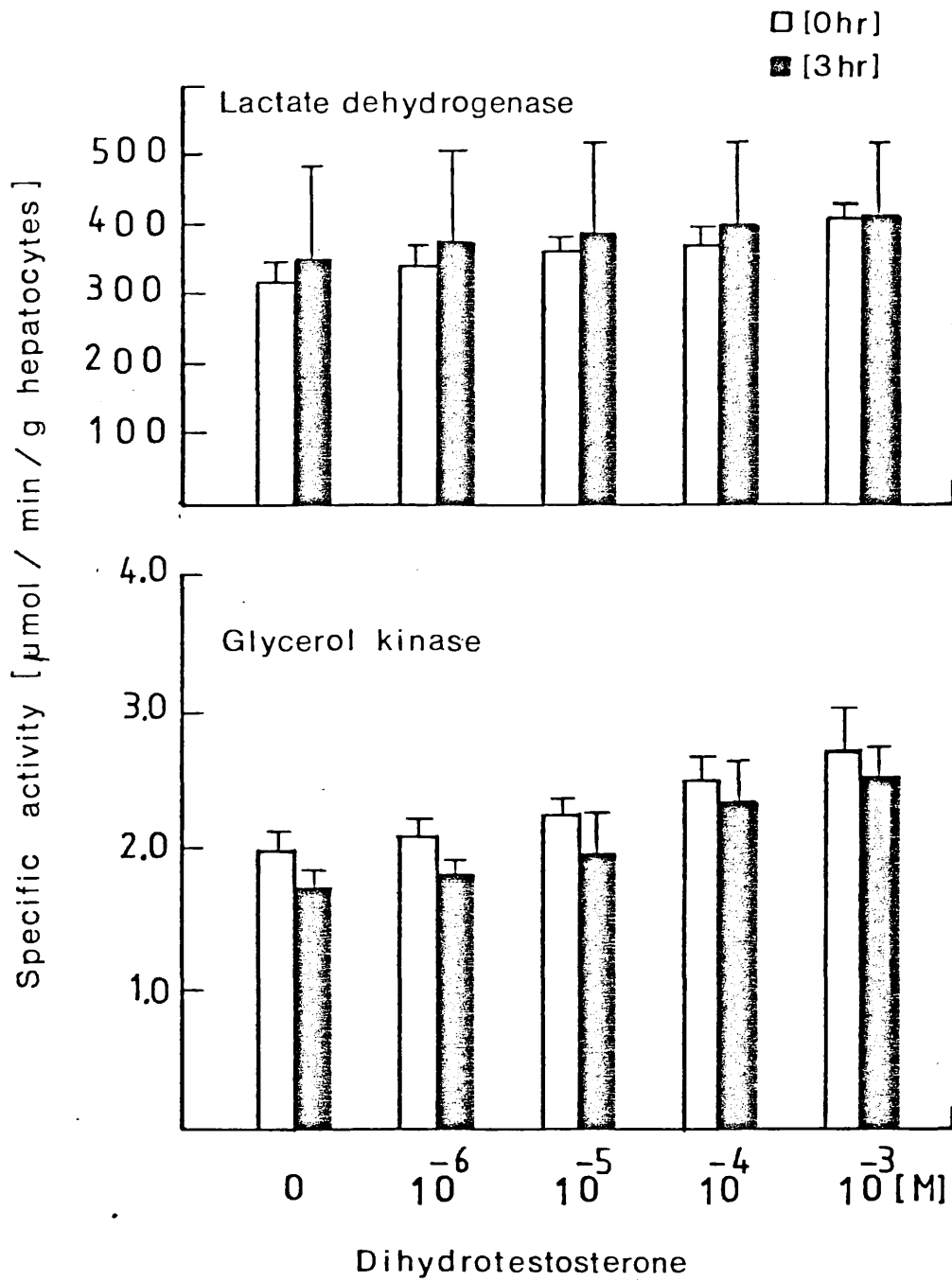


Fig. 14. The effect of incubating hepatocytes from female rats with different concentrations of dihydrotestosterone on the activities of glycerol kinase and lactate dehydrogenase. For experimental details see Section VI.B.5. (p. 145).  
(see Appendix 5 for numerical data)

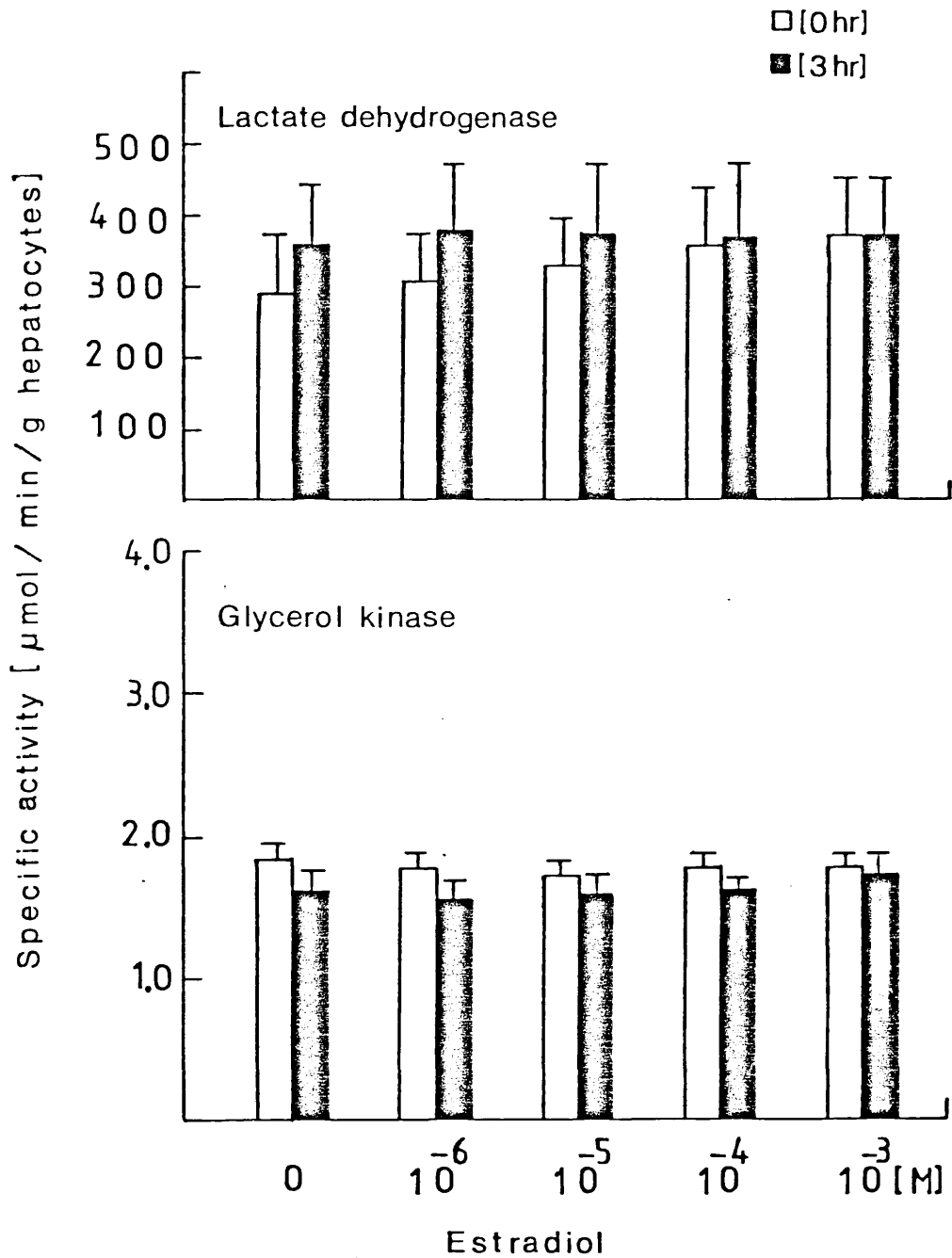
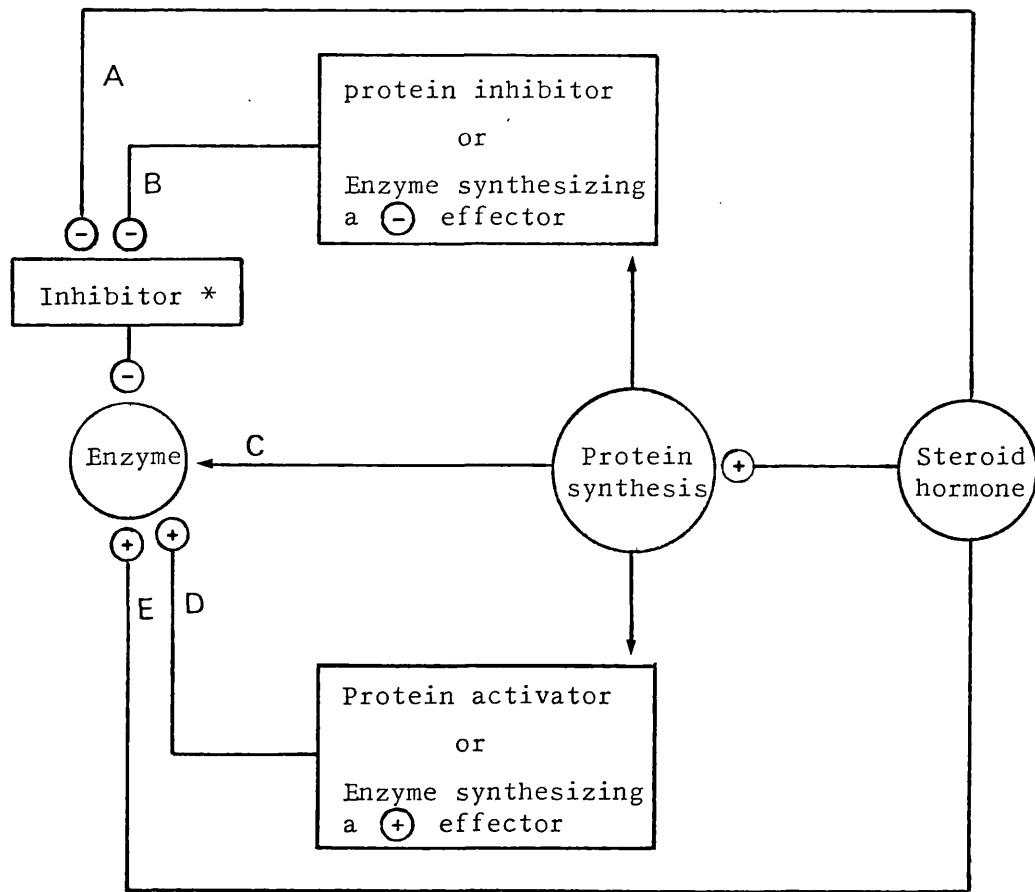


Fig. 15. The effect of incubating hepatocytes from female rats with different concentrations of estradiol on the activities of glycerol kinase and lactate dehydrogenase. For experimental details see Section VI.B.5. (p. 145). (see Appendix 6 for numerical data)

Male hepatocytes were, therefore, sonicated and the preparation centrifuged at 100,000 x g for 1 hr. Dihydrotestosterone was added to the supernatant at various concentrations and assays of glycerol kinase then carried out both immediately and after a 3hr incubation at 37<sup>o</sup>. The results (Table XII) showed no changes in activity resulting from the direct addition of steroid. It appears, therefore, that the apparent activation of glycerol kinase by androgen is a function of the steroid acting on intact hepatocytes and does not result from a direct interaction of the hormone with the enzyme protein.

In theory, a steroid hormone might increase enzyme activity in a number of ways which are summarized in the scheme below:



( \* enzyme or non-enzyme protein)

TABLE XII. The effect of 5 $\alpha$ -dihydrotestosterone on glycerol kinase activity in a soluble supernatant fraction from male rat hepatocytes

Enzyme	Incubation time [hr]	Specific activity [ $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes]				
		5 $\alpha$ -Dihydrotestosterone concentration				
	0	0	10 <sup>-6</sup> M	10 <sup>-5</sup> M	10 <sup>-4</sup> M	10 <sup>-3</sup> M
Glycerol kinase	0	1.55 $\pm$ 0.08	1.50 $\pm$ 0.02	1.52 $\pm$ 0.04	1.59 $\pm$ 0.02	1.56 $\pm$ 0.06
	3	1.44 $\pm$ 0.04	1.39 $\pm$ 0.03	1.40 $\pm$ 0.03	1.45 $\pm$ 0.07	1.47 $\pm$ 0.06

Specific activity values are means  $\pm$  S.D. of three separate observations

The in vitro study described above does not support a mechanism involving a direct activation of the enzyme by dihydrotestosterone (E) or inactivation of a theoretical inhibitor of the kinase (A) unless it is assumed that breaking the hepatocytes in some way changes the enzyme or the inhibitor (perhaps a conformational change) so that the steroid cannot combine.

The other postulated controls in the Scheme above all rely on dihydrotestosterone activating the machinery of protein synthesis. In (C) enzyme protein is synthesized in response to the hormone, in (D) a protein is synthesized which in turn activates the enzyme and in (B) a protein is synthesized which either directly or indirectly inhibits the enzyme inhibitor.

Sex hormones in vivo are believed to normally influence the rate of biochemical reactions by stimulating protein synthesis in the target tissue. The process starts immediately after the entry of the hormone into the cell where it is bound to a specific hormone receptor in the cytosol. The resulting hormone-receptor complex is then translocated to the nucleus in an 'activated' form. Once in the nucleus, it undergoes an interaction with the DNA-nucleoprotein complex (chromatin) and the target cells respond by synthesizing mRNA (transcription). The mRNA is then moves into the cytosol where it stimulates the polysomes (translation) and the process ends by the release of newly synthesized protein into the cell (453).

Most of the studies concerning sex hormone receptors and their role in macromolecular synthesis have been limited primarily to the reproductive tissues and until recently, hepatic tissue was generally considered as a 'non-target' for sex hormones. However, in 1975 androgen uptake by the liver was demonstrated after intraperitoneal administration of tritiated testosterone to castrated male rats (368). Cytosolic sex hormone receptors were also later identified in livers from male and female rats (see also p. 69) (318, 319, 320).

The apparent activation of glycerol kinase by incubating hepatocytes with dihydrotestosterone occurred very rapidly: with the 'zero time' incubations the hormone was only in contact with the intact cells for about 10 min. In addition, no amino acids or other exogenous factors which are normally required for protein synthesis, were present in the hepatocyte incubation media. It seems unlikely, therefore, that protein synthesis giving rise to increased levels of glycerol kinase was responsible for steroid activation of the enzyme.



Nevertheless, it was decided to try and eliminate the possibility that dihydrotestosterone was stimulating glycerol kinase synthesis either directly or indirectly.

For this study, hepatocytes from male rats were prepared and incubated with 5 $\alpha$ -dihydrotestosterone ( $10^{-3}$ M) in the presence and absence of specific protein synthesis inhibitors (cycloheximide or puromycin). The inhibitors were added to the cells during the pre-incubation period, i.e. 1hr prior to the addition of the hormone, to allow sufficient time for penetration of the tissues by the inhibitors which could have been slow, relative to the rate of entry of testosterone. Amino acid mixtures, including [ $^3$ H]isoleucine, were also added to the incubation media. Glycerol kinase activities were then assayed and also total protein synthesis by the method of Mans and Novelli (454) which is based on measuring the incorporation of label from [ $^3$ H]isoleucine into trichloroacetic acid-insoluble material (see Section VI.B.5.e. p. 148).

