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The Acute Biochemical Response of the Starved Rabbit Liver *in situ* to Glucose Infusion

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Abstract

Increasing the blood glucose levels from 88 to 400 mg/100 ml in rabbit liver *in situ* during 5-min time intervals resulted in a decrease in the production of CO_2 from C-1 of liver glucose together with a slight increase in the oxidation of C-6 of glucose; this was caused by a high glucose-induced decrease in the activity of the oxidative pentose phosphate pathway. Increasing the glucose concentrations also resulted in a threefold increase in the levels of palmitoyl- and stearoyl-CoA esters at a glucose load of 0·8 g; this is consistent with a specific feed-back inhibition of the production of NADPH by reactions of the oxidative pentose phosphate pathway by long-chain fatty acyl-CoAs. A decrease in plasma free fatty acids occurred when blood glucose levels were raised; this was associated with an increase in the concentration of free fatty acids in liver.

There was a decreased glucose output by liver following an increased glucose load. In liver there were increases in AMP levels, decreased ATP levels, a twofold increase in fructose 1,6-diphosphate and a fall in glucose 6-phosphate and fructose 6-phosphate concentrations. These changes are indicative of the decreased gluconeogenic flux caused through inhibition of hexosediphosphatase by AMP, and the elevated levels of the long-chain fatty acyl-CoA esters probably acted to inhibit adenine nucleotide translocation across the mitochondrial membrane. The formation of *sn*-glycero-3-phosphate appears to be the rate-limiting step for relieving the inhibitions caused by the accumulation of long-chain fatty acyl-CoAs.

Introduction

In animal tissues control of fatty acid synthesis is exerted at the levels of acetyl-CoA carboxylase (EC 6.4.1.2) and the fatty acid synthetase system, as well as the supporting pathways that provide the carbon precursors and reducing equivalents. Considerable evidence has been obtained which identifies the regulatory status of some key enzymes of fatty acid synthesis by monitoring the changes of enzyme levels in response to altered physiological states (Lane and Moss 1971). Animals in the fasted state are characterized by a rapid decline in fatty acid synthesis which is associated with decreased levels in the activities of acetyl-CoA carboxylase, the fatty acid synthetase system, and the enzymes that generate carbon precursors and reducing equivalents for lipogenesis. Under these conditions there is marked elevation of the concentrations of both plasma free fatty acids (Fredrickson and Gordon 1958) and long-chain fatty acyl-CoA esters in liver (Bortz and Lynen 1963), and it has been proposed that the elevated concentration of fatty acyl-CoA esters would reduce lipogenesis from short-chain precursors in liver by inhibition of acetyl-CoA carboxylase and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (Taketa and Pogell 1966).

Upon refeeding a high-carbohydrate fat-free diet to starved animals, long-chain fatty acid oxidation in both liver and extra hepatic tissue is markedly decreased

(Lossow and Chaikoff 1955), with a corresponding decrease in the concentration of plasma free fatty acids (Gordon 1957), but there is a marked and coordinate elevation of the levels of all the key enzymes of lipogenesis (Muto and Gibson 1970). However, it has been observed that under these nutritional conditions lipogenesis does not commence until at least 4 h after the feeding of the high-carbohydrate diet to the starved animal (Baker *et al.* 1968).

An explanation of this lag phase in lipogenesis following refeeding after starvation is that there is initially insufficient *sn*-glycero-3-phosphoric acid* to allow for rapid production of triglycerides (Fallon and Kemp 1968). This deficiency in *sn*-glycero-3-phosphate would be particularly important in the liver if long-chain fatty acyl-CoA derivatives act as the *in vivo* metabolic inhibitors of acetyl-CoA carboxylase and glucose-6-phosphate dehydrogenase (Taketa and Pogell 1966), because an initial period of triglyceride synthesis would be required to remove the inhibitory concentrations of long-chain fatty acyl-CoA esters and thus allow optimal activation of the lipogenic pathway.

This paper reports results of a study of liver metabolism *in situ* (Williams *et al.* 1971*a*) where after administration of glucose initial events of glucose metabolism in livers of rabbits starved for 40 h result in elevated concentrations of long-chain fatty acyl-CoA esters in liver which act to inhibit the reactions of the oxidative segment of the pentose pathway.

