The Effect of Chronic Diabetes, Induced by Streptozotocin, on the Activities of some Enzymes of Glycerolipid Synthesis in Rat Liver

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1. Rats were injected with a single dose of 35 mg of streptozotocin/kg body wt. They exhibited a diabetes that was characterized by glycosuria, polyuria, polydipsia, hyperphagia, hyperglycaemia, increased concentrations of unesterified fatty acids, glycerol and triacylglycerols in the serum and an increased activity of glucose 6-phosphatase in the liver. 2. After 10 weeks the hepatic activities of the microsomal glycerol phosphate acyltransferase, phosphatidate phosphohydrolase, phosphatidate cytidyltransferase, diacylglycerol acyltransferase, choline phosphotransferase, CDP-diacylglycerol–inositol phosphatidyltransferase and the soluble phosphatidate phosphohydrolase were measured. 3. The only significant changes were an increase in the activity of the soluble phosphatidate phosphohydrolase and a decrease in that of the CDP-diacylglycerol–inositol phosphatidyltransferase in the diabetic rats. 4. These results are discussed in relation to the control of glycerolipid synthesis.

Diabetes mellitus is characterized by hyperglycaemia, which is often accompanied by glycosuria, and by an increase in the consumption of food and water and in urine output. In severe cases there is ketonaemia, ketonuria and hypertriglyceridaemia. The last is usually caused by an accelerated secretion of very-low-density lipoprotein, and the severity of the hypertriglyceridaemia can be taken as an indication of the adequacy of the control of the diabetes (Nikkilä & Kekki, 1973; Nikkilä, 1974).

In the present study rats were made chronically diabetic by a single injection of streptozotocin. These animals were then used to investigate whether the diabetic state is accompanied by changes in the relative activities of the enzymes responsible for the synthesis of glycerolipids in the liver. It is hoped that these results will add to our understanding of how glycerolipid synthesis is controlled.

Materials and Methods

Materials

The sources of most of these have been described (Sánchez *et al.*, 1973; Sturton & Brindley, 1977). CDP-diacylglycerol (prepared from eggs) was obtained from Miles Laboratories, Stoke Poges,

Slough SL1 3NW, Bucks., U.K. All radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Streptozotocin was a gift from the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, U.S.A. *sn*-1,2-Diacylglycerol was prepared essentially by the method described by Mangiapane *et al.* (1973). The starting material was crude egg lecithin, which was obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. This was purified twice by chromatography on alumina columns as described by Singleton *et al.* (1965).

Induction of experimental diabetes

Male Wistar rats (260–270 g) were obtained from the University of Nottingham Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K. They were injected intraperitoneally with 35 mg of streptozotocin/kg body wt. The streptozotocin was dissolved in 50 mM-sodium citrate buffer, adjusted to pH 4.5 with HCl. Control rats were injected with an equivalent volume of this buffer. Both groups of animals were allowed free access to the 41B diet (Brindley *et al.*, 1976) and to water, and were maintained for about 10 weeks before being used in the experiments. The progress of the diabetes was monitored at weekly intervals. The rats were placed in Metabowls (Jencons, Hemel Hempstead, Herts., U.K.) and their weight changes, food and water intakes and urine

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productions measured over a 24h period. Blood samples were obtained by clipping the end of the tails. These samples were used to measure the blood glucose concentrations by the method of Werner *et al.* (1970) with a glucose oxidase kit [Boehringer Corp. (London) Ltd., Lewes, Sussex BN7 1LG, U.K.], and the concentration of unesterified fatty acids by the method of Elphick (1975). Free glycerol and triacylglycerol concentrations in the blood were measured by the method of Eggstein (1966).

Preparation of the microsomal and particle-free supernatant fractions

These were prepared as described by Mangiapane *et al.* (1973). The samples of the particle-free supernatant fractions, which were used to measure the phosphatidate phosphohydrolase activity (EC 3.1.3.4), were dialysed for about 16h at 4°C against 0.3M-sucrose, adjusted to pH 7.4 with KHCO_3 and containing 0.5mM-dithiothreitol. This removed phosphate from the preparations. Samples of the microsomal fraction used for the phosphohydrolase assay were dialysed in the same way except that 2.5mM-EDTA was added to the dialysis medium.