[With similar preparations of male hepatocytes it has been shown that the rates of total protein synthesis and albumin and  $\alpha_{2u}$ -globulin synthesis are linear for periods up to 6hr: the rate of  $\alpha_{2u}$ -globulin synthesis was reported to be 0.15 $\mu$ g/hr/ $10^6$  cells (D. Settachan, unpublished results). This latter value is comparable to the rates found by other workers using perfused liver systems or intact animals (455, 456)].

The experiment showed that  $10^{-3}$  M dihydrotestosterone activated glycerol kinase in the presence and absence of the two protein synthesis inhibitors (Table XIII). In addition, both cycloheximide and puromycin inhibited total protein synthesis by 82% and 96%, respectively (Table XIV) and, hence, particularly with reference to cycloheximide, it is unlikely that protein synthesis is involved in the activation phenomenon.

From Figs. 11, 12 and 14 it could be concluded that the apparent activation of glycerol kinase was related to the stability of the enzyme. For example, glycerol kinase inside the hepatocytes could be more stable than outside and dihydrotestosterone could perhaps stabilize the cells and allow less cellular disruption during incubations. Thus, incubations in the absence of dihydrotestosterone could lead to more denaturation of glycerol kinase as it is released into the medium, than incubations in the presence of the hormone. However, Fig. 9 suggests that there is little difference in the viabilities and hence the stabilities, of hepatocytes incubated for 3hr

TABLE XIII. The effect of incubating hepatocytes from male rats with 5 $\alpha$ -dihydrotestosterone [10<sup>-3</sup> M] on the activity of glycerol kinase in the presence and absence of cycloheximide or puromycin.

Enzyme	Incubation time [hr]	Specific activity [ $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes]		
		Control [No 5 $\alpha$ -DHT]	5 $\alpha$ -dihydrotestosterone	
			No inhibitor	+ Cycloheximide* + Puromycin*
Glycerol kinase	3	1.80	2.85	2.75
	3	1.92	2.98	3.02

Values represent total specific activity of glycerol kinase in the cell and the incubation medium. Hepatocytes were prepared from an adult male rat (320g body weight) and preincubated for 1hr with the inhibitors.

\*The final concentration of cycloheximide was 70 $\mu\text{M}$  and puromycin, 40 $\mu\text{M}$  (249, 276).

TABLE XIV. The rate of incorporation of [<sup>3</sup>H]isoleucine into protein in isolated hepatocytes

Hepatocyte incubation mixture *	Relative incorporation rate ** dpm/10 <sup>6</sup> cells/hr	% Inhibition
Hepatocytes (amino acids omitted)		
No inhibitors	4623	0
+ Cycloheximide (70μM)	130	97
+ Puromycin (40μM)	684	85
Hepatocytes (amino acids added)		
No inhibitors	5668	0
+ Cycloheximide	212	96
+ Puromycin	1028	82

\* Hepatocytes were preincubated for 1hr in the presence or absence of the protein inhibitor prior to the addition of [<sup>3</sup>H]isoleucine (276)

\*\* The incorporation rates are means of four samples taken from each incubation mixture

in the presence or absence of dihydrotestosterone at concentrations up to  $10^{-3}$  M. Therefore, presumably, the rate of leakage of glycerol kinase from the cells would be similar in both cases. Fig. 16 also shows that there is only a small total loss of glycerol kinase activity when hepatocytes are incubated for 2-3 hr and the proportion of the activity in the cells and in the medium remains the same. None of this data, therefore, supports the idea that the apparent activation of glycerol kinase by dihydrotestosterone is merely due to a decrease in the denaturation of the enzyme particularly if it is remembered that the apparent activation was observed after only a few minutes exposure of the hepatocytes to the hormone.

The mode of activation of glycerol kinase by dihydrotestosterone is, therefore, not clear. Intact hepatocytes appear to be a prerequisite so it is possible that some intermediary glycerol kinase effector, which is lost or which changes conformation when the cells are disrupted, is needed. The phenomenon also exhibits a degree of specificity in that estradiol is not an activator of the enzyme. From a physiological point of view the observed effect of dihydrotestosterone may be unimportant in view of the high concentrations of hormone ( $10^{-4}$  -  $10^{-3}$  M) needed to bring about activation. However, in vivo, localized high concentrations of hormone within cells or particular parts of cells are always possible.

The present investigation and studies by other workers in the field (discussed in Section IV.B. p.72) when considered together (see Fig. 17) now provide data on the activities of the majority of the enzymes in male and female rat livers involved in triose synthesis from fructose and many of the enzymes concerned with the conversion of trioses to triglycerides.

A first, important point to recognize is that sex differences in enzyme activities may be diet-dependent. Although no reversal of such differences has been observed in the present study there were instances where a sex difference found with animals on one diet was nullified in the case of those on another diet. Thus with glycerol 3-phosphate dehydrogenase, for example (Table VIIa), no sex differences were apparent with animals ingesting Dixon 86 diet or CDD[R] supplemented with sucrose but with CDD[R] alone or supplemented with glucose, enzyme activity was significantly higher in the female livers. Other dietary effects were observed with sorbitol dehydrogenase (Table Va),

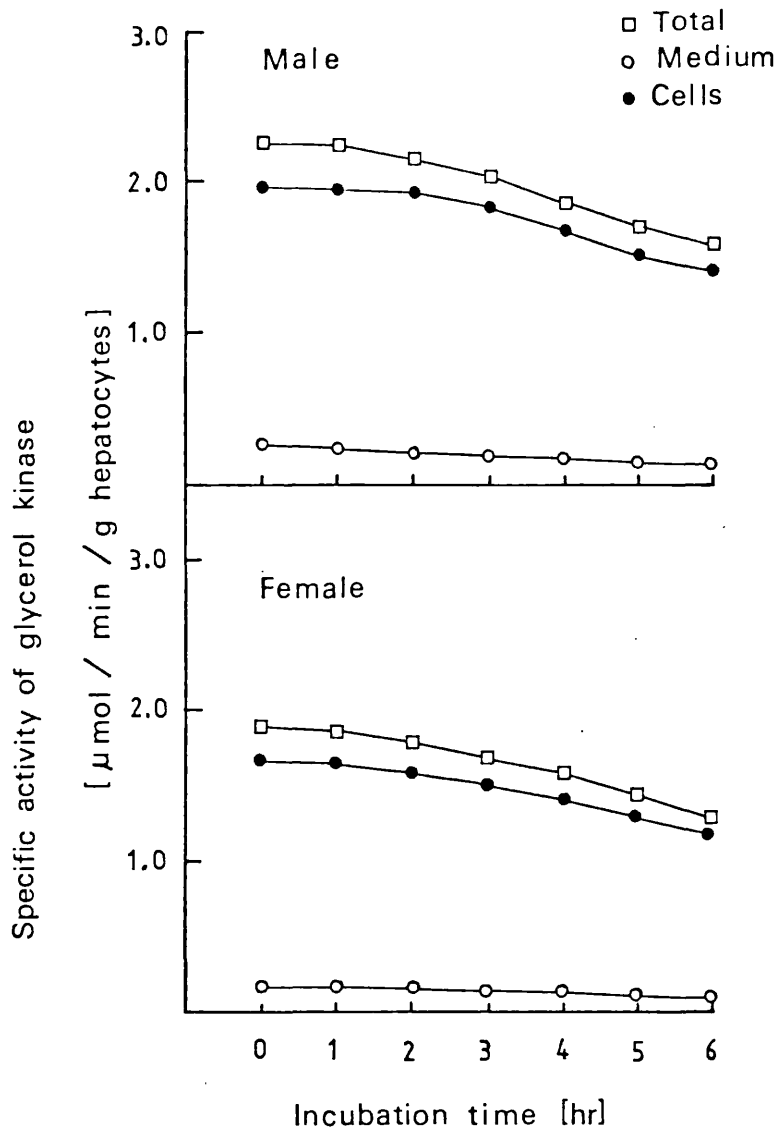


Fig. 16. The effect of incubating hepatocytes from male and female rats at 37° on the activity of glycerol kinase.

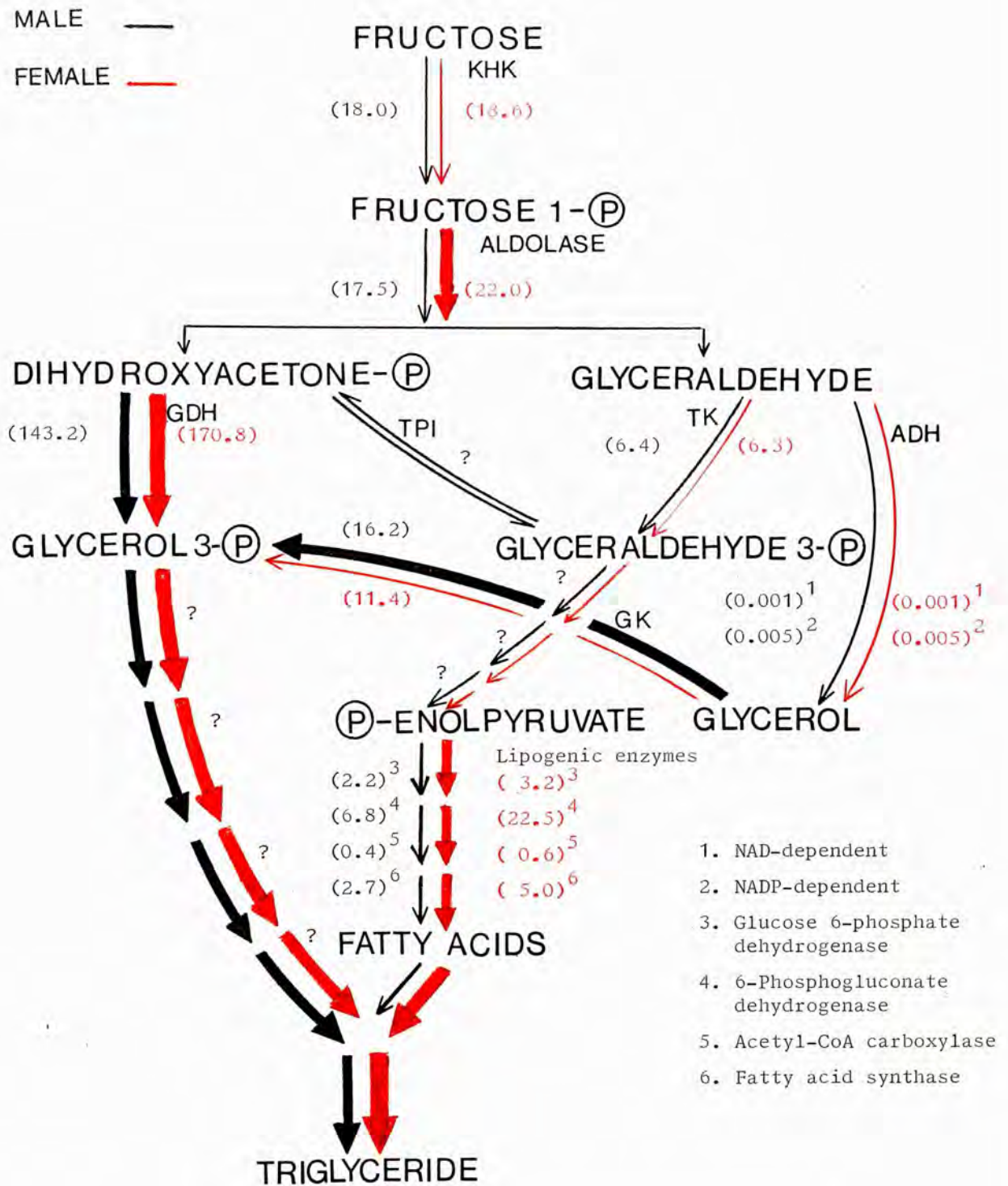


Fig. 17. Sex differences in enzymes involved in the conversion of fructose to triglyceride

Figures in parenthesis are specific activities (nmol/min/mg protein) of the liver enzymes from rats fed Dixon CDD[R] diet (258, 322)

triokinase (Table VIa) and glycerol kinase (Table VIIIa). These results were, of course, obtained by long-term feeding and the effect of short-term carbohydrate supplement or intermittent carbohydrate loading may be different.

The general conclusion to be gained from liver perfusion studies (Section IV.C. p.74) is that female rat livers have a greater potential for producing triglyceride than male livers although no comparisons using fructose as a substrate have been made. This ability is firstly reflected in the higher activities in the female liver of a number of key enzymes involved in fatty acid synthesis from triose i.e. pyruvate kinase, glucose 6-phosphate and 6-phosphogluconate dehydrogenases, acetyl-CoA carboxylase and fatty acid synthase.

The sex differential in these activities can, at least in part, be accounted for by differing hormonal controls of the enzymes in male and female rats involving sex steroids and pancreatic hormones. In the first place, insulin and glucagon, have been shown to effect lipogenesis. This process is enhanced by insulin in hepatocytes (470, 471) and in perfused liver (308, 310, 472, 473). Several reports also show that glucagon antagonizes the effect of insulin with respect to lipogenesis (470, 471, 473, 474) and triglyceride output (303, 308). Insulin may achieve these effects by increasing the activities of pyruvate kinase (349, 351), acetyl-CoA carboxylase and fatty acid synthase (397, 475), glucose 6-phosphate dehydrogenase (416) and, possibly, 6-phosphogluconate dehydrogenase (420). Estrogen injections have also been shown to activate fatty acid synthase, acetyl-CoA carboxylase (403, 421) and 6-phosphogluconate dehydrogenase (385). Glucagon, on the other hand, has been reported to inhibit pyruvate kinase (349, 351), fatty acid synthase (476) and glucose 6-phosphate dehydrogenase (417) in rats and acetyl-CoA carboxylase (471) in mouse hepatocytes.

Mandour, Kissebah and Wynn (421) showed that estrogen increases the insulin/glucagon ratio in ovariectomized female rats and this observation suggests that sex differences in the levels of the lipogenic enzymes may, to some degree, be a function of sex hormone control mediated by insulin and glucagon. In this connection, it is interesting to note that female rats have higher insulin levels than males both in a fasting state and particularly after feeding a carbohydrate-enriched diet (59). (Women have also been reported to have a higher insulin level than men (381)).

All of the lipogenic enzymes listed above would be involved in fatty acid production from trioses arising from fructose and, thus even in the absence of sex differences in the rate of triose formation, female livers would presumably convert fructose more efficiently to triglyceride than male livers. However, there do appear to be quantitative sex differences in the way triose is produced from fructose in rat liver.

Inspection of the enzyme activities in Fig. 17 suggests that the passage of carbon from fructose to triglyceride-glycerol, at least, may be more facile in the female rat liver. For example, with fructose 1-phosphate aldolase, the specific activity of the hepatic enzyme in the female is about 25% higher than in the male (S.A. Hashemi, unpublished results) and this difference has been observed with rats on diets of chow with or without carbohydrate supplementation. The greatest sex difference was found with sucrose supplementation of the diet. This enzyme-catalyzed step may also be rate-limiting and controlled by ATP degradation products (see Section III, p.61).