Materials and Methods

Chemicals and Enzymes

All enzymes, coenzymes and substrates were obtained from Calbiochem Aust. Pty Ltd, Sydney. Substrates were in the form of their sodium salts. Inorganic and organic reagents were of A.R. grade (E. Merck A.G., Darmstadt, Germany). D-[1-^{14}C]Glucose (40 mCi/mmol), D-[6-^{14}C]glucose (45 mCi/mmol) and ^{63}Ni (10 mCi/10 mg Ni) were obtained from the Radiochemical Centre, Amersham, England. Ethylene glycol succinate, silicic acid and acid-washed Chromosorb G (80–100 mesh) were obtained from Varian Pty Ltd, Sydney. Heparin (Pularin) was obtained from Evans Medical Ltd, U.K.

Experimental Animals

New Zealand white female rabbits (*Oryctologus cuniculus*), whose average body weight was 2·6± 0·2 kg, were obtained from the Animal Breeding Unit, Prince Henry Hospital, Sydney. Rabbits were deprived of food but not water for 40 h prior to experimentation.

Surgical Procedure

Rabbits starved for 40 h were anaesthetized with diethyl ether–carbogen gas mixture (CO_2 : O_2 = 5 : 95 v/v) and the liver was surgically exposed as described by Williams *et al.* (1971*a*). Six minutes after the commencement of anaesthesia, glucose (0–1·6 g dissolved in 4 ml of 0·9% NaCl) was injected into the inferior vena cava over a period of 15–20 s. Precisely 6 min after the administration of glucose, radioactive substrate contained in 1 ml of water was infused into the portal vein at the rate of 4·0 ml/min for 15 s. The duration and rate of infusion ensured that there was an even distribution of isotopically labelled substrate throughout all liver lobes (Williams *et al.* 1971*a*). At the conclusion of the 15-s infusion, blood flow from the hepatic veins to the vena cava was arrested (Williams *et al.* 1971*a*). After 5 min of partial ischaemia (Williams *et al.* 1973) the liver was rapidly removed (8 s) and crushed in liquid nitrogen (Williams *et al.* 1971*a*). In order to determine the effect of the 'glucose load' on the circulating levels of blood glucose and free fatty acids, 1-ml

* This nomenclature for glycerophosphate follows the stereospecific numbering system for lipids as recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (see Blackmore *et al.* 1973).

samples of blood were collected in heparinized syringes from the portal vein 1 min before the infusion of radioactive substrate and just prior to the removal of the liver. Blood samples were also removed from the hepatic veins (Williams *et al.* 1973) 1 min prior to glucose infusion and again immediately prior to the removal of the liver.

Determination of Glycolytic Intermediates

Glycolytic intermediates were extracted from frozen powdered rabbit liver and assayed using the procedures detailed by Irving *et al.* (1972). $NADP^+$ and NADPH were determined by the method of Klingenberg (1963). Blood glucose was determined on perchlorate-treated heparinized blood by the method of Hugget and Nixon (1957).

Isolation of CO_2 and Determination of Specific Radioactivity

CO_2 was isolated from frozen liver powder using the method of Williams *et al.* (1971*a*).

Isolation and Determination of Free Fatty Acids

Free fatty acids were quantitatively extracted from plasma by a modification of the method of Dole and Meinertz (1960) and determined by a modification of the method of Ho (1970). Plasma (50 μl) was mixed thoroughly with 100 μl of extraction medium (0·5M H_2SO_4–n-heptane–isopropanol, 2 : 20 : 78 v/v). n-Heptane (100 μl) and 100 μl of water were added and the solution thoroughly mixed, then centrifuged (5000 *g* for 5 min). To 50 μl of the upper heptane phase was added 10 mg of silicic acid and 50 μl of chloroform. After mixing, the solution was centrifuged as before. To 50 μl of the heptane phase was added 100 μl of chloroform–n-heptane (55 : 45 v/v) and 10 μl of ^{63}Ni working solution (10^7 c.p.m./ml) (Ho 1970), and the solution thoroughly mixed. A portion (100 μl) of the upper phase was added to 5 ml of scintillation mixture and the radioactivity determined. The scintillation mixture was composed of toluene, 350 ml; dioxane, 350 ml; methanol, 210 ml; naphthalene, 73 g; 2,5-diphenyloxazole, 4·45 g; and 1,4-bis(5-phenyloxazol-2-yl)benzene, 73 mg. The concentration of free fatty acid in the final aliquot was determined from a standard curve prepared by the identical treatment of 50-μl samples containing 25–100 μmol of palmitic acid.