Enzyme assays

The activity of each enzyme was determined at several protein concentrations under optimum conditions. This ensured that the rates were proportional to the enzyme concentration. The assays for the activities of glycerol phosphate acyltransferase (EC 2.3.1.15) and of phosphatidate phosphohydrolase were performed on fresh preparations, whereas those for the other enzymes were performed on samples that had been frozen at -20°C until they were used. Freezing and thawing did not adversely affect these enzyme activities.

Glycerol phosphate acyltransferase. This was determined by measuring the rate of incorporation of *sn*-[1,3- ^3H]glycerol 3-phosphate into lipids by using palmitoyl-CoA generated from palmitate (Lloyd-Davies & Brindley, 1975). The incorporation of radioactivity into the neutral lipid fraction (diacylglycerol and triacylglycerol) was also measured, since this gives an indication of the activity of phosphatidate phosphohydrolase towards newly synthesized phosphatidate under these conditions.

Phosphatidate phosphohydrolase. The determination is based on that described by Bowley *et al.* (1977). Each assay contained in a volume of 0.25 ml: 20mM-Tris/maleate buffer, pH 6.4; 1mM-dithiothreitol; 2mg of fatty acid-poor bovine serum albumin; 2mM- MgCl_2 ; 1.5mM-potassium phosphatidate. The incubation was started by adding either 200–500 μg of soluble protein or 50–200 μg of microsomal protein. The incubations were performed at 37°C and were

stopped after 20 min for the soluble protein or after 15 min for the microsomal enzyme.

Diacylglycerol acyltransferase (EC 2.3.1.20). This activity was measured by following the incorporation of ^3H from [^3H]palmitoyl-CoA into triacylglycerol. Each assay contained (in 0.25 ml): 25mM-Tris buffer, adjusted to pH 7.4 with HCl; 5mM-dithiothreitol; 10mM- MgCl_2 ; 50 μM -[^3H]palmitoyl-CoA (2.5 $\mu\text{Ci}/\mu\text{mol}$); 0.37mg of fatty acid-poor bovine serum albumin; 43 μg of Tween 20; 4mM-diacylglycerol. The reactions were started with 30–60 μg of microsomal protein and, after incubation at 37°C for 8 min, they were stopped with 1.88 ml of chloroform/methanol (1:2, v/v). Acylglycerols were extracted and purified by alumina chromatography as described for the assay of monoacylglycerol acyltransferase (EC 2.3.1.22) with palmitoyl-CoA as an acyl-donor (Short *et al.*, 1974).

Choline phosphotransferase (EC 2.7.8.2). This activity was measured by determining the rate of incorporation of ^{14}C from CDP-[methyl- ^{14}C]choline into phosphatidylcholine. The assays contained (final vol. 0.25 ml): 25mM-Tris buffer adjusted to pH 7.4 with HCl; 5mM-dithiothreitol; 20mM- MgCl_2 ; 300 μM -CDP-[methyl- ^{14}C]choline (0.5 $\mu\text{Ci}/\mu\text{mol}$); 0.37mg of fatty acid-poor bovine serum albumin; 87 μg of Tween 20; 4mM-diacylglycerol; 50–150 μg of microsomal protein. Reactions were stopped after 8 min at 37°C by adding 1.88 ml of chloroform/methanol (1:2, v/v). Lipids were extracted by the method of Bligh & Dyer (1959) and the bottom phases were washed twice with 2 ml of synthetic top phase. This was prepared by mixing 1 litre of chloroform, 1 litre of methanol and 900 ml of water.

Phosphatidate cytidyltransferase (EC 2.7.7.41). This activity was determined by using an emulsion of phosphatidate as described by Sturton & Brindley (1977).

CDP-diacylglycerol-inositol phosphatidyltransferase (EC 2.7.8.11). The method was that of Salway *et al.* (1968).

Arylesterase (EC 3.1.1.2). This activity was determined by the method of Shephard & Hübscher (1969), by using an ϵ_{368} of 3000 litre $\cdot$ mol $^{-1}$ $\cdot$ cm $^{-1}$ for indoxyl.

Glucose 6-phosphatase (EC 3.1.3.9). The assay conditions were those described by Hübscher & West (1965), but the activity was measured by determining the rate of glucose release with a glucose oxidase kit (Boehringer).