In addition, one of the products of aldolase, dihydroxyacetone phosphate, can presumably be rapidly metabolized to glycerol 3-phosphate, an intermediate in triglyceride synthesis, as there are relatively high levels of glycerol 3-phosphate dehydrogenase in rat liver. This enzyme has a low  $K_m$  (0.075mM) for its substrate dihydroxyacetone phosphate, and, again, the specific activity in female liver is significantly higher than in male (Table VIIa). Hence, relatively low levels of dihydroxyacetone phosphate which may be produced from fructose by the coupled ketohexokinase-aldolase system can probably be more readily converted in the female than the male to triglyceride via the glycerol 3-phosphate dehydrogenase-catalyzed reaction.

Of course, other factors besides enzyme levels such as availability of coenzymes, other reactions utilizing fructose metabolites such as glycolysis and gluconeogenesis, and hormone effects will also influence the rate of formation of triglyceride-glycerol. In addition, glycerol 3-phosphate dehydrogenase levels are diet-dependent and long-term feeding, at least, of a sucrose supplement appears to nullify the sex difference.

Dihydroxyacetone phosphate can also be converted to triglyceride-fatty



acid via triosephosphate isomerase which initially yields glyceraldehyde 3-phosphate and this in turn can then be metabolized by the glycolytic and lipogenic pathways. This is probably a relatively minor pathway as significantly less carbon from [ $^{14}\text{C}$ ]fructose is incorporated into triglyceride-fatty acid than into triglyceride-glycerol (see p.58). There is no information concerning possible sex differences in triosephosphate isomerase and glycolytic enzymes leading as far as phosphoenol pyruvate.

Regarding glyceraldehyde, the second product of aldolase cleavage of fructose 1-phosphate, it may be phosphorylated by triokinase to glyceraldehyde 3-phosphate in the rat (see Section III, p.62) and then there is a facile route to dihydroxyacetone phosphate and thereby triglyceride-glycerol, via triosephosphate isomerase. Triokinase is present in the liver at relatively low levels and there are no apparent sex differences in activity (except, possibly, when animals are ingesting a low-fat diet when the male level is 40% higher than the female (Table VIa)). It is probably, therefore, that the triokinase-triosephosphate isomerase pathway does not change the sex difference in glycerol 3-phosphate production which is attributed to glycerol 3-phosphate dehydrogenase variance. Triokinase, however, with a high  $K_m$  for ATP may not function efficiently at low ATP levels perhaps, for example, when fructose phosphorylation is occurring (see Section III, p.61 (111, 232)) and in this case the glyceraldehyde-glycerol-3-phosphate pathway might operate in the rat leading glyceraldehyde carbon by a second route to triglyceride-glycerol. However, the low activities of all the enzymes on this pathway presumably means that contributions to the pool of glycerol 3-phosphate are smaller from glyceraldehyde than from dihydroxyacetone phosphate. If this is so then the sex difference ( $\delta$  45% >  $\eta$ ) in hepatic glycerol kinase activity which, in theory, would reduce the ability of the female liver relative to the male to produce triglyceride-glycerol would be of little importance with respect to lipogenesis from fructose.

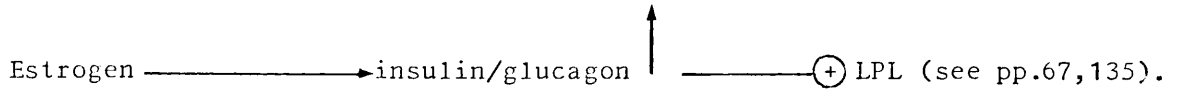
Triglyceride-fatty acid formation from fructose (which, from isotopic tracer studies may be interpreted as occurring less readily than triglyceride-glycerol production) can occur from dihydroxyacetone phosphate and glyceraldehyde. With regard to sex variations in the rates of triglyceride-fatty acid formation from these trioses the lipogenic enzymes alone would appear to control these with little part being played by the triose-metabolizing enzymes.

All the data on male/female differences in enzyme levels so far considered support the observations that triglyceride production by the liver of female rat (and probably other mammals) is higher than that of the male organ. If this is the case then one would expect the liver output to be reflected in the serum triglyceride levels but this is not so. Hence, the 'clearing' rates, catalyzed by lipoprotein lipase, should presumably play an important role in maintaining female serum triglycerides at a lower level than that of male.

The specific activity of lipoprotein lipase in human plasma is reported to be higher in women than men by about 33% (413). In addition, as women normally possess a significantly higher adipose tissue/body weight ratio than men (61) the related increased total adipose lipoprotein lipase activity in relation to body weight in the former should allow a more efficient triglyceride-clearing system (cf 413). This assumes that the ratio of other lipoprotein lipase-contributing tissues to body weight does not differ significantly in males and females.

In rats, the serum triglyceride concentration is, as expected, indirectly proportional to the specific activity of the adipose lipoprotein lipase (396) and differences in the levels of lipoprotein lipase in males and females have also been reported. The data of Hamosh and Hamosh (415), for example, show that the specific activity of the enzyme (U/g adipose tissue) is higher (ca 30%) in females than males, both feeding on chow. A smaller differential (ca 7%) was reported by Bruckdorfer et al. (396) with animals ingesting on a low-fat diet supplemented with sucrose. As with humans, the adipose tissue/body weight ratio is higher in female rats than in male (431).

Undoubtedly further work on lipoprotein lipase levels in rats is needed but if the 'clearing' efficiency in females is greater than males, as seems to be the case in humans, it may again relate to the insulin/glucagon ratio which, if high, favours lipoprotein lipase activity (see p. 67). Female rats have higher levels of plasma insulin than males (see p. 135) and, hence, it could be postulated that the sex difference in lipoprotein lipase activity is a function of estrogen control mediated by the insulin/glucagon ratio through the following chain of events, as appeared to be the case with the lipogenic enzymes (see p. 135):



However, this postulated sequence of events is jeopardized by reports that ovariectomy of rats increases the lipoprotein lipase level (415) and estrogen injection into intact female rats lowers the activity of the lipase (412). In the latter case, of course, introduction of exogenous hormone would have presumably raised the endogenous hormone concentration beyond the physiological level in which case toxic effects may have ensued.

In conclusion and in the absence of comparative quantitative data on lipogenesis and 'clearing' rates, the postulate must remain that the high level of triglyceride in the serum of male rats relative to female is very largely controlled by the relatively high total lipoprotein lipase activity that the female tissues possess which is in excess of the capacity for hepatic lipogenesis.

In the future an accurate determination of the total lipoprotein lipase activity in male and female animals in relation to body weight is needed for comparison with a measure of total secretion of hepatic triglycerides into the serum. In addition, a clearer understanding of the effects of hormones and diet on these two processes is required.

MATERIALS AND METHODS

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A. MATERIALS

1. Chemicals

All the chemicals used were of Analar grade and all solutions were prepared in glass distilled, deionized water. Substrates and purified enzymes were purchased from Boehringer Corporation (London) Ltd., Lewes, Sussex, U.K., unless otherwise stated.

2. Rats

Male and female rats used in this study were of the Wistar strain. The rats were either obtained from Tucks and Son, Rayleigh, Essex, U.K. or bred in our laboratory.

3. Diets

Two types of commercial diets were used, Dixon 86 and Dixon CDD[R]. The composition of these diets is shown in Table XVa. The calculated percentage composition of these diets and their calorific values are presented in Table XVb.

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

Dixon 86	cwt/ton	Dixon CDD[R]	cwt/ton
Wheat	10	Wheat	10.3
Barley	5	Maize	1.5
Concentrated meat meal	1.5	Soya meal	2.5
White fish meal	1.4	White fish meal	3
Grass	1	Milk powder	0.5
Yeast	1	Yeast	0.39
Molasses	1	Shredded suet fat	1.2
Salt	0.2	Salt	0.16
Vitamins	0.022	Vitamins	0.223
		Mineral salt	0.25

\*Values were obtained from the manufacturer: E. Dixon & Sons (Ware) Ltd., Crane Mead Mills, Ware, Herts, U.K.

TABLE XVb. Calculated percentage partial compositions and calorie values of Dixon 86 and Dixon CDD[R] diets

Composition	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
<u>Kcal/kg</u>		
Gross energy	3,942	3,931
Metabolizable energy	3,548	3,538

#### 4. Liver Perfusion Cabinet

The perfusion cabinet consisted of a metal cabinet with glass front doors, a fan heater connected to a thermostat for the control of temperature inside the cabinet, a glass 'lung' made to specification (240), a Watson-Marlow H.R. flow-inducer (Watson-Marlow, Ltd., Marlow, Bucks, U.K.), to pump the perfusion solution, and Swinnex-13 filter units (Buc, France) to filter the circulating perfusate.

## B. METHODS

### 1. Long Term Feeding Experiments

Male and female Wistar rats (initial body weight  $50 \pm 5$ g) were fed for 110 days ad lib. on Dixon 86 or Dixon CDD[R] diets with water to drink or Dixon CDD[R] diet supplemented with either 5% (w/v) sucrose or glucose in the drinking water. After 110 days the animals were sacrificed by cervical dislocation without fasting and the livers were immediately (within 45 sec) removed, washed twice in ice-cold saline (0.154 M-NaCl), dried, weighed and stored at (-70<sup>o</sup>).

### 2. Liver Enzyme Preparations and Enzyme Assays

Livers were homogenized in appropriate ice-cold buffer solutions (1:3, w/v), using a Potter-Elvehjem glass homogenizer with a teflon pestle (clearance 0.48mm), and 100,000 x g (1hr) soluble supernatants were then prepared (see, however, triokinase Section VI.2.c., p. 144).

In the case of enzymes assayed spectrophotometrically, a Pye-Unicam SP.1800 ultraviolet spectrophotometer was normally used. The reaction mixtures were contained in quartz cuvettes (1cm light path) thermostatically maintained at 30<sup>o</sup>.

Assays, unless otherwise stated, were initiated by the addition of the substrate after preincubation of the homogenate to ensure removal of endogenous substrates. The reaction rate as a function of substrate concentration for each assay was measured and the  $K_m$  value calculated and compared with literature values: only minor differences were observed. All assay reactions were shown to be linear with time and proportional to the enzyme concentration.

(a) Ketohexokinase preparations were made in 25mM-phosphate buffer (pH 7.4) containing 5mM-EDTA and 0.5mM-dithiothreitol (152). The enzyme was assayed by the method of Hers (432, 433) which is based on the measurement of fructose disappearance. Fructose was estimated by colorimetric method developed by Roe et al. (434) and later modified by Hers et al. (435).

The reaction mixture contained: 2mM-fructose, 5mM-ATP, 5mM-magnesium acetate, 120mM-potassium acetate. 50mM-Sodium fluoride and 50mM-N-acetyl D-glucosamine were also added prior to the addition of the enzyme (0.2ml). The total volume of the reaction mixture was 1ml. The reaction was started by the addition of liver extract to well shaken, preheated (37<sup>o</sup> for 20 min) reaction mixtures. After 10 min

incubation at 37<sup>o</sup> the reaction was stopped by successive addition of 0.15M-zinc sulphate (3ml) and 0.3N-barium hydroxide (3ml) solutions. The mixture was shaken and then filtered. Phosphate esters are retained on the barium sulphate/zinc hydroxide precipitate and the free fructose can be estimated in the filtrate without interference. To the filtrate (2ml), resorcinol-thiourea reagent (1ml) and 30% HCl (7ml) were added, the solution was mixed and placed for 15 min in a water bath at 75<sup>o</sup>. After cooling, absorbance was measured at 520nm using Pye-Unicam SP.500 ultraviolet spectrophotometer.

(b) Sorbitol dehydrogenase was prepared in 0.2M-triethanolamine-HCl buffer (pH 7.4) (439). The enzyme was measured by the method of Gerlach and Hiby (439) using fructose as substrate. The assay mixture (final volume 2ml) consisted of: enzyme preparation (0.1ml), 107mM-triethanolamine-HCl buffer (pH 7.4), 0.4mM-NADH and 400mM-fructose. Loss of NADH was measured at 340nm.

(c) Triokinase was obtained by homogenizing rat liver in 0.154 M-KCl (pH 7.2) and centrifuging at 4,000 x g (20 min). The resultant supernatant was again centrifuged (37,000 x g; 2hr) and the supernatant dialysed overnight against 0.154 M-KCl (112). Enzyme activity was estimated spectrophotometrically by measuring the amount of NADH oxidized at 340nm using triosephosphate isomerase and glycerol 3-phosphate dehydrogenase coupled reactions as adopted by Heinz and Lamprecht (112). The reaction mixture (final volume 3ml) consisted of: 37mM-triethanolamine-HCl buffer (pH 7.5), 0.45mM-NADH, 2.75mM-ATP, 13mM-magnesium chloride, 14.8mM-D-glyceraldehyde, glycerol 3-phosphate dehydrogenase (50  $\mu\text{g} \hat{=} 1.8$  U), triosephosphate isomerase (50  $\mu\text{g} \hat{=} 22.5$  U) and liver extract (0.1ml). The reaction was started by the addition of ATP to the mixture.

(d) Glycerol 3-phosphate dehydrogenase preparations were obtained in 100mM-Tris (pH 7.2), 1mM-EDTA, 1mM-2-mercaptoethanol and 1% ethylene glycol (258). The enzyme was assayed by the method reported by Lee and Craine (265) using glycerol 3-phosphate as a substrate. Since, at neutral pH the equilibrium of the reaction favours the formation of glycerol 3-phosphate, the reaction was carried out at pH 9.8 and a trapping agent (hydrazine) for the dihydroxyacetone phosphate produced, was added. The reaction mixture contained: 160mM-glycine, 400mM-hydrazine, 0.5mM-NAD, 10mM-glycerol 3-phosphate and



liver extract (0.02ml). The final volume was 2.0ml and the pH 9.8. The formation of NADH during the reduction of glycerol 3-phosphate to dihydroxyacetone phosphate was measured at 340nm.

(e) Glycerol kinase was prepared in 100mM-Tris(pH 7.2), 1mM-EDTA, 1mM-2-mercaptoethanol and 1% ethylene glycol (258) and the enzyme was assayed by the method of Bublitz and Wieland (459). The formation of NADH produced by the oxidation of glycerol 3-phosphate was followed spectrophotometrically at 340nm by adding to the reaction mixture excess of  $\text{NAD}^+$  and glycerol 3-phosphate dehydrogenase. The reaction mixture contained: 160mM-glycine, 400mM-hydrazine, 1.6mM-magnesium chloride, 0.67mM-NAD, 2.5mM-ATP, glycerol 3-phosphate dehydrogenase ( $100\mu\text{g} \hat{=} 4\text{U}$ ), liver extract (0.02ml) and 1.66mM-glycerol. The final volume of the reaction mixture was 3.0ml and the final pH 9.8.

(f) Lactate dehydrogenase preparations were obtained as in the case of glycerol kinase and the activity was assayed by measuring the conversion of NADH to  $\text{NAD}^+$  in the presence of pyruvate as a substrate according to the method of Bergmeyer and Bernt (460). The reaction mixture (final volume 3.0ml) contained: 48mM-phosphate buffer (pH 7.0), 0.76mM-sodium pyruvate, 0.2mM-NADH and liver extract (0.02ml). The formation of  $\text{NAD}^+$  was measured at 340nm.