The free fatty acid content of liver was determined on a 0·5-g sample of the frozen tissue. Each sample of tissue was homogenized in 2 ml of extraction medium (Dole and Meinertz 1960). To the homogenate was added 2 ml n-heptane and 2 ml water. After thorough mixing the heptane was separated from the mixture and retained. To the remaining aqueous phase was added a further 2 ml of n-heptane and the homogenization repeated. The homogenization was repeated four times in all, and the combined heptane phases evaporated to dryness under an atmosphere of nitrogen. The residue was dissolved in 10 ml diethyl ether and the free fatty acids separated from other lipid material by application to a silicic acid column and elution with 2% (v/v) formic acid in diethyl ether as described by McCarthy and Duthie (1962). The eluate containing the free fatty acids was evaporated to dryness and the methyl esters of the fatty acids prepared using the method of Hammerstrand and Bonelli (1968). The methyl esters were separated and quantitatively determined by gas–liquid chromatography. The column used was a 3 m by 6 mm pyrex glass tube and contained 6% ethylene glycol succinate absorbed onto Chromosorb G (80–100 mesh). A Packard Gas Chromatograph (model 846, 7300 series) was used and operated with an oven temperature of 190°C.

Determination of Long-chain Fatty Acyl-CoA Esters in Liver

The long-chain fatty acyl-CoAs were extracted from a 20-g portion of frozen rabbit liver by the method of Bortz and Lynen (1963). Any contaminating fatty acids were quantitatively removed from the extracted fatty acyl-CoAs by mild alkaline hydrolysis (Bortz and Lynen 1963). The separation and quantitation of long-chain fatty acyl-CoAs was carried out by gas–liquid chromatography as described in the previous paragraph. Determinations of long-chain fatty acyl-CoA were made using the phosphotransacetylase method of Stadtman (1955).

Results

The data in Fig. 1 show the effect of increasing blood glucose concentration (sampled from the portal vein) on the oxidation of [1-^{14}C]- and [6-^{14}C]glucose to $^{14}CO_2$ in the liver of 40-h starved rabbits *in situ*. The relative molar specific radio-

activity of the $^{14}CO_2$ from the oxidation of [1-^{14}C]glucose decreased sixfold, whereas there was a slight increase in relative molar specific activity of $^{14}CO_2$ from the oxidation of [6-^{14}C]glucose, when the blood glucose concentration was increased from 88 to

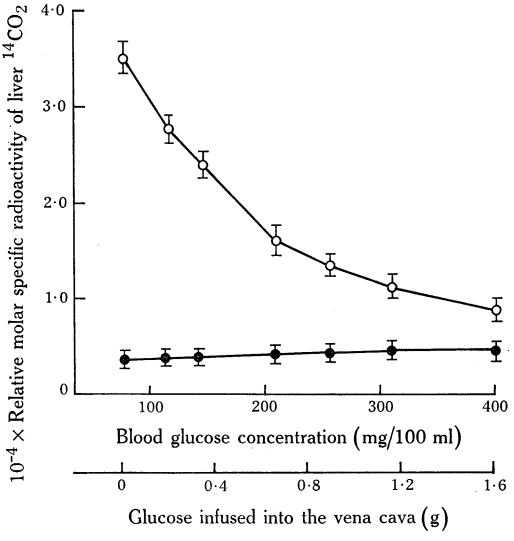


Fig. 1. Effect of administered glucose on the oxidation to $^{14}CO_2$ of [1-^{14}C]- and [6-^{14}C]glucose by the liver of rabbits starved for 40 h. The livers of the rabbits were surgically exposed and glucose (0–1·6 g) was infused into the inferior vena cava as described in the Methods. After a period of 6·0 min, 10 μCi (0·3 mM, in 1 ml of water) of either [1-^{14}C]glucose (○) or [6-^{14}C]glucose (●) were infused into the portal vein over a 15-s period. The blood flow from the hepatic veins was then arrested and metabolism of the radioactive substrate allowed to proceed for exactly 5·0 min. The liver was then removed into liquid nitrogen and rapidly crushed. The isolation of CO_2 and glucose from the liver powder and the determination of their specific radioactivities was carried out as described in the Methods. Glucose was determined on 1-ml samples of blood removed from the portal vein immediately preceding [^{14}C]glucose infusion. The relative molar specific radioactivity of the CO_2 was determined for each animal by the ratio: molar specific radioactivity of CO_2/molar specific radioactivity of glucose. Vertical lines indicate standard deviations from at least three experiments.