Determination of DNA and protein

The methods are described by Hübscher *et al.* (1965).

Determination of radioactivity

Samples of ^3H - and ^{14}C -labelled lipids were dissolved in 10 ml of xylene containing 4 g of 2,5-di-

phenyloxazole/litre and 0.1g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre and their activities determined by liquid-scintillation counting. External-standard ratios or channel ratios were used for quench correction.

Results and Discussion

The injection of a single dose of 35 mg of streptozotocin/kg body wt. to rats produced a persistent and constant hyperglycaemia and glycosuria after 24 h and polyuria after 7 days. The diabetic rats lost weight compared with the controls, although they ate more food and drank more water (Table 1). The concentrations of unesterified fatty acids, glycerol and triacylglycerols were higher in the plasma of the diabetic rats (Table 1), and this probably results from the increased mobilization of adipose-tissue triacylglycerols (Blackshear & Alberti, 1974). The liver weights were lower in the diabetic rats, but these values were higher when expressed relative to the body weight (Table 1). These results are probably partly accounted for by the observed loss of liver glycogen, which is accompanied by an increase in the concentration of hepatic glucose (Palmano *et al.*, 1977). The increased activity of glucose 6-phosphatase in the liver is consistent with these observations (Table 1) and agrees with the work of Chang & Schneider (1971). The injection of 35 mg of streptozotocin/kg body wt. only increases the concentration of serum ketone bodies by 3–4-fold above control values (Palmano *et al.*, 1977). These animals therefore provide a suitable and stable model for studying metabolism in the diabetic state. Higher doses of

streptozotocin (75 mg or over/kg body wt.) quickly produce a diabetic ketoacidosis in Wistar rats (Blackshear & Alberti, 1974), a situation that is likely to produce an increased mortality.

Since the induction of diabetes alters the weight of the liver and its protein content, but not that of DNA (Table 1), it was decided to express enzyme activities relative to DNA. To do this for the microsomal activities, it was necessary to correct for the recovery of endoplasmic-reticulum membranes in this fraction relative to the homogenate in which the DNA concentration was measured (Mangiapane *et al.*, 1973). This was done by using both arylesterase and glucose 6-phosphatase as endoplasmic-reticulum markers. The recoveries on the basis of these assays were not significantly different. The average recovery of these markers in the microsomal fractions was higher ($P < 0.05$) in the eight diabetic rats [64 ± 8 (S.D.)%] than in the eight control rats [56 ± 6 (S.D.)%].

The microsomal activities of glycerol phosphate acyltransferase, phosphatidate phosphohydrolase, phosphatidate cytidyltransferase, diacylglycerol acyltransferase and choline phosphotransferase, and the rate of conversion of glycerol phosphate into neutral lipids, were not significantly different in the livers of the diabetic and control rats (Table 2). Young & Lynen (1969) reported that the hepatic activities of choline phosphotransferase and diacylglycerol acyltransferase were not affected by non-ketotic diabetes in rats. They did, however, observe an increased activity (related to protein) of both enzymes in ketotic diabetes. The only enzyme that showed an increased activity in the present experiments, in which mildly ketotic rats were used (Palmano *et al.*, 1977), was the soluble phosphatidate

Table 1. *Characterization of some of the changes produced by streptozotocin-induced diabetes in rats*

The values shown in the Table were obtained 10 weeks after injecting rats with either streptozotocin (eight diabetic rats) or with citrate buffer (eight control rats) as described in the Materials and Methods section. The significance of the difference between the groups is calculated by Student's *t* test.