### 3. Protein Determination

The protein contents of all enzyme preparations were measured spectrophotometrically (Pye-Unicam SP.500 ultraviolet spectrophotometer) by the Biuret method (127). Bovine serum albumin was used as a standard.

### 4. Scintillation Counting

Radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer. Aqueous samples were assayed in a (1:2, v/v) mixture of Triton X-100 and toluene scintillant (5g PPO/1 toluene).

### 5. In Vitro Experiments

Hepatocytes were prepared according to the method which was originally described by Berry and Friend (467) and later modified by Krebs *et al.* (468).

In the procedure, rats (fed on Dixon CDD[R] diet ad lib.) were anaesthetized with nembatal [sodium pentobarbitone (60mg/ml, May & Baker Ltd., Dagenham, Essex, U.K.) 0.1ml/100g body weight] the peritoneal cavity was then opened by two lateral incisions starting

at the midline of the lower abdomen and proceeding both anteriorly and posteriorly exposing the peritoneal cavity. The gut was reclined to the left to expose the portal vein and inferior vena cava. Two loose ligatures were applied around the portal vein, one anterior to the superior mesenteric vein and the other just posterior to the splenic vein. A third ligature was then placed loosely around the inferior vena cava anterior to the right renal vein. The portal vein was then cannulated between the two ligatures with a sterile Luer cannula (Braunula sterile Luer; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.). The two ligatures around the portal vein were then tied and the liver perfused with Krebs-Ringer bicarbonate (KRB) buffer (177) without  $\text{Ca}^{2+}$  ions (37<sup>o</sup>, equilibrated with  $\text{O}_2$  and  $\text{CO}_2$  (95:5, v/v)) at a rate of approximately 25ml/min. The inferior vena cava was immediately severed below the right kidney to prevent the liver from swelling and to allow the perfusate to flow freely through the organ. Immediately after this step the rib cage was cut open, exposing the thoracic cavity, and a cannula was introduced into the inferior vena cava through the right atrium of the heart. A tight ligature was placed around the inferior vena cava to hold the cannula in place and the ligature around the inferior vena cava in the peritoneal cavity was then tied.

When the liver appeared pale and the perfusate was free from blood (usually after 2-5 min), a second perfusion with collagenase [(Boehringer Corporation (London) Ltd., Lewes, Sussex, U.K.) 20mg suspended in 100ml of KRB (without  $\text{Ca}^{2+}$  ions)] was carried out until the organ was smooth and swollen and the leakage of the medium from the liver was rapid (usually after 15-20 min). The liver was then removed, placed in a plastic beaker (250ml capacity) containing KRB (50ml) buffer and finely cut up with scissors and gassed gently with  $\text{O}_2$  and  $\text{CO}_2$  (95:5, v/v) for 1-2 min. A plastic funnel with a nylon mesh (Nybolt, No. 10; 132 micron; J. Staniar & Co., Manchester, U.K.) was then used to filter the cell suspensions into centrifuge tubes (50ml capacity). The cells were sedimented at 50 x g (1 min) in a bench centrifuge and the pellet then suspended in KRB buffer and the centrifugation repeated. The pellet was resuspended in an appropriate volume of KRB buffer containing 0.22mM calcium chloride, gentamycin sulphate (20µg/ml) and bovine serum albumin (20mg/ml) (fraction V, Sigma (London) Chemical Co. Ltd., Poole, Dorset, U.K.). The hepatocytes were counted under the microscope using a haematocrit chamber

and the concentration of the cells then adjusted accordingly.

The hepatocytes used for both the gluconeogenesis and the hormonal studies were preincubated for 1hr at 37° to restore the ATP/ADP ratio to normal (69).

(b) Gluconeogenesis from L-[U-<sup>14</sup>C]lactate

Male rats (200-300g) fed CDD[R] diet with water to drink ad lib. were used. Hepatocytes were prepared (see Section VI.B. 5.a.) and preincubated for 1hr (37° with continuous gassing O<sub>2</sub> and CO<sub>2</sub> (95:5, v/v)). The cell suspension (3.2 x 10<sup>6</sup> cells/ml; 1ml) was added to L-[U-<sup>14</sup>C]lactate (0.025μCi/μmol, 5μl; Radiochemical Centre, Ltd., Amersham, Buckinghamshire, U.K.) in plastic tubes (glucagon, final concentration 10nM, was also added in some cases) and the tubes were incubated in a shaking water bath at 37° (200 cycles/min) for appropriate times. The reactions were then stopped by addition of 0.15 M-zinc sulphate (0.5ml) and 0.3 N-barium hydroxide (0.5ml). Water (2ml) was added and the precipitate removed by centrifugation. [<sup>14</sup>C]Glucose was separated from charged molecules by the procedure of Exton and Park (70): moist Dowex 50W-X8 (H<sup>+</sup> form, 200-400 mesh; 200mg) and moist Duolite ES 561 (Dia-prosim, Vitry, Chauny, France; 800mg) were mixed with the protein free supernatant and shaken for 1hr. This resin treatment was repeated. The resulting supernatants were then added to Triton X-100 - toluene scintillant (1:2, v/v; 10ml) and analysed for [<sup>14</sup>C] glucose in a scintillation counter. This method for measuring gluconeogenesis was originally described by Claus et al. (69).

(c) Preparation of hormone solutions

Steroid hormones were dispersed in KRB buffer by following a method outlined for testosterone and estradiol by Schweppe and Jungman (120). Hormones were dissolved in propan 2-ol to give the required concentration. Prior to each experiment the appropriate amount of hormone solution was pipetted into a 25ml Erlenmeyer flask and after evaporation of the alcohol at 37° under a continuous stream of nitrogen gas, incubation medium (4ml) (KRB buffer containing 0.22mM-calcium chloride and bovine serum albumin 2.25mg/ml) was added to each flask. The flasks were shaken at 4° for 2hr and then left overnight in the cold room (0-4°). Prior to the addition of the hepatocytes, the flasks were equilibrated with O<sub>2</sub> and CO<sub>2</sub> (95:5, v/v) at 37° for 30 min.

(d) Hormone studies with isolated hepatocytes

Hepatocytes ( $8-10 \times 10^6$  cells/ml; 1ml), after preincubation for 1hr, were added to the hormone solution and the mixture incubated in a shaking water bath at  $37^\circ$  (200 cycles/min) with continuous gassing with  $O_2$  and  $CO_2$  (95:5, v/v) for 3hr. The cells are then centrifuged down in the cold ( $0-4^\circ$ ) at  $6,000 \times g$  (10 min) and the supernatants (incubation media) stored at  $-70^\circ$ . Prior to the determination of the enzyme activities the cells were resuspended in buffer solution (1ml) containing 100mM-Tris (pH 7.2), 1mM-EDTA, 1mM-2-mercaptoethanol and 1% ethylene glycol. The cells were sonicated and the supernatants obtained by centrifugation at  $100,000 \times g$  (1hr). The stated total enzyme activities of hepatocytes are the sums of the activities in the cells plus the incubation media.

(e) Estimation of total protein synthesis in isolated hepatocytes

Total protein synthesis was estimated by determining the incorporation of radioactivity into trichloroacetic acid (TCA)-insoluble material. Hepatocytes ( $8-10 \times 10^6$  cells/ml; 1ml) after being incubated in the presence of [ $^3H$ ]isoleucine ( $1\mu Ci/50\mu mol$ ,  $10\mu l$ ) for 3hr, with and without protein inhibitors, were sonicated and centrifuged at  $6,000 \times g$  (10min). When amino acid mixtures were added, the final concentrations used were as in Waymouth's medium (477). Aliquots ( $100\mu l$ ) were spotted onto 2.1cm diameter discs of Whatman grade 1 paper which were dried and dropped into ice-cold 10% (w/v) TCA for 15 min as described by Mans and Novelli (454). The discs were then placed in 5% TCA for 30 min at  $90^\circ$ , washed in fresh, cold 5% TCA and placed in ethanol/ether (3:1, v/v) for 30 min at  $37^\circ$ . After washing in fresh ethanol/ether, the discs were placed in ether for 15 min at room temperature and then dried. They were then put into glass scintillation vials, incubated overnight at  $37^\circ$  with NCS solubilizer and then toluene scintillant (10ml) was added to each vial and the radioactivity measured.

(f) Estimation of  $K^+$  levels in isolated hepatocytes

Hepatocytes ( $8-10 \times 10^6$  cells/ml; 1ml) were centrifuged at  $6,000 \times g$  (10 min) and the  $K^+$  level measured in the cell free media by flame photometry (EEL, flame photometer) (204).

6. Statistical Analysis

The following formula (Zivin and Bartko, 478) was used to calculate the statistical significance of the data:

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

Where  $\bar{X}$  = Mean of arithmetic average  $\Sigma X/N$

SD = Standard deviation

N = Number of observations

df = Degrees of freedom (df = n-2)

Appendix 1. The specific activities of some carbohydrate-metabolizing enzymes expressed in terms of liver weight.

REGIME ENZYMES	DIXON CDD[R] DIET									
	CONTROL				SUCROSE				GLUCOSE	
	SEX		Male	Female	Male	Female	Male	Female	Male	Female
Ketoheokinase (EC 2.7.1.3)	SEX		2.64 ± 0.21 (n = 8)	2.63 ± 0.25 (n = 8)	6.25 ± 0.79 (n = 4)	6.84 ± 0.25 (n = 4)	6.21 ± 0.29 (n = 4)	6.50 ± 0.42 (n = 4)		
	SEX		34.9 ± 4.4 (n = 8)	48.9 ± 9.2 (n = 8)	30.42 ± 2.2 (n = 5)	35.32 ± 2.4 (n = 5)	26.2 ± 2.4 (n = 5)	26.0 ± 2.4 (n = 5)		
Sorbitol dehydrogenase (EC 1.1.1.14)	SEX		0.985 ± 0.19 (n = 8)	0.860 ± 0.05 (n = 8)	1.142 ± 0.09 (n = 5)	1.077 ± 0.09 (n = 5)	0.761 ± 0.19 (n = 5)	0.632 ± 0.19 (n = 5)		
	SEX		23.14 ± 3.95 (n = 8)	28.24 ± 4.19 (n = 8)	28.42 ± 2.18 (n = 5)	28.69 ± 2.29 (n = 5)	16.97 ± 2.83 (n = 5)	23.43 ± 3.32 (n = 5)		
Glycerol-3-phosphate dehydrogenase (cytosolic) (EC 1.1.1.8)	SEX		2.59 ± 0.32 (n = 8)	1.89 ± 0.28 (n = 8)	2.86 ± 0.38 (n = 5)	1.51 ± 0.66 (n = 5)	2.75 ± 0.51 (n = 5)	2.504 ± 0.13 (n = 5)		
	SEX		527.1 ± 68.4 (n = 8)	582.5 ± 174.5 (n = 8)	578.5 ± 71.3 (n = 5)	511.9 ± 34.9 (n = 5)	578.04 ± 43.5 (n = 5)	569.18 ± 90.3 (n = 5)		

Enzyme activity expressed as  $\mu\text{mol min}^{-1} \text{g}^{-1}$  liver.

Values are Means ± S.D.

Figures in parenthesis represent the number of animals examined.

Rats (initial weight  $50 \pm 5\text{g}$ ) were fed for 110 days ad lib. on Dixon CDD[R] diet alone or CDD[R] diets supplemented with either 5% (w/v) sucrose or glucose in the drinking water.

Appendix 2. The effect of incubating hepatocytes from male rats with different concentrations of 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) on the activities of glycerol kinase and lactate dehydrogenase (see also Fig. 11).

	Incubation time [hr]	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes				
		Control [no 5 $\alpha$ -DHT]	5 $\alpha$ -dihydrotestosterone			
			10 $^{-6}$ M	10 $^{-5}$ M	10 $^{-4}$ M	10 $^{-3}$ M
Glycerol kinase	0	2.29 $\pm$ 0.23	2.12 $\pm$ 0.31	2.21 $\pm$ 0.23	2.86 $\pm$ 0.27	3.10 $\pm$ 0.63
	3	1.92 $\pm$ 0.25	1.88 $\pm$ 0.21	2.17 $\pm$ 0.22	2.93 $\pm$ 0.48	3.20 $\pm$ 0.63
Lactate dehydrogenase	0	388.78 $\pm$ 55.5	384.26 $\pm$ 57.83	397.82 $\pm$ 54.1	418.25 $\pm$ 55.8	430.44 $\pm$ 58.3
	3	389.28 $\pm$ 139.4	399.46 $\pm$ 118.7	411.43 $\pm$ 119.3	398.11 $\pm$ 91.7	423.20 $\pm$ 101.5

Enzyme activities are means  $\pm$  S.D. of four separate experiments  
 Rats were fed on Dixon CDD[R] diet with water to drink ad lib.  
 Mean weights of animals were 390  $\pm$  30 g.

Appendix 3. The effect of incubating hepatocytes from castrated male rats with different concentrations of 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) on the activities of glycerol kinase and lactate dehydrogenase (see also Fig. 12).

Enzyme	Incubation time [hr]	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes			
		Control [no 5 $\alpha$ -DHT]	5 $\alpha$ -dihydrotestosterone		
			10 $^{-6}$ M	10 $^{-5}$ M	10 $^{-4}$ M
Glycerol kinase	0	1.56 $\pm$ 0.22	1.56 $\pm$ 0.22	2.17 $\pm$ 0.14	2.43 $\pm$ 0.25
	3	1.49 $\pm$ 0.09	1.64 $\pm$ 0.16	2.30 $\pm$ 0.41	2.84 $\pm$ 0.26
Lactate dehydrogenase	0	356.88 $\pm$ 21.9	385.41 $\pm$ 25.4	396.64 $\pm$ 17.6	415.46 $\pm$ 13.1
	3	415.58 $\pm$ 20.6	429.1 $\pm$ 16.6	441.62 $\pm$ 6.57	451.99 $\pm$ 38.8

Enzyme activities are means  $\pm$  S.D. of three separate experiments  
 Rats were fed on Dixon CDD[R] diet with water to drink ad lib.  
 Mean weights of animals were 290  $\pm$  10 g.



Appendix 4. The effect of incubating hepatocytes from male rats with different concentrations of estradiol-17 $\beta$  on the activities of glycerol kinase and lactate dehydrogenase (see also Fig. 13).

Enzyme	Incubation time [hr]	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes				
		Control [no estradiol]	Estradiol-17 $\beta$			
			10 <sup>-6</sup> M	10 <sup>-5</sup> M	10 <sup>-4</sup> M	10 <sup>-3</sup> M
Glycerol kinase	0	2.19 $\pm$ 0.09	2.33 $\pm$ 0.08	2.41 $\pm$ 0.17	2.42 $\pm$ 0.17	2.40 $\pm$ 0.19
	3	1.92 $\pm$ 0.34	1.91 $\pm$ 0.20	1.96 $\pm$ 0.31	1.82 $\pm$ 0.36	1.55 $\pm$ 0.51
Lactate dehydrogenase	0	321.04 $\pm$ 51.3	311.24 $\pm$ 41.8	334.61 $\pm$ 26.9	359.1 $\pm$ 39.3	370.53 $\pm$ 30.8
	3	419.01 $\pm$ 85.9	401.66 $\pm$ 85.54	408.45 $\pm$ 85.1	408.5 $\pm$ 79.6	389.62 $\pm$ 88.5

Enzyme activities are means  $\pm$  S.D. of four separate experiments  
Rats were fed on Dixon CDD[R] diet with water to drink ad lib.  
Mean weights of animals were 400  $\pm$  44 g.