400 mg/100 ml of blood. These results suggest that inhibition of the reactions of the oxidative segment of the pentose pathway, which produces $^{14}CO_2$ from [1-^{14}C]glucose, occurs as a result of the increased glucose load on the liver.

Glucose production *in situ* by livers of rabbits starved for 40 h was assessed from the difference in blood glucose concentration between the portal vein and the hepatic vein (Williams *et al.* 1971*a*, 1973). The data in Table 1 show that the amount of glucose released by the liver decreased as the glucose load increased, suggesting a decreased rate of gluconeogenesis (Williams *et al.* 1973).

Variations in the concentrations of liver metabolites are shown in Table 2. There was a marked increase in the concentration of glucose in the liver tissue as the quantity of administered glucose was increased. It has been shown (Williams *et al.* 1971*a*) that the small volume of blood remaining in rabbit liver following extirpation does not influence measurements of hepatic concentrations of glucose and glycogen. Although there was a decreased rate of hepatic gluconeogenesis as a result of increased whole body glucose administration (Table 1), there was an increase in the hepatic glucose concentration (Table 2). This effect may be attributed not only to the influence of the elevated portal blood glucose concentration (Cahill *et al.* 1958) but also to the inhibitory and retarding effect of elevated intracellular glucose on the rate of glycolytic flux (Newsholme and Gevers 1967). In support of this view the concentrations of both fructose 1,6-diphosphate and *sn*-glycero-3-phosphate increased twofold while the concentrations of glucose 6-phosphate and fructose 6-phosphate decreased by 50% after the administration of 1·6 g of glucose. The adenylate pro-

portion [ATP]/[ADP][HPO_4^{2-}] decreased, which is consistent with the lowered concentration of total ATP in the liver, while the cytoplasmic redox potential ([free NAD^+]/[free NADH]), although initially low as a result of diethyl ether anaesthesia, remained constant as the level of administered glucose was increased. The concentration of $NADP^+$ increased and was accompanied by a parallel decrease in the concentration of NADPH.

Table 1. Effect of administered glucose on glucose production *in situ* by the liver of rabbits starved for 40 h

Each rabbit was anaesthetized, the liver surgically exposed and glucose (0–1·6 g in 4 ml of 0·9% NaCl) infused into the inferior vena cava as described in the Methods section. After a period of 6·0 min the hepatic blood flow was arrested. After a further 5·0 min [partial ischaemic period (P.I.)] the liver was removed and blood sampled from the portal vein and hepatic veins. Blood glucose concentration was determined as described in the Methods. Values, expressed as mg/100 ml, are means±S.D. for three individual experiments

Glucose infused (g)	Glucose in portal vein: 5 min after infusion[A]	After 5·0 min P.I. (P)	Glucose in hepatic veins after 5·0 min P.I. (H)	H−P
0	118±8	108±5	220±6	112
0·2	153±12	139±15	230±10	91
0·4	171±15	221±20	282±13	61
0·8	253±5	261±6	307±20	46
1·2	344±9	315±15	365±12	50
1·6	400±8	403±15	440±20	37

[A] The glucose concentration in the portal vein prior to the administration of glucose was 88±15 mg/100 ml blood ($n = 14$).

The effect of administered glucose upon the levels of the free fatty acids in plasma from rabbits starved for 40 h is shown in Fig. 2. Plasma concentrations of free fatty acids decreased by almost 50% following the administration of 0·8 g of glucose, and analysis by gas–liquid chromatography indicated that this change was confined to fatty acids with chain length greater than 14 carbon atoms. In contrast the hepatic concentrations of linoleic, oleic and palmitic acids increased threefold (Fig. 3) but the concentration of stearic acid did not increase significantly as the glucose load was increased. The concentration of CoA derivatives of all of the above four fatty acids showed a threefold increase as a result of the administration of 0·8 g of glucose (Fig. 4).