Appendix 5. The effect of incubating hepatocytes from female rats with different concentrations of 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) on the activities of glycerol kinase and lactate dehydrogenase (see also Fig. 14).

Enzyme	Incubation time [hr]	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes			
		Control [no 5 $\alpha$ -DHT]	5 $\alpha$ -dihydrotestosterone		
			10 <sup>-6</sup> M	10 <sup>-5</sup> M	10 <sup>-4</sup> M
Glycerol kinase	0	1.88 $\pm$ 0.16	2.14 $\pm$ 0.14	2.39 $\pm$ 0.19	2.59 $\pm$ 0.34
	3	1.65 $\pm$ 0.08	1.92 $\pm$ 0.28	2.25 $\pm$ 0.30	2.43 $\pm$ 0.25
Lactate dehydrogenase	0	300.72 $\pm$ 41.7	327.21 $\pm$ 31.4	345.23 $\pm$ 29.1	388.96 $\pm$ 23.3
	3	330.88 $\pm$ 137.7	362.44 $\pm$ 129.1	369.98 $\pm$ 128.8	394.72 $\pm$ 112.4

Enzyme activities are means  $\pm$  S.D. of four separate experiments  
Rats were fed on Dixon CDD[R] diet with water to drink ad lib.  
Mean weights of animals were 310  $\pm$  20 g.

Appendix 6. The effect of incubating hepatocytes from female rats with different concentrations of estradiol-17 $\beta$  on the activities of glycerol kinase and lactate dehydrogenase (see also Fig. 15).

Enzyme	Incubation time [hr]	Specific activity $\mu\text{mol min}^{-1} \text{g}^{-1}$ hepatocytes				
		Control [no estradiol]	Estradiol-17 $\beta$			
			10 <sup>-6</sup> M	10 <sup>-5</sup> M	10 <sup>-4</sup> M	10 <sup>-3</sup> M
Glycerol kinase	0	1.83 $\pm$ 0.10	1.74 $\pm$ 0.07	1.72 $\pm$ 0.06	1.77 $\pm$ 0.04	1.77 $\pm$ 0.11
	3	1.59 $\pm$ 0.16	1.53 $\pm$ 0.13	1.57 $\pm$ 0.12	1.60 $\pm$ 0.04	1.71 $\pm$ 0.13
Lactate dehydrogenase	0	275.37 $\pm$ 78.9	298.21 $\pm$ 70.1	317.39 $\pm$ 77.2	353.91 $\pm$ 86.5	365.32 $\pm$ 81.6
	3	351.03 $\pm$ 91.8	367.55 $\pm$ 97.9	369.57 $\pm$ 97.4	360.17 $\pm$ 111.8	366.71 $\pm$ 89.6

Enzyme activities are means  $\pm$  S.D. of four separate experiments

Rats were fed on Dixon CDD[R] diet with water to drink ad lib.

Mean weights of animals were 300  $\pm$  10 g.

B I B L I O G R A P H Y

1. World Health Organization (1958) Tech.Rep.Ser. 143, Geneva, Switzerland
2. Department of Health and Social Security Report on Health and Social Subjects (1974) 7, Diet and Coronary Heart Disease HMSO, London
3. McGill, H.C., Jr., Geer, J.C. and Strong, J.P. (1963) In: Atherosclerosis and its Origin, Sandler, M. and Bourne, G., Eds., pp. 39-65, Academic Press, London and New York
4. Smith, E.B., Slater, R.S. and Chu, P.K. (1968) J.Atheroscler. Res. 8, 399-419
5. Timiras, P.S. (1972) In: Developmental Physiology and Aging, Timiras, P.S., Ed., pp. 477-501, Macmillan Co., New York
6. Boyd, G.S. (1970) In: Biochemical Disorders in Human Disease, Thompson, R.H.S. and Wootton, I.D.P., Eds., pp. 635-659, J. & A. Churchill, London
7. Hagerup, L.M. (1974) Acta Med.Scand.Suppl. 557, 32-37
8. Strong, J.P., Eggen, D.A. and Oalman, M.C. (1972) In: The Pathogenesis of Atherosclerosis, Wissler, R.W. and Geer, J.C., Eds., pp. 20-40, The Williams and Wilkins Co., Baltimore
9. Suzangar, M. and Dickson, J.A. (1970) Exp.Cell Res. 63, 353-364
10. Bottcher, C.J.F. and Van Gent, C.M. (1961) J.Atheroscler.Res. 1, 36-46
11. Wissler, R.W. (1965) In: Comparative Atherosclerosis, Roberts, J.C. and Strauss, R., Eds., pp. 342-357, Hoeber, Medical Division, Harper and Row, New York
12. Von Rokitansky, C. (1852) In: A Manual of Pathological Anatomy 4, Rokitansky, C., Ed., pp. 265-275, Translated by G.E. Day, Sydenham Society, London
13. Virchow, R. (1856) Gesammelte Abhandlungen Zur Wiessenschaftlichen Medicin, Frankfurt, 499-513
14. Leary, T. (1941) Arch.Path. 32, 507-555
15. Duguid, J.B. (1946) J.Path.Bact. 58, 207-212
16. Wilens, S.L. (1951) Amer.J.Path. 27, 825-839
17. Weber, G., Convery, H.J.H., Lea, M.A. and Stamm, N.B. (1966) Science 154, 1357-1360

18. Saltykow, S. (1913) Virchow Arch. [Path.Anat.] 213, 8-17
19. Anitschkow, N.N. and Chalatov, S. (1913) Zbl.Allg.Path. 24, 1-9
20. Weber, G., Singhal, R.L., Stamm, N.B., Lea, M.A. and Fisher, E.A. (1966) Adv.Enz.Reg. 4, 59-81
21. Haust, M.D. and More, R.H. (1972) In: The Pathogenesis of Atherosclerosis, Wissler, R.W. and Geer, J.C., Eds., pp. 1-19, The Williams and Wilkins Co., Baltimore
22. Macdonald, I. (1973) Progr.Biochem.Pharmacol. 8, 216-241
23. Robinson, R.W., Higano, N. and Cohen, W.D. (1963) J.Chron.Dis. 16, 155-161
24. Kekki, M. and Nikkila, E.A. (1971) Metabolism 20, 878-889
25. Smith, E.B. and Slater, R.S. (1970) In: Atherosclerosis, Proceedings of the 2nd International Symposium, Jones, R.J., Ed., pp. 42-49, Springer-Verlag, New York
26. Geer, J.C. and Guidry, M.A. (1964) J.Exp.Mol.Path. 3, 485-499
27. Ruffer, M.A. (1911) J.Path.Bact. 15, 453-462
28. Leibowitz, J.O. (1970) Berkeley and Los Angeles, University of California press, 1-4
29. Carew, T.E., Koschinsky, T., Hayes, S.B. and Steinberg, D. (1976) Lancet I, 1315-1317
30. Herman, R.H. (1974) In: Sugars in Nutrition, Sipple, H.L. and McNutt, K.W., Eds., pp. 145-172, Academic Press, New York and London
31. Born, G.V.R. (1962) Nature (London) 194, 927-929
32. Born, G.V.R. and Cross, M.J. (1963) Nature (London) 197, 974-976
33. Williams, T.F., Exton, J.H., Park, C.R. and Regen, D.M. (1968) Amer.J.Physiol. 215, 1200-1209
34. Baur, H. and Heldt, H.W. (1976) In: Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies, Tager, J.M., Soling, H.D. and Williamson, J.R., Eds., pp. 357-362, North-Holland publishing Co., Amsterdam, The Netherlands
35. Kannel, W.B., Dawber, T.R., Friedman, G.D., Glennon, W.E. and McNamara, P.M. (1964) Ann.Int.Med. 61, 888-899

36. Chapman, J.M. and Massey, F.J. (1964) J.Chron.Dis. 17, 933-949
37. Kannel, W.B., Castelli, W.P. and McNamara, P.M. (1967) J.Occup. Med. 2, 611-619
38. Wilhelmsen, L., Wedel, H. and Tibblin, G. (1973) Circulation 48, 950-958
39. Rosenman, R.H., Brand, R.J., Jenkins, D., Friedman, M., Straus, R. and Wurm, M. (1974) JAMA 233, 872-877
40. Aronow, W.S., Allen, W.H., Christofaro, D.D. and Ungermann, S. (1975) Circulation 51, 1038-1045
41. Gertler, M.M. and White, P.D. (1954) Harvard University Press, Cambridge, Mass., 23-54
42. Rose, G.A. (1964) Br.J.Prev.Social Med. 18, 75-78
43. Engel, H.J., Page, H.L. and Campbell, W.B. (1974) JAMA 230, 1531-1534
44. Jensen, J., Blankenhorn, D.H. and Kornerup, V. (1967) Circulation 36, 77-82
45. Heinz, F. (1972) Acta Med.Scand.Suppl. 542, 27-36
46. Adelman, R.C., Spolter, P.D. and Weinhouse, S. (1966) J.Biol.Chem 241, 5467-5472
47. Zakim, D., Pardini, R.S., Herman, R.H. and Sauberlich, H.E. (1967) Biochim.Biophys.Acta 144, 242-251
48. Mulcahy, R., Hickey, N. and Maurer, B. (1967) Circulation 36, 577-586
49. Pereira, J.N. and Jangaard, N.O. (1971) Metabolism 20, 392-400
50. Mahley, R.W., Weisgraber, K.H., Innerarity, T., Brewer, H.B. and Assmann, G. (1975) Biochemistry 14, 2817-2823
51. Albrink, M.J. and Man, E.B. (1959) Arch.Int.Med. 103, 4-8
52. Cohen, A.M. and Teitelbaum, A. (1968) Life Sci. 7, 23-29
53. Bar-On, H. and Stein, Y. (1968) J.Nutr. 94, 95-105
54. Baur, H., Kasperek, S. and Pfaff, E. (1975) Hoppe-Seyler's Z. Physiol.Chem. 356, 827-838

55. Tuovinen, C.G.R. and Bender, A.E. (1975) Nutr.Metabol. 19, 1-9
56. Welborn, T.A., Cumpston, G.N., Cullen, K.J., Curnow, D.H., McCall, M.G. and Stenhouse, N.S. (1969) Amer.J.Epidem. 89, 521-536
57. Kannel, W.B., Le Bauer, E.J., Dawber, T.R. and McNamara, P.M. (1967) Circulation 35, 734-744
58. Landau, B.R. and Merlevede, W. (1963) J.Biol.Chem. 238, 861-867
59. Sugawa-Katayama, Y. and Morita, N. (1977) J.Nutr. 107, 534-538
60. Santen, R.J., Willis, P.W. and Fajans, S.S. (1972) Arch.Int.Med. 130, 833-843
61. Albrink, M.J. (1967) Text Book of Medicine, Vol.II, Beeson and McDermoth, Eds., pp.1164-1174, Saunder's Co., Philadelphia
62. Nikkila, E.A. and Pelkonen, R. (1966) Proc.Soc.Exp.Biol.Med. 123, 91-94
63. Nikkila, E.A. and Ojala, K. (1965) Life Sci. 4, 937-943
64. Goldenberg, S., Aley, M. and Blumenthal, H.T. (1958) Diabetes 7, 98-108
65. Lin, W.J. and Anderson, J.W. (1977) J.Nutr. 107, 584-595
66. Rondier, J., Truffert, J., Lego, A., Cayla, J., Hila, A., De Gennes, J.L. and Delbarre, F. (1977) Annals Clin.Res. 9, 239-245
67. Macdonald, I. (1975) Adv.Exp.Biol.Med. 60, 57-64
68. Heinz, F. and Lamprecht, W. (1967) Hoppe-Seyler's Z.Physiol.Chem. 348, 855-863
69. Claus, T.H., Pilkis, S.J. and Park, C.R. (1975) Biochim.Biophys. Acta 404, 110-123
70. Exton, J.H. and Park, C.R. (1967) J.Biol.Chem. 242, 2622-2636
71. Exton, J.H., Corbin, J.C. and Harper, S.C. (1972) J.Biol.Chem. 247, 4996-5003
72. Levy, R.I. (1971) Fed.Proc. 30, 829-834
73. Zahlten, R.N., Kneer, N.M., Stratman, F.W. and Lardy, H.A. (1974) Arch.Biochem.Biophys. 161, 528-535
74. Salel, A.F., Riggs, K., Masson, D.T., Amsterdam, E.A. and Zelis, R. (1974) Amer.J.Med. 57, 897-903



75. Miller, G.J. and Miller, N.E. (1975) Lancet I, 16-19
76. Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967) New Engl. J.Med. 276, 148-156
77. Palafox, A.L. (1977) Nutr.Rep.Internatl. 15, 355-360
78. Christiansen, N.C. and Horder, M. (1970) Danish Med.Bull. 17, 54-59
79. Keys, A., Taylor, H.L., Blackburn, H., Brozek, J., Anderson, J.T. and Simonson, E. (1963) Circulation 28, 381-395
80. Kannel, W.B. (1974) Giorn.Ital.Cardiol. 4, 123-137
81. Zakim, D. and Herman, R.H. (1968) Biochim.Biophys.Acta 165, 374-379
82. Nikkila, E.A. and Ojala, K. (1964) Life Sci. 3, 1021-1023
83. Hyams, L. and Loop, A. (1969) Amer.J.Epidem. 90, 93-102
84. Albrink, M.J., Meigs, J.W. and Mann, E.B. (1961) Amer.J.Med. 31, 4-23
85. Brown, D.F., Heslin, A.S. and Doyle, J.T. (1961) New Engl.J.Med. 264, 733-737
86. Slack, J. (1969) Lancet II, 1380-1382
87. Pilkis, S.J., Claus, T.H., Johnson, R.A. and Park, C.R. (1975) J.Biol.Chem. 250, 6328-6336
88. Lamprecht, W. and Heinz, F. (1959) Z.Naturforschg.13, 464-465
89. Schwartz, D., Patois, E. and Beaumont, J.L. (1967) J.Atheroscler. Res. 7, 537-548
90. Schaefer, L.E. (1964) Amer.J.Med. 36, 262-268
91. Gale, M.M. and Crawford, M.A. (1969) Metabolism 18, 1021-1025
92. Kannel, W.B., Castelli, W.P. and McNamara, P.M. (1968) Natl.Cancer Instit.Monograph 28, 9-20
93. Doyle, J.T., Dawber, T.R., Kannel, W.B., Kinch, S.H. and Kahn, H.A. (1964) JAMA 190, 886-890
94. Tibblin, G. (1970) Adv.Cardiol. 4, 123-130
95. Moore, B.W. (1959) J.Amer.Chem.Soc. 81, 5837-5838

96. Jenkins, D.C., Rosenman, R.H. and Zyznski, S.J. (1968) Circulation 38, 1140-1155
97. Van Den Berghe, G. (1978) Curr.Top.Cell Regul. 13, 97-135
98. Glock, G.E. and McLean, P. (1955) Biochem.J. 61, 390-397
99. Kormann, A.W., Hurst, R.O. and Flynn, T.G. (1972) Biochim. Biophys.Acta 258, 40-55
100. Toews, C.J. (1967) Biochem.J. 105, 1067-1073
101. Topping, D.L. (1977) Atherosclerosis 26, 129-137
102. Lew, E.A. (1967) In: The Epidemiology of Hypertension, Stamler, J., Stamler, R. and Pullman, T.N., Eds., pp. 392-397, Crane and Stratton, New York and London.
103. Kahn, H.A. (1963) Amer.J.Publ.Health 53, 1058-1067
104. Yudkin, J. (1957) Lancet II, 155-162
105. McDonough, J.R., Kusumi, F. and Bruce, R.A. (1970) Circulation 41, 743-751
106. Riley, C.P., Oberman, A., Lampton, T.D. and Hurst, D.C. (1970) Circulation 42, 43-52
107. Oscai, L.B., Patterson, J.A., Bogard, D.L., Beck, R.J. and Rothermel, B.L. (1972) Amer.J.Cardiol. 30, 775-780
108. Konttinen, A. and Nikkila, E.A. (1967) In: Physical Activity and the Heart, Karvonen, M.J. and Barry, A.J., Eds., pp. 208-215, C.S. Thomas, Springfield, Ill., U.S.A.
109. Gertler, M.M., Garn, S.M. and Levine, S.A. (1951) Ann.Int.Med. 34, 1421-1431
110. Hansen, O.E. (1966) Amer.Heart J. 72, 570-573
111. Frandsen, E.K. and Grunnet, N. (1971) Eur.J.Biochem. 23, 588-592
112. Heinz, F. and Lamprecht, W. (1961) Hoppe-Seyler's Z.Physiol.Chem. 324, 88-100
113. Hers, H.G. (1962) In: The Enzymes 5, Boyer, Lardy and Myrback, Eds., pp. 75-83, Academic Press, New York and London
114. Mikkelsen, W.M., Dodge, H.J. and Valkenberg, H.A. (1965) Amer.J.Med. 39, 242-251

115. Benedek, T.G. (1967) Ann.Int.Med. 66, 851-861
116. Lopez-S, A., Vial, R., Balart, L. and Arroyave, G. (1974) Atherosclerosis 20, 1-9
117. Bennett, A.E., Doll, R. and Howell, R.W. (1970) Lancet I, 1011-1014
118. Quigley, J.P. and Gotterer, G.S. (1969) Biochim.Biophys.Acta 173, 469-476
119. White, L.W. and Landau, B.R. (1965) J.Clin.Invest. 44, 1200-1213
120. Jungmann, R.A. and Schweppe, J.S. (1971) Steroids 17, 541-557
121. Sigrist-Nelson, K. and Hopfer, U. (1974) Biochim.Biophys.Acta 367, 247-254
122. Mavrias, D.A. and Mayer, R.J. (1973) Biochim.Biophys.Acta 291, 531-537
123. Keys, A., Anderson, J.T. and Grande, F. (1957) Lancet I, 66-68
124. Mavrias, D.A. and Mayer, R.J. (1973) Biochim.Biophys.Acta 291, 538-544
125. Marr, J.W. (1973) Health Trends 5, 37-39
126. Dahlqvist, A. (1978) Internatl.Rev.Biochem. 16, 179-206
127. Layne, E. (1957) In: Methods in Enzymology, III, Colowick, S.P. and Kaplan, N.O., Eds., pp. 447-454, Academic Press, New York
128. Keys, A. (1963) Atherosclerosis and its origin, Sandler, M. and Bourne, G., Eds., pp. 263-299, Academic Press, London and New York
129. Grande, F. (1975) In: World Review of Nutrition and Dietetic 22, Karger and Basel, Eds., pp. 248-269, Academic Press, New York
130. Keys, A. (1970) Amer.Heart Assoc.Monogr. 29, American Heart Association, New York
131. Honegger, P. (1973) Ph.D. dissertation, University of Zurich.
132. Keys, A. (1971) Atherosclerosis 14, 193-202
133. Yudkin, J. (1969) Nutr.News 32, 9-12
134. Walker, A.R.P. (1971) Atherosclerosis 14, 137-152
135. Wretlind, A. (1974) In: Sugars in Nutrition, Sipple, H.L. and McNutt, K.W., Eds., pp. 81-92, Academic Press, London and New York

136. Ashton, W.L. (1965) Lancet I, 653-654
137. Yudkin, J. and Morland, J. (1967) Amer.J.Clin.Nutr. 20, 503-506
138. Yudkin, J. and Roddy, J. (1964) Lancet II, 6-8
139. Medical Research Council Working Party Report (1970) Lancet II, 1265-1271
140. Ahrens, E.H. Jr., (1961) Trans.Ass.Amer.Phys. 74, 134-146
141. Kaufmann, N.A. (1970) Atherosclerosis 11, 365-367
142. Macdonald, I. and Braithwaite, D.M. (1964) Clin.Sci. 27, 23-30
143. Macdonald, I. (1966) Amer.J.Clin.Nutr. 18, 369-372
144. Macdonald, I. (1967) Amer.J.Clin.Nutr. 20, 185-190
145. Macdonald, I. (1967) Amer.J.Clin.Nutr. 20, 345-351
146. Grande, F., Anderson, J.T. and Keys, A. (1965) J.Nutr. 86, 313-317
147. Keys, A., Anderson, J.T. and Grande, F. (1960) J.Nutr. 70, 257-266
148. Antar, M.A. and Ohlson, M.A. (1965) J.Nutr. 85, 329-337
149. Antar, M.A., Little J.A., Lucas, C., Buckley, G.C. and Csima, A. (1970) Atherosclerosis 11, 191-201
150. Kuo, P.T. and Bassett, D.R. (1965) Ann.Int.Med. 62, 1199-1212
151. Kaufmann, N.A., Poznanski, R., Blondheim, S.H. and Stein, Y. (1966) Amer.J.Clin.Nutr. 18, 261-269
152. Weiser, M.M. and Quill, H. (1971) Analyt.Biochem. 43, 275-281
153. Herman, R.H., Zakim, D. and Stifel, F.B. (1970) Fed.Proc. 29, 1302-1307
154. Hill, P. (1970) Lipids 5, 621-627
155. Coltart, T.M. and Macdonald, I. (1971) Br.J.Nutr. 25, 323-331
156. Chevalier, M., Wiley, J.H. and Leveille, G.A. (1972) Proc.Soc. Exp.Biol.Med. 139, 220-222
157. Laube, H., Klor, H.U., Fussganger, R. and Pfeiffer, E.F. (1973) Nutr.Metabol. 15, 273-280

158. Bruckdorfer, K.R., Kang, S.S., Khan, I.H., Bourne, A.R. and Yudkin, J. (1974) Horm.Metabol.Res. 6, 99-106
159. Vrana, A., Fabry, P. and Kazdova, L. (1976) Nutr.Rep.Internatl. 14, 593-596
160. Takemoto, T.I. (1975) Tohoku J.Exp.Med. 115, 213-222
161. Szanto, S. and Yudkin, J. (1969) Postgrad.Med.J. 45, 602-607
162. Mann, J.I., Watermeyer, G.S., Manning, E.B., Randles, J. and Truswell, A.S. (1973) Clin.Sci. 44, 601-604
163. Katzen, H.M. and Schimke, R.T. (1965) Proc.Natl.Acad.Sci.USA 54, 1218-1225
164. Macdonald, I. and Roberts, J.B. (1967) Metabolism 16, 572-579
165. Stamler, J., Kjelsberg, M. and Hall, Y. (1960) J.Chron.Dis. 12, 440-455
166. Pick, R., Stamler, J., Rodbard, S. and Katz, L.N. (1952) Circulation 6, 276-280
167. Stamler, J. (1963) In: Atherosclerosis and its Origin, Sandler, M. and Bourne, G., Eds., pp. 231-262, Academic Press, London and New York
168. Stamler, J., Pick, R. and Katz, L.N. (1956) Ann.N.Y.Acad.Sci. 64, 596-619
169. Pick, R., Stamler, J. and Katz, L.N. (1959) In: Hormones and Atherosclerosis, Pincus, G., Eds., pp. 173-195, Academic Press, New York
170. Pick, R., Stamler, J., Rodbard, S. and Katz, L.N. (1959) Circulation Res. 7, 202-204
171. Hartroft, W.S. and Thomas, W.A. (1963) In: Atherosclerosis and its Origin, Sandler, M. and Bourne, G., Eds., pp. 439-457, Academic Press, London and New York
172. Uzunova, A., Ramey, E. and Ramwell, P.W. (1976) Nature (London) 261, 712-713
173. Alvares, F.L. and Nordlie, R.C. (1977) J.Biol.Chem. 252, 8404-8413
174. Dahlqvist, A. (1972) Acta Med.Scand.Suppl. 542, 13-18
175. Blair, D.G.R., Yakimets, W. and Tuba, J. (1963) Canad.J.Biochem. Physiol. 41, 917-929

176. Deren, J.J., Broitman, S.A. and Zamcheck, N. (1967) J.Clin.Invest. 46, 186-195
177. Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z.Physiol. Chem. 210, 33-66
178. Gray, G.M. and Ingelfinger, F.J. (1965) J.Clin.Invest. 44, 390-398
179. Dahlqvist, A. and Borgstrom, B. (1961) Biochem.J. 81, 411-418
180. Crane, R.K. (1960) Physiol.Rev. 40, 789-825
181. Guy, M.J. and Deren, J.J. (1971) Amer.J.Physiol. 221, 1051-1056
182. Kiyasu, J.Y., Katz, J. and Chaikoff, I.L. (1956) Biochim. Biophys.Acta 21, 286-290
183. Fitch, W.M., Hill, R. and Chaikoff, I.L. (1959) J.Biol.Chem. 234, 2811-2813
184. Kiyasu, J.Y. and Chaikoff, I.L. (1957) J.Biol.Chem. 224, 935-939
185. Cadenas, E. and Sols, A. (1960) Biochim.Biophys.Acta 42, 460-468
186. Olsen, W.A. (1974) The Med.Clin.of North America 58, 1387-1395
187. Sols, A. (1956) Biochim.Biophys.Acta 19, 144-152
188. Tepperman, H.M. and Tepperman, J. (1963) Adv.Enz.Reg. 1, 121-136
189. Hers, H.G. (1955) J.Biol.Chem. 214, 373-381
190. Ginsburg, V. and Hers, H.G. (1960) Biochim.Biophys.Acta 38, 427-434
191. Herman, R.H., Stifel, F.B., Greene, H.L. and Herman, Y.F. (1972) Acta Med.Scand.Suppl. 542, 19-25
192. Rosensweig, N.S., Stifel, F.B., Herman, R.H. and Zakim, D. (1968) Biochim.Biophys.Acta 170, 228-234
193. Stifel, F.B., Rosensweig, N.S., Zakim, D. and Herman, R.H. (1968) Biochim.Biophys.Acta 170, 221-227
194. Salas, J., Salas, M., Vinuela, E. and Sols, A. (1965) J.Biol.Chem. 240, 1014-1018
195. Schwarz, G.P. and Basford, R.E. (1967) Biochemistry 6, 1070-1078
196. Katzen, H.M. (1966) Biochem.Biophys.Res.Comm. 24, 531-536
197. Underwood, A.H. and Newsholme, E.A. (1965) Biochem.J. 95, 868-875

198. Heinz, F. (1968) Hoppe-Seyler's Z. Physiol.Chem. 349, 399-404
199. Rosensweig, N.S. and Herman, R.H. (1968) J.Clin.Invest. 47,  
2253-2262
200. Weber, G., Lea, M.A. and Stamm, N.B. (1967) Life.Sci. 6,  
2441-2452
201. Adelman, R.C. (1970) J.Biol.Chem. 245, 1032-1035
202. Kemp, R.G. (1970) J.Biol.Chem. 246, 245-252
203. Brock, D.J.H. (1969) Biochem.J. 113, 235-242
204. Ballentine, R. and Burford, D.D. (1957) In: Methods in Enzymology, Vol.III, Colowick, S.P. and Kaplan, N.O., Eds., pp. 1030-1035, Academic Press, New York
205. Cahill, G.F., Jr., Ashmore, J., Earle, A.S. and Zottu, S. (1958) Amer.J.Physiol. 192, 491-496
206. Warkentin, D.L. and Fondy, T.P. (1973) Eur.J.Biochem. 36,  
97-109
207. Lange, C.F. and Kohn, P. (1961) J.Biol.Chem. 236, 1-5
208. Vinuela, E., Salas, M. and Sols, A. (1963) J.Biol.Chem. 238,  
1175-1177
209. Morrison, G.R. (1967) Arch.Biochem.Biophys. 122, 569-573
210. Lee, Y.P., Craine, J.E. and Lee, A.S.T. (1973) J.Biol.Chem. 248,  
5610-5615
211. Katzen, H.M. (1968) Adv.Enz.Reg. 5, 335-356
212. Ling, K.H., Marcus, F. and Lardy, H.A. (1965) J.Biol.Chem. 240,  
1893-1899
213. Pilkis, S.J. (1968) Proc.Soc.Exp.Biol.Med. 129, 681-684
214. Heinz, F. (1973) Progr.Biochem.Pharmacol. 8, 1-56
215. Leuthardt, F. and Testa, E. (1951) Helv.Chim.Acta 34, 931-938
216. Cori, G.T., Ochoa, S., Slein, M.W. and Cori, C.F. (1951) Biochim.Biophys.Acta 7, 304-317
217. Froesch, E.R., Prader, A., Wolf, H.P. and Labhart, A. (1959) Helv.Paediatr.Acta 14, 99-112

218. Heinz, F., Lamprecht, W. and Kirsch, J. (1968) J.Clin.Invest 47, 1826-1832
219. Heinz, F. and Weiner, F. (1969) Comp.Biochem.Physiol. 31, 283-296
220. Heinz, F. and Lamprecht, W. (1967) Hoppe-Seyler's Z.Physiol. Chem. 348, 1179-1182
221. Heinz, F. and Lamprecht, W. (1968) Comp.Biochem.Physiol. 27, 319-327
222. Sestoft, L. (1974) Biochim.Biophys.Acta 343, 1-16
223. Vestling, C.S., Mylroie, A.K., Irish, U. and Grant, N.H. (1950) J.Biol.Chem. 185, 789-801
224. Kuyper, C.M.A. (1959) Koninkl.Ned.Akad.Wetensch. 62, 137-144
225. Parks, R.E., Ben-Gershom, E. and Lardy, H. (1957) J.Biol.Chem. 227, 231-242
226. Adelman, R.C., Ballard, F.J. and Weinhouse, S. (1967) J.Biol.Chem. 242, 3360-3365
227. Sanchez, J.J., Gonzalez, N.S. and Pontis, H.G. (1971) Biochim. Biophys.Acta 227, 67-78
228. Sanchez, J.J., Gonzalez, N.S. and Pontis, H.G. (1971) Biochim. Biophys.Acta 227, 79-85
229. Tygstrup, N., Winkler, K. and Lundqvist, F. (1965) J.Clin.Invest. 44, 817-830
230. Craig, J.W., Drucker, W.R., Miller, M., Owens, J.E., Woodward, H.Jr., Borfman, B. and Pritchard, W.H. (1951) Proc.Soc.Exp.Biol.Med. 78, 698-702
231. Walker, D.G. (1963) Biochem.J. 87, 576-581
232. Woods, H.F., Eggleston, L.V. and Krebs, H.A. (1970) Biochem.J. 119, 501-510
233. Schapira, F., Schapira, G. and Drefus, J.C. (1961) Enzym.Biol.Clin. 1, 170-175
234. Hers, H.G. and Joassin, G. (1961) Enzym.Biol.Clin. 1, 4-14
235. Hue, L. and Hers, H.G. (1972) Eur.J.Biochem. 29, 268-275