Discussion

The data in Fig. 1 indicate that when blood glucose levels were elevated from 88 to 400 mg/100 ml the production of CO_2 from C-1 of glucose was greatly decreased, whereas the oxidation of C-6 of glucose was slightly increased. Similar effects upon the output of respiratory CO_2 were observed by Tepperman *et al.* (1960) when fasted rats were fed glucose and [1-^{14}C]glucose, and hence these data for liver *in situ* agreed in this respect with similar data for the whole animal. The observed decrease in the C-1 : C-6 ratio as blood glucose concentration was increased clearly reflects a decrease in the activity of the reactions of the oxidative segment of the pentose phosphate pathway.

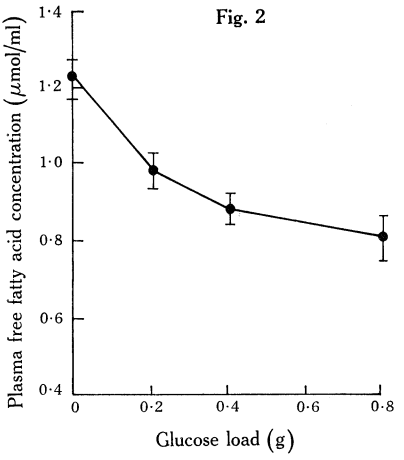
Table 2. Effects of administered glucose on metabolite concentrations in livers of rabbits starved for 40 h

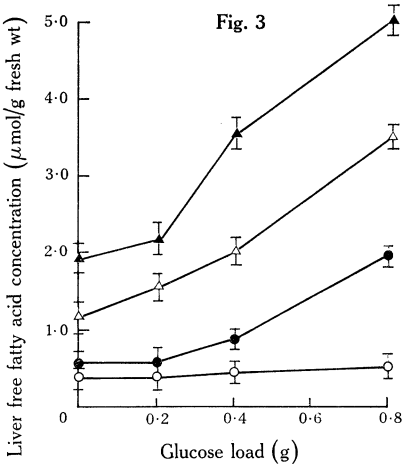
The experimental conditions were as for Table 1. Values are metabolite concentrations (determined as described in Methods) expressed as μmol/g fresh wt of liver. Each value is the mean±S.E.M. for three or five individual experiments

Metabolite	Applied glucose load (g): 0	0·2	0·4	0·8	1·2	1·6
Glucose	13·5±0·2	15·1±0·2	16·5±0·3	20·0±0·2	23·5±0·02	24·7±0·2
Lactate	5·09±0·60	5·76±0·65	4·65±0·45	6·28±0·61	5·45±0·57	5·53±0·67
Pyruvate	0·056±0·012	0·057±0·008	0·053±0·007	0·054±0·008	0·053±0·009	0·051±0·008
ATP	0·51±0·06	0·38±0·04	0·27±0·04	0·24±0·04	0·21±0·04	0·23±0·04
ADP	0·65±0·04	0·68±0·05	0·64±0·04	0·57±0·04	0·62±0·04	0·52±0·05
AMP	0·52±0·058	0·67±0·053	0·76±0·049	0·91±0·064	0·88±0·055	0·98±0·064
P_i	6·70±0·08	6·50±0·05	6·32±0·06	5·20±0·06	5·10±0·07	5·12±0·07
Glucose 6-phosphate	0·27±0·01	0·28±0·01	0·22±0·01	0·20±0·01	0·11±0·01	0·14±0·01
Fructose 6-phosphate	0·035±0·003	0·029±0·003	0·036±0·003	0·024±0·003	0·019±0·002	0·026±0·003
Fructose 1,6-diphosphate	0·007±0·0002	0·007±0·0002	0·008±0·0003	0·012±0·002	0·014±0·002	0·012±0·002
$NADP^+$	0·10±0·01	0·12±0·01	0·15±0·01	0·17±0·01	0·16±0·01	0·23±0·01
NADPH	0·59±0·02	0·58±0·02	0·51±0·01	0·43±0·02	0·44±0·02	0·33±0·02
sn-Glycero-3-phosphoric acid	0·34±0·01	0·39±0·01	0·42±0·01	0·51±0·02	0·60±0·02	0·61±0·02
[free NAD^+]/[free NADH][A]	99±38	89±13	103±15	77±12	88±15	83±15
$10^3 \times$ [ATP]/[ADP][HPO_4^{2-}][B]	195±24	143±16	111±17	135±21	111±22	144±26
[ATP]+[ADP]+[AMP]	1·68	1·73	1·67	1·72	1·71	1·73

[A] Calculated as described by Williamson *et al.* (1967). [B] Concentration of HPO_4^{2-} at pH 7·0 was taken to be 60% of the total P_i.