236. Sillero, M.A.G., Sillero, A. and Sols, A. (1969) Eur.J.Biochem. 10, 345-350
237. Perez, N., Clark, T.L., Rabajille, E. and Niemyer, H. (1964) J.Biol.Chem. 239, 2420-2426
238. Kornbluth, R.A., Ostro, M.J., Rittman, L.S. and Fondy, T.P. (1974) FEBS Lett. 29, 190-194
239. Lin, E.C.C. (1977) Ann.Rev.Biochem. 46, 765-795
240. Miller, L.L. (1973) In: Isolated Liver Perfusion and its Applications, Bartosek, I., Guaitani, A. and Miller, L.L., Eds., pp. 11-52, Raven Press, New York
241. Vernon, R.G. and Walker, D.G. (1970) Biochem.J. 118, 531-536
242. Munz, J.A. (1953) Arch.Biochem.Biophys. 42, 435-455
243. McLoughlin, D.J. and MacQuarrie, R. (1978) Biochim.Biophys.Acta 527, 204-211
244. Lehmann, V.F.G. and Pfleiderer, G. (1968) Hoppe-Seyler's Z. Physiol.Chem. 349, 1777-1785
245. Underwood, A.H. and Newsholme, E.A. (1967) Biochem.J. 104, 296-299
246. Krzanowski, J. and Matschinsky, F.M. (1969) Biochem.Biophys.Res. Comm. 34, 816-823
247. Robinson, J. and Newsholme, E.A. (1967) Biochem.J. 104, 2C-4C
248. Tildon, J.T., Stevenson, J.H., Jr., and Ozand, P.T. (1976) Biochem.J. 157, 513-516
249. Pestka, S. (1974) In: Methods in Enzymology, Vol. XXX, Moldave, K. and Grossman, L., Eds., pp. 261-282, Academic Press, New York
250. Thenen, S.W. and Mayer, J. (1975) Proc.Soc.Exp.Biol.Med. 148, 953-957
251. Bubnitz, C. and Kennedy, E.P. (1954) J.Biol.Chem. 211, 951-961
252. Wieland, O. and Suyter, M. (1957) Biochem.Z. 329, 320-331
253. Grunnet, N. and Lundquist, F. (1967) Eur.J.Biochem. 3, 78-84
254. Robinson, J. and Newsholme, E.A. (1969) Biochem.J. 112, 455-464
255. Grunnet, N. (1970) Biochem.J. 119, 927-928
256. Kampf, S.C., Seitz, H.J. and Tarnowski, W. (1970) Hoppe-Seyler's Z.Physiol.Chem. 351, 32-40

257. Kida, K., Kobayashi, K., Kimura, H. and Yugari, Y. (1973) J.Biochem. 73, 299-306
258. Fathipour, A. and Pridham, J.B. (1974) Biochem.Soc.Trans. 2, 1116-1118
259. Young, H.L. and Pace, N. (1958) Arch.Biochem.Biophys. 75, 125-141
260. McGinnis, J.F. and De Vellis, J. (1974) Biochem.Biophys.Res. Comm. 60, 186-195
261. McGinnis, J.F. and De Vellis, J. (1974) Biochim.Biophys.Acta 364, 17-27
262. Fondy, T.P., Solomon, J. and Ross, C.R. (1971) Arch.Biochem. Biophys. 145, 604-611
263. Ross, C.R., Curry, S., Schwartz, A.W. and Fondy, T.P. (1971) Arch.Biochem.Biophys. 145, 591-603
264. Vijayvargiya, R. and Singhal, R.L. (1970) Proc.Soc.Exp.Biol. Med. 133, 670-673
265. Lee, Y.P. and Craine, J.E. (1971) J.Biol.Chem. 246, 7616-7622
266. Fitch, W.M. and Chaikoff, I.L. (1960) J.Biol.Chem. 235, 554-557
267. Daae, L.N.W. (1973) Biochim.Biophys.Acta 306, 186-193
268. McGinnis, J.F. and De Vellis, J. (1977) Arch.Biochem.Biophys. 179, 682-689
269. Malakhova, E.A., Bazasian, G.G., Levchuk, T.P. and Yakovlev, V.A. (1978) Thrombosis Res. 12, 209-218.
270. Lamers, J.M.J. and Hulsmann, W.C. (1973) Biochim.Biophys.Acta 313, 1-8
271. Raivio, K.O., Kekomaki, M.P. and Maenpaa, P.H. (1969) Biochem. Pharmacol. 18, 2615-2624
272. Manning, R. and Brindley, D.N. (1972) Biochem.J. 130, 1003-1012
273. Hajra, A.K. (1968) J.Biol.Chem. 243, 3458-3465
274. Bode, J.Ch., Zelder, O., Rumpelt, H.J. and Wittkamp, U. (1973) Eur.J.Clin.Invest. 3, 436-441
275. Soling, H.D. and Bernhard, G. (1971) FEBS Lett. 13, 201-203

276. Kleisen, C.M. and Borst, P. (1975) Biochim.Biophys.Acta 390, 78-81
277. Eggleston, L.V. and Woods, H.F. (1970) FEBS Lett. 6, 43-45
278. Robinson, D.S. and French, J.E. (1960) Pharmacol.Rev. 12, 241-263
279. Burch, H.B., Lowry, O.H., Meinhardt, L., Max, P., Jr., and Chyu, K. (1970) J.Biol.Chem. 245, 2092-2102
280. Stewart, J.E. and Schotz, M.C. (1971) J.Biol.Chem. 246, 5749-5753
281. Eisenberg, S. and Levy, R.I. (1975) Adv.Lipid Res. 13, 1-89
282. Mansour, T.E. (1963) J.Biol.Chem. 238, 2285-2292
283. Mansour, T.E. and Ahlfors, C.E. (1968) J.Biol.Chem. 243, 2523-2533
284. Alaupovic, P. (1971) Atherosclerosis 13, 141-146
285. Lea, M.A. and Weber, G. (1968) J.Biol.Chem. 243, 1096-1102
286. Simard-Duquesne, N. (1968) Biochem.Biophys.Res.Comm. 33, 874-876
287. Knöpp, R.H., Boroush, M.A. and O'Sullivan, J.B. (1975) Metabolism 24, 481-493
288. Garfinkel, A.S., Nilsson-Ehle, P. and Schotz, M.C. (1976) Biochim.Biophys.Acta 424, 264-273
289. Robinson, D.S. and Wing, D.R. (1971) Biochem.Soc.Symposia 33, Smellic, R.M.S., Ed., pp. 123-135, Academic Press, London and New York
290. Eaton, R.P. (1971) J.Lipid Res. 12, 491-497
291. Dutt, M. (1974) Indian J.Med.Res. 62, 1011-1016
292. Robinson, D.S. and Wing, D.R. (1970) In: Adipose Tissue, Levine and Pfeiffer, Eds., pp. 41-46, Academic Press, London and New York.
293. La Rosa, J.C., Levy, R.I., Windmueller, H.G. and Fredrickson, D.S. (1972) J.Lipid Res. 13, 356-363
294. Krauss, R.M., Windmueller, H.G., Levy, R.I. and Fredrickson, D.S. (1973) J.Lipid Res. 14, 286-295
295. Greten, H., Sniderman, A.D., Chandler, J.G., Steinberg, D. and Brown, W.V. (1974) FEBS Lett. 42, 157-160

296. Assmann, G., Krauss, R.M., Fredrickson, D.S. and Levy, R.I. (1973) J.Biol.Chem. 248, 1992-1999
297. Pollock, R.J., Hajra, A.K. and Agranoff, B.W. (1975) Biochim. Biophys.Acta 380, 421-435
298. Bruchovsky, N. and Wilson, J.D.(1968) J.Biol.Chem. 243, 2012-2021
299. Bruchovsky, N. and Wilson, J.D.(1968) J.Biol.Chem. 243, 5953-5960
300. Lloyd-Davies, K.A. and Brindley, D.N. (1975) Biochem.J. 152, 39-49
301. Lamb, R.G. and Fallon, H.J. (1974) Biochim.Biophys.Acta 348, 166-178
302. Heimberg, M., Dunkerley, A. and Brown, T.O. (1965) Biochem. Pharmacol. 14, 890-893
303. Heimberg, M., Weinstein, I. and Kohout, M. (1969) J.Biol.Chem. 244, 5131-5139
304. Hajra, A.K. and Agranoff, B.W. (1968) J.Biol.Chem. 243, 3542-3555
305. La Belle, E.F., Jr., and Hajra, A.K. (1972) J.Biol.Chem. 247, 5835-5841
306. Scrutton, M.C. and Utter, M.F. (1968) Ann.Rev.Biochem. 37, 249-302
307. Rognstad, R., Clark, D.G. and Katz, J. (1974) Biochem.J. 140, 249-251
308. Mayes, P.A. (1976) Biochem.Soc.Trans. 4, 575-580
309. Tanaka, T., Harano, Y., Sue, F. and Morimura, H. (1967) J.Biochem.TOKYO 67, 71-91
310. Topping, D.L. and Mayes, P.A. (1972) Biochem.J. 126, 295-311
311. Gardner, R.S. and Mayes, P.A. (1974) Biochem.Soc.Trans. 2, 1127-1128
312. Zawalich, W.S., Rognstad, R., Pagliara, A.S. and Matschinsky, F.M. (1977) J.Biol.Chem. 252, 8519-8523
313. Williams, P., Robinson, D. and Bailey, A. (1979) Lancet I, 72-75
314. Jourdan, M.H. (1969) Proc.Nutr.Soc. 28, 10A-11A
315. Cryer, A., Riley, S.E., Williams, E.R. and Robinson, D.S. (1974) Biochem.J. 140, 561-563

316. Macdonald, I. (1970) Br.J.Nutr. 24, 537-543
317. Hansson, V., Tvester, K.J. and Attramadal, A. (1971) Acta Endocrinol. 67, 384-392
318. Aten, R.F., Dickson, R.B. and Eisenfeld, A.J. (1978) Endocrinology 103, 1629-1635
319. Aten, R.F., Weinberger, M.J. and Eisenfeld, A.J. (1978) Endocrinology 102, 433-442
320. Eisenfeld, A.J., Aten, R.F., Weinberger, M.J., Haselbacker, G., Halpern, K. and Krakoff, L. (1976) Science 191, 862-864
321. Watkins, M.L., Fizette, N. and Heimberg, M. (1972) Biochim. Biophys.Acta 280, 82-85
322. Hashemi, S.A. reported by Pridham, J.B. and Davies, D.R. (1979) In: Sugar: Science and Technology, Birch, G.G. and Parker, K.J., Eds., pp. 437-454, Applied Science Publishers Limited, London
323. Robel, P. (1971) Karolinska Symposia on Research Methods in Reproductive Endocrinology, 3rd Symposium, pp. 279-294
324. Ching-Hui, W., Hoshi, M. and Shreeve, W.W. (1974) Metabolism 23, 1125-1140
325. Mann, J.I., Truswell, A.S. and Pimstone, B.L. (1971) Clin.Sci. 41, 123-129
326. Kupke, I.R. (1973) Progr.Biochem.Pharmacol. 8, 57-124
327. Yu, H.L. and Burton, D.N. (1974) Arch.Biochem.Biophys. 161, 297-305
328. Bloch, K. and Vance, D. (1977) Ann.Rev.Biochem. 46, 263-298
329. Cahlin, E., Jonsson, J., Persson, B., Stakeberg, H., Bjorntorp, P., Gustafson, A. and Schersten, T. (1973) Scand.J.Clin.Lab.Invest. 32, 21-33
330. Carlson, C.A. and Kim, K.H. (1974) Arch.Biochem.Biophys. 164, 478-489
331. Allred, J.B. and Roehrig, K.L. (1973) J.Biol.Chem. 248, 4131-4133
332. Flory, W., Peczon, B.D., Koeppe, R.E. and Spivey, H.O. (1974) Biochem.J. 141, 127-131
333. Bruckdorfer, K.R. and Yudkin, J. (1975) Nutr.Metabol. 19, 225-232

334. Macdonald, I. (1962) J.Physiol. 162, 334-344
335. Cohen, A.M., Briller, S. and Shafrir, E. (1972) Biochim. Biophys.Acta 279, 129-138
336. Casal, J.J. and Holman, R.T. (1965) J.Amer.Oil Chem.Soc. 42, 1134-1137
337. Macdonald, I., Rebello, T. and Keyser, A. (1975) Nutr. Metabol. 18, 283-293
338. Carminatti, H., De Asua, L.J., Recondo, E., Passeron, S. and Rozengurt, E. (1968) J.Biol.Chem. 243, 3051-3056
339. Cardenas, J.M. and Dyson, R.D. (1973) J.Biol.Chem. 248, 6938-6944
340. Mukherjee, S., Basu, M. and Trivedi, K. (1969) J.Atheroscler.Res. 10, 261-272
341. Maruhama, Y. (1970) Metabolism 19, 1085-1093
342. Waddell, M. and Fallon, H.J. (1973) J.Clin.Invest. 52, 2725-2731
343. Kutzbach, C., Bischofberger, H., Hess, B. and Telschow, Z. (1973) Hoppe-Seyler's Z.Physiol.Chem. 354, 1473-1489
344. Tanaka, T., Sue, F. and Morimura, H. (1967) Biochem.Biophys. Res.Comm. 29, 444-449
345. Weber, G., Lea, M.A., Convery, H.J.U. and Stamm, N.B. (1967) Adv.Enz.Reg. 5, 257-298
346. Walker, D.G. (1966) Biochem.J. 100, 17P
347. Sharma, C., Manjeshwar, R. and Weinhouse, S. (1963) J.Biol.Chem. 238, 3840-3845
348. Chevalier, M.M., Wiley, J.H. and Leveille, G.A. (1972) J.Nutr. 102, 337-342
349. Taunton, O.D., Stifel, F.B., Greene, H.L. and Herman, R.H. (1972) Biochem.Biophys.Res.Comm. 48, 1663-1670
350. Clark, M.G., Kneer, N.M., Bosch, A.L. and Lardy, H.A. (1974) J.Biol.Chem. 249, 5695-5703
351. Taunton, O.D., Stifel, F.B., Greene, H.L. and Herman, R.H. (1974) J.Biol.Chem. 249, 7228-7239

352. Stifel, F.B., Taunton, O.D., Greene, H.L. and Herman, R.H. (1974) J.Biol.Chem. 249, 7240-7244
353. Carbonell, J., Feliu, J.E., Marco, R. and Sols, A. (1973) Eur.J.Biochem. 37, 148-156
354. Marie, J., Kahn, A. and Boivin, P. (1976) Biochim.Biophys.Acta 438, 393-406
355. Engstrom, L. (1978) Curr.Top.Cell Reg. 131, 29-51
356. Susor, W.A. and Rutter, W.J. (1968) Biochem.Biophys.Res.Comm. 30, 14-20
357. Exton, J.H. and Park, C.R. (1968) J.Biol.Chem. 243, 4189-4196
358. Exton, J.H. and Park, C.R. (1969) J.Biol.Chem. 244, 1424-1433
359. Reed, L.J. (1974) Accounts Chem.Res. 7, 40-46
360. Tsai, C.S., Burgett, M.W. and Reed, L.J. (1973) J.Biol.Chem. 248, 8348-8352
361. Portenhauser, R. and Wieland, O. (1972) Eur.J.Biochem. 31, 308-314.
362. Hucho, F., Randall, D.D., Roche, T.E., Bergett, M.W., Pelley, J.W. and Reed, L.J. (1972) Arch.Biochem.Biophys. 151, 328-340
363. Pettit, F.H., Roche, T.E. and Reed, L.J. (1972) Biochem.Biophys. Res.Comm. 49, 563-571
364. Roy, A.K., Millin, B.S. and McMinn, D.M. (1974) Biochim.Biophys. Acta 354, 213-232
365. Irwin, J.F., Lane, S.E. and Neuhaus, O.W. (1971) Biochim.Biophys. Acta 252, 328-334
366. Roy, A.K. and Neuhaus, O.W. (1967) Nature (London) 214, 618-620
367. Gustafsson, J.A., Pousette, A., Stenberg, A. and Wrangé, O. (1975) Biochemistry 14, 3942-3948
368. Gustafsson, J.A. and Pousette, A. (1975) Biochemistry 14, 3094-3101
369. Czech, M.P. (1977) Ann.Rev.Biochem. 46, 359-384
370. Martin, B.R., Denton, R.M., Pask, H.T. and Randle, P.J. (1972) Biochem.J. 129, 763-773

371. Taylor, S.I., Mukherjee, C. and Jungas, R.L. (1973) J.Biol.Chem. 248, 73-81
372. Wieland, O., Patzelt, C. and Loffler, G. (1972) Eur.J. Biochem. 26, 426-433
373. Garrison, J.C. (1978) J.Biol.Chem. 253, 7091-7100
374. Brown, L.M. and Harrison, M.F. (1951) Nature (London) 168, 83-85
375. Fishman, W.H. and Farmelant, M.H. (1953) Endocrinology 52, 536-542
376. Braidman, I.P. and Rose, D.P. (1971) Endocrinology 89, 1250-1255
377. Braidman, I.P. and Rose, D.P. (1971) Biochem.Pharmacol. 20, 973-975
378. Gurtoo, H.L. and Parker, N.B. (1976) Biochem.Biophys.Res.Comm. 72, 216-222
379. Denef, C. and De Moor, P. (1972) Endocrinology 91, 374-384
380. Hay, A.W.M. and Pridham, J.B. (1973) Proc.Nutr.Soc. 32, 33A
381. Boyns, D.R., Crossley, J.N., Abrams, M.E., Jarrett, R.J. and Keen, H. (1969) Brit.Med.J. 1, 595-598
382. Walker, D.G. (1963) Biochem.J. 88, 17P
383. Walker, D.G. and Rao, S. (1964) Biochem.J. 90, 360-368
384. Glock, G.E. and McLean, P. (1953) Biochem.J. 55, 400-408
385. Huggins, C. and Yao, F-O (1959) J.Exp.Biol.Med. 110, 899-919
386. Stifel, F.B., Herman, R.H. and Rosensweig, N.S. (1969) Biochim Biophys.Acta 184, 495-502
387. Stifel, F.B., Herman, R.H. and Rosensweig, N.S. (1970) Biochim. Biophys. Acta 208, 368-372
388. Lufkin, E.G., Stifel, F.B., Herman, R.H. and Rosensweig, N.S. (1972) J.Clin.Endocrinol. 34, 586-591
389. Schwarzlose, W. and Heim, F. (1973) Life Sci. 12, 107-115
390. Kerr, J.S. and Frankel, H.M. (1976) Internatl.J.Biochem. 7, 455-460



391. Lane, M.D., Moss, J. and Polakis, S.E. (1974) Curr.Top. Cell Reg. 8, 139-195
392. Macdonald, I. (1976) Proc.Nutr.Soc. 35, 155-157
393. Rossner, S., Larsson, C.V., Carlson, L.A. and Boberg, J. (1971) Acta Med.Scand. 190, 301-305
394. Gustafsson, J.-A. and Stenberg, A. (1974) J.Biol.Chem. 249, 711-718
395. Gustafsson, J.-A. and Stenberg, A. (1974) J.Biol.Chem. 249, 719-723
396. Bruckdorfer, K.R., Kang, S.S. and Yudkin, J. (1972) Proc. Nutr.Soc. 31, 11A
397. Volpe, J.J. and Vagelos, P.R. (1973) Ann.Rev.Biochem. 42, 21-60
398. Jefferys, D.B. and White, I.R. (1973) Nutr.Rep.Internatl. 8, 201-209
399. Groener, J.E.M. and Macdonald, I. (1975) Nutr.Metabol. 19, 212-222
400. Weinstein, I., Selzer, M. and Belitsky, R. (1974) Biochim. Biophys.Acta 348, 14-22
401. Dahlen, J.V., Kennan, A.L. and Porter, J.W. (1968) Arch. Biochem.Biophys. 124, 51-57
402. Soler-Argilaga, C. and Heimberg, M. (1976) J.Lipid Res. 17, 601-615
403. Afolabi, S.K., Tulloch, B.R., Kissebah, A.H., Vydellingum, N. and Fraser, T.R. (1976) Clin.Endocrinology 5, 203-208
404. Farber, F. (1967) In: Advances in Lipid Research 5, Paoletti, R. and Kritchevsky, D., Eds., pp.119-183, Academic Press, London and New York.
405. Bar-On, H., Stein, O. and Stein, Y. (1972) Biochim.Biophys.Acta 270, 444-452
406. Hosotani, T. and Yoshida, A. (1974) J.Nutr.Sci.Vitaminol. 20, 215-225
407. Bonsignore, A. and De Flora, A. (1972) Curr.Top.Cell Reg. 6, 21-62

408. Matsuda, T. and Yugari, Y. (1967) J.Biochem. 61, 535-540
409. Yugari, Y. and Matsuda, T. (1967) J.Biochem. 61, 541-549
410. Avigad, G. (1966) Proc.Natl.Acad.Sci. USA 56, 1543-1547
411. Huttunen, J.K., Ehnholm, C., Kekki, M. and Nikkila, E.A. (1976) Clin.Sci.Mol.Med. 50, 249-260
412. Kim, H.J. and Kalkhoff, R.K. (1975) J.Clin.Invest. 56, 888-896
413. Nikkila, E.A. and Kekki, M. (1971) Acta Med.Scand. 190, 49-59
414. Glueck, C.J., Fallat, R.W. and Scheel, D. (1975) Metabolism 24, 537-545
415. Hamosh, M. and Hamosh, P. (1975) J.Clin.Invest. 55, 1132-1135
416. Rudack, D., Chisholm, E.M. and Holten, D. (1971) J.Biol.Chem. 246, 1249-1254
417. Rudack, D. and Holten, D. (1975) J.Biol.Chem. 250, 3960-3965
418. Rudack, D., Davie, B. and Holten, D. (1971) J.Biol.Chem. 246, 7823-7824
419. Proscal, D. and Holten, D. (1972) Biochemistry 11, 1310-1314
420. Proscal, D., Winberry, L. and Holten, D. (1976) J.Biol.Chem. 251, 3539-3544
421. Mandour, T., Kissebah, A. and Wynn, V. (1977) Eur.J.Clin. Invest. 7, 181-187
422. Weidenhagen, R. (1932) Ergebnisse Der Enzymforsch. 1, 168-208
423. Soling, H.D., Willms, B. and Janson, G. (1970) FEBS Lett. 11, 324-327
424. Shiff, T.S., Roheim, P.S. and Eder, H.A. (1971) J.Lipid Res. 12, 596-603
425. Maruhama, Y. and Macdonald, I. (1972) Metabolism 21, 835-842
426. Zucker, I. (1969) Physiol.Behav. 4, 595-602
427. Wade, G.N. and Zucker, I. (1969) Physiol.Behav. 4, 935-943
428. Wade, G.N. and Zucker, I. (1970) J.Comp.Physiol.Psych. 70, 213-220

429. Kakolewski, J.W., Cox, V.C. and Valenstein, E.S. (1968) Psych.Rep. 22, 547-554
430. Bull, L.S., Hurley, W.L., Kennett, W.S., Tamplin, C.B. and Williams, W.F. (1974) J.Nutr. 104, 968-975
431. Tay, B.S. (1977) Ph.D. Thesis, Royal Holloway College (University of London)
432. Hers, H.G. (1955) In: Methods in Enzymology, Vol. I, Colowick, S.P. and Kaplan, N.O., Eds., pp. 286-290, Academic Press, New York.
433. Hers, H.G. (1952) Biochim.Biophys.Acta 8, 416-423
434. Roe, J.H., Epstein, J.H. and Goldstein, N.P. (1949) J.Biol. Chem. 178, 839-845
435. Hers, H.G., Beaufays, H. and De Duve, C. (1953) Biochim. Biophys.Acta 11, 416-426
436. Weiser, M.M. and Quill, H. (1975) In: Methods in Enzymology, Vol. XLI, Colowick, S.P. and Kaplan, N.O., Eds., pp. 61-63, Academic Press, New York
437. Blakley, R.L. (1951) Biochem.J. 49, 257-271
438. Williams-Ashman, H.G. and Banks, J. (1954) Arch.Biochem.Biophys. 50, 513-515
439. Gerlach, V. and Hiby, W. (1974) In: Methods of Enzymatic Analysis 2, Bergmeyer, H.U., Ed., pp. 569-573, Academic Press, New York.
440. Gabbay, K.H. and O'Sullivan, J.P. (1968) Diabetes 17, 239-243
441. Smith, M.G. (1962) Biochem.J. 83, 135-144
442. Asada, M. and Galambos, J.T. (1963) Gastroenterology 44, 578-587
443. Hers, H.G. (1956) Biochim.Biophys.Acta 22, 202-203
444. Exton, J.H. and Edson, N.L. (1964) Biochem.J. 91, 478-483
445. Adcock, L.H. and Gray, C.H. (1957) Biochem.J. 65, 554-560
446. Heinz, F. and Wittneben, H-E. (1970) Hoppe-Seyler's Z.Physiol. Chem. 351, 1215-1220

447. Renold, A.E., Hastings, A.B. and Nesbett, F.B. (1954) J.Biol.Chem. 209, 687-696
448. Baquer, N.Z., Cascales, M., McLean, P. and Greenbaum, A.L. (1976) Eur.J.Biochem. 68, 403-413
449. Harding, J.W., Pyeritz, E.A., Copeland, E.S. and White, H.B. (1975) Biochem.J. 146, 223-229
450. Tepperman, H.M. and Tepperman, J. (1968) Amer.J.Physiol. 214, 67-72
451. De Gomez Dumm, I.N.T., De Alaniz, M.J.T. and Brenner, R.R. (1970) J.Lipid Res. 11, 96-101
452. Allen, R.J.L. and Leahy, J.S. (1966) Br.J.Nutr. 20, 339-347
453. Chan, L. and O'Malley, B.W. (1976) N.Eng.J.Med. 294, 1322-1328
454. Mans, R.J. and Novelli, G.D. (1961) Arch.Biochem.Biophys. 94, 48-53
455. Lane, S.E. and Neuhaus, O.W. (1972) Biochim.Biophys.Acta 257, 461-470
456. Roy, A.K., McMinn, D.M. and Biswas, N.M. (1975) Endocrinology 97, 1501-1508
457. Gabbay, K.H. (1974) In: Sugars in Nutrition, Sipple, H.L. and McNutt, K.W., Eds., pp. 469-482, Academic Press, London and New York
458. Newsholme, E.A., Robinson, J. and Taylor, K. (1967) Biochim. Biophys. Acta 132, 338-346
459. Bublitz, C. and Wieland, O. (1962) In: Methods in Enzymology, Vol.V, Colowick, S.P. and Kaplan, N.O., Eds., pp. 354-361, Academic Press, London and New York
460. Bergmeyer, H.U. and Bernt, E. (1974) In: Methods of Enzymatic Analysis, Vol. II, Bergmeyer, H.U., Ed., pp. 574-579, Academic Press, London and New York
461. Jeejeebhoy, K.N. and Phillips, M.J. (1976) Gastroenterology 71, 1086-1096
462. Rous, P. and Beard, J.W. (1934) J.Exp.Med. 59, 577-591
463. Schneider, W.C. and Potter, V.R. (1943) J.Biol.Chem. 149, 217-227

464. Branster, M.V. and Morton, R.K. (1957) Nature(London) 180, 1283-1284
465. Rodbell, M. (1964) J.Biol.Chem. 239, 375-380
466. Howard, R.B., Christiansen, A.K., Gibbs, F.A. and Pesch, L.A. (1967) J.Biol.Chem. 35, 675-684
467. Berry, M.N. and Friend, D.S. (1969) J.Cell Biol. 43, 506-520
468. Krebs, H.A., Cornell, N.W., Lund, P. and Hems, R. (1973) In: Regulation of Hepatic Metabolism, Alfred Benzon Symposium VI, Lundquist, F. and Tygstrup, N., Eds., pp. 726-750, Academic Press, New York.
469. Wagle, S.R. and Ingebretsen, W.R. Jr., (1975) In: Methods in Enzymology, Vol.XXXV, Lowenstein, J.M., Ed., pp. 579-594, Academic Press, London and New York
470. Geelen, M.J.H. and Gibson, D.M. (1976) In: Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies, Tager, J.M., Soling, M.D. and Williamson, J.R., Eds., pp. 219-232, North-Holland Publishing Co., Amsterdam
471. Muller, P., Singh, A., Orci, L. and Jeanrenaud, B. (1976) Biochim.Biophys.Acta 428, 480-494
472. Assimacopoulos-Jeannet, F., Karakash, C., Le Marchand, Y. and Jeanrenaud, B. (1977) Biochim.Biophys.Acta 498, 91-101
473. Hems, D.A. (1977) FEBS Lett. 80, 237-245
474. Cook, G.A. Nielson, R.C., Hawkins, R.A., Mehlman, M.A., Lakshmanan, M.R. and Veech, R.L. (1977) J.Biol.Chem. 252, 4421-4424
475. Nakanishi, S., Tanabe, T., Horikawa, S. and Numa, S. (1976) Proc.Natl.Acad.Sci.USA 73, 2304-2307
476. Lakshmanan, M.R., Nepkroeff, C.M. and Porter, J.W. (1972) Proc.Natl.Acad.Sci.USA 69, 3516-3519
477. Waymouth, C. (1959) J.Natl.Cancer Inst. 22, 1003-1017
478. Zivin, J.A. and Bartko, J.J. (1976) Life Sci. 18, 15-26