We have been unable to demonstrate any direct inhibitory effects of increasing glucose concentration (up to 400 mg per 100 ml) on the activities of either glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase (EC 1.1.1.44)





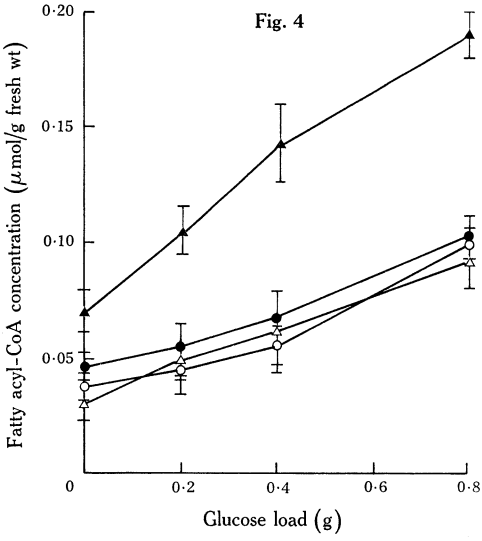


Fig. 2. Effect of administered glucose on plasma free fatty acid of rabbits starved for 40 h. The livers of the rabbits were surgically exposed and glucose (0–0·8 g) infused into the inferior vena cava as described in the Methods. Blood was sampled from the portal vein 6·0 min after glucose administration and plasma free fatty acids determined as described in the Methods. The vertical lines indicate standard deviations from at least three experiments.

Fig. 3. Effect of administered glucose on the concentration of free fatty acids in the liver of rabbits starved for 40 h. General experimental conditions were as described in Table 1. Free fatty acids were determined in liver removed into liquid nitrogen immediately following the 5·0-min partial ischaemic period, as described in the Methods. Values are means of three individual experiments; vertical lines indicate ±S.D. ● Palmitate; ○ stearate; △ oleate; ▲ linoleate.

Fig. 4. Effect of administered glucose on the concentration of long-chain fatty acyl-CoA esters in the liver of rabbits starved for 40 h. Experimental details as for Fig. 3. Values are means of three individual experiments; vertical lines indicate ±S.D. Acyl-CoA derivatives are: ● palmitoyl; ○ stearoyl; △ oleoyl; ▲ linoleoyl.

isolated from rabbit liver (Williams, unpublished data). There are, however, a variety of metabolites which influence the activity of these dehydrogenases. NADPH and inorganic phosphate are both inhibitors of these enzymes (Glasser and Brown 1955), but the concentration of each of these metabolites decreased in the liver as the level

of administered glucose was increased. Fructose 1,6-diphosphate is also an inhibitor of 6-phosphogluconate dehydrogenase (Dyson and D'Orazio 1971), but the twofold increase in the concentration of this metabolite would not be expected to bring about a sixfold decrease in the C-1 : C-6 ratio, particularly when C-1 : C-6 ratios of 3 have been obtained in rabbit liver where the concentration of fructose 1,6-diphosphate was far greater than those reported here (Williams *et al.* 1971*b*).

The CoA derivatives of palmitic and stearic acids have been shown to be potent inhibitors of glucose-6-phosphate dehydrogenase (Taketa and Pogell 1966). The data in Fig. 4 show that there was a threefold increase in the concentrations of both palmitoyl- and stearoyl-CoA for an administered glucose load of 0·8 g, which was accompanied by a decrease in the C-1 : C-6 ratio to one-third of the normal value (Fig. 1). Taketa and Pogell (1966) have shown that inhibition of glucose-6-phosphate dehydrogenase by palmitoyl-CoA was competitive with respect to glucose 6-phosphate and of the mixed type with respect to $NADP^+$, and the observed decrease in the oxidation of C-1 of glucose when the concentrations of palmitoyl- and stearoyl-CoA increased as a result of the increased glucose load is consistent with a specific inhibition of glucose-6-phosphate dehydrogenase by long-chain fatty acyl-CoA esters. Since these observations were made using liver *in situ* the effect may not be attributed to a non-specific inhibition by the detergent properties of these CoA thioesters, as has been suggested by Srere (1965). The decreased concentration of glucose 6-phosphate in the liver following glucose administration would potentiate inhibition of glucose-6-phosphate dehydrogenase by long-chain fatty acyl-CoA esters, while the increased concentration of $NADP^+$ (Table 2) would only be expected to effect a marginal activation of this enzyme (Eger-Neufeldt *et al.* 1965).

The experimental results reported in this paper reflect the early metabolic events that occur in rabbit liver during the transition from the starved state towards the fed condition. Upon the administration of glucose to the starved animal there was a decrease in the concentration of plasma free fatty acids (Fig. 2). This decrease in plasma free fatty acids was accompanied by an accumulation of free fatty acids and their acyl-CoA derivatives in the liver (Figs 3 and 4).

The data provide evidence that gluconeogenesis was diminished as the glucose load was increased. Williams *et al.* (1973) showed that a 5-min period of partial ischaemia did not affect the rate of glucose production in livers of rabbits starved for 40 h. There were smaller increases in the glucose accumulation in hepatic veins during the period of glucose administration via the portal vein (Table 1). There was a marked decrease in ATP concentration and a rise in AMP concentration in the liver on the administration of a small glucose load (Table 2). The rapid decrease in the concentration of ATP may be due to either a decrease in fatty acid oxidation or to inhibition of adenine nucleotide translocation across the liver mitochondria by the elevated levels of long-chain fatty acyl-CoA esters (Vaartjes *et al.* 1972). The increased concentration of AMP would act to further decrease the rate of gluconeogenesis by inhibition of hexosediphosphatase (EC 3.1.3.11) (Taketa and Pogell 1965), and the data in Table 2 show an increase in the concentration of liver fructose 1,6-diphosphate together with a decrease in glucose 6-phosphate and fructose 6-phosphate which is consistent with inhibition at this point.

The events that occur in the brief period of metabolism reported in this paper are transient. Restoration of the metabolic fluxes associated with the fed state, viz.

glycolysis and fatty acid synthesis by the liver, has not occurred during the short experimental interval. In part this would only be achieved when the inhibitory levels of CoA esters of long-chain fatty acids declined. This would act to relieve the inhibition of acetyl-CoA carboxylase and allow fatty acid synthesis to proceed. Such a situation would create a self-negating system unless there was a mechanism for removal of the fatty acyl-CoA esters. The data in Table 2 show that there was a small but consistent rise in the concentration of *sn*-glycero-3-phosphate as the quantity of administered glucose was increased. The increasing concentrations of this metabolite would be expected to stimulate monoglyceride phosphate formation from the CoA esters of fatty acids catalysed by the enzyme glycerolphosphate acetyltransferase (EC 2.3.1.15). The inhibition of fatty acid synthesis and the reactions of the oxidative segment of the pentose phosphate pathway would thus be relieved. The increases in the concentration of *sn*-glycero-3-phosphate during the brief period of glucose administration examined in this paper are probably insufficient to stimulate a rapid glyceride formation in view of the observations of Tzur *et al.* (1964). These authors calculated that for optimum rates of microsomal triglyceride synthesis to occur it was necessary to have *sn*-glycero-3-phosphate concentrations of 3–5 μmol/g wet wt of liver, although optimum rates of mitochondrial triglyceride synthesis only require 0·3–0·5 μmol/g wet wt of liver (Tzur *et al.* 1964). It would thus appear that formation of *sn*-glycero-3-phosphate may be the rate-limiting step for relieving the inhibitory effects of long-chain fatty acyl-CoA esters upon refeeding of fasted animals, since it has been observed (Howard and Lowenstein 1964) that 2 h after the administration of glucose to starved rats, the concentration of *sn*-glycero-3-phosphate had only increased to 60% of the level found in fed animals. The synthesis of *sn*-glycero-3-phosphate is dependent upon the cytoplasmic [free NAD^+]/[free NADH] ratio by virtue of the reactions involving glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), and as the cytoplasmic redox potential does not change significantly during this initial period of refeeding (Table 2) this may explain some of the delay in relieving the inhibitory effects of long-chain fatty acyl-CoA esters.

Acknowledgments

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