	Control	Diabetic	Significance of difference
Body weight (g)	289 $\pm$ 4	202 $\pm$ 32	$P < 0.001$
Liver weight (g)	11.0 $\pm$ 0.5	8.7 $\pm$ 1.4	$P < 0.001$
Liver weight Body weight $\times 100$	3.8 $\pm$ 0.2	4.3 $\pm$ 0.4	$P < 0.01$
Food intake (g/24h)	9 $\pm$ 4	26 $\pm$ 2	$P < 0.001$
Water intake (ml/24h)	14 $\pm$ 6	96 $\pm$ 8	$P < 0.001$
Urine production (ml/24h)	8 $\pm$ 3	88 $\pm$ 10	$P < 0.001$
Total protein (mg/g of liver)	168 $\pm$ 23	194 $\pm$ 14	$P < 0.02$
Total microsomal protein (mg)	502 $\pm$ 84	361 $\pm$ 64	$P < 0.005$
DNA P (μ mol/g of liver)	5.9 $\pm$ 0.7	7.2 $\pm$ 1.7	Not significant (0.1 $> P > 0.05$)
Blood glucose (mM)	6.8 $\pm$ 1	27.5 $\pm$ 8	$P < 0.001$
Serum unesterified fatty acids (mM)	0.54 $\pm$ 0.2	0.94 $\pm$ 0.2	$P < 0.001$
Serum triacylglycerols (mM)	1.58 $\pm$ 0.4	2.83 $\pm$ 0.5	$P < 0.001$
Serum glycerol (mM)	0.07 $\pm$ 0.02	0.20 $\pm$ 0.04	$P < 0.001$

Table 2. *Effect of streptozotocin-induced diabetes on the hepatic activities of arylesterase, glucose 6-phosphatase and of some enzymes involved in glycerolipid metabolism*

The activities are shown as means $\pm$ S.D. (eight rats in each group) and are expressed relative to the DNA concentration in the corresponding homogenates as described in the Results and Discussion section. All of the activities were measured in the microsomal fractions except for the activity of the soluble phosphatidate phosphohydrolase.

Activity	Activity (nmol of substrate converted/min per μ mol of DNA P)		Significance of difference
	Control	Diabetic	
Glycerol phosphate incorporated into total lipids	56 $\pm$ 24	52 $\pm$ 21	Not significant ($P > 0.7$)
Glycerol phosphate incorporated into neutral lipids	3.2 $\pm$ 1.5	4.2 $\pm$ 1.2	Not significant ($P > 0.2$)
Microsomal phosphatidate phosphohydrolase	51 $\pm$ 14	57 $\pm$ 8	Not significant ($P > 0.3$)
Soluble phosphatidate phosphohydrolase	10.2 $\pm$ 1.5	14.3 $\pm$ 2.9	$P < 0.005$
Phosphatidate cytidyltransferase	16 $\pm$ 3	16 $\pm$ 3	Not significant ($P > 0.9$)
CDP-diacylglycerol-inositol phosphatidyltransferase	4.5 $\pm$ 1.0	3.5 $\pm$ 0.5	$P < 0.02$
Diacylglycerol acyltransferase	51 $\pm$ 11	44 $\pm$ 8	Not significant ($P > 0.1$)
Choline phosphotransferase	102 $\pm$ 22	106 $\pm$ 26	Not significant ($P > 0.7$)
Arylesterase	25 $\pm$ 5	13 $\pm$ 7	$P < 0.001$
Glucose 6-phosphatase	0.3 $\pm$ 0.1	0.7 $\pm$ 0.3	$P < 0.001$

phosphohydrolase (Table 2). There was also a decrease in the activity of CDP-diacylglycerol-inositol phosphatidyltransferase in the diabetic rats.

When the enzyme activities for all 16 rats were compared with each other by linear regression analysis there were no significant correlations between the activities of the endoplasmic-reticulum markers (glucose 6-phosphatase and arylesterase) and the enzymes of glycerolipid metabolism. There was a highly significant negative correlation between the activities of glucose 6-phosphatase and arylesterase ($r = -0.83$; $P < 0.001$).

No significant correlations were found when comparing the activities of glycerol phosphate acyltransferase with either diacylglycerol acyltransferase ($r = 0.48$) or with the soluble phosphatidate phosphohydrolase ($r = 0.15$). The activity of CDP-diacylglycerol-inositol phosphatidyltransferase showed no correlation either with the activities of the soluble phosphatidate phosphohydrolase ($r = 0.08$) or with those of phosphatidate cytidyltransferase ($r = 0.50$). All of the other pairs of enzymes involved in glycerolipid metabolism showed a significant correlation ($r > 0.52$; $P < 0.05$).

The differences in the response of the microsomal and soluble phosphatidate phosphohydrolases to diabetes are probably explained by the fact that these activities seemed to be produced by different enzymes. The microsomal activity consists of at least two different enzyme activities (Caras & Shapiro, 1975;

M. Bowley, J. Cooling, S. L. Burditt & D. N. Brindley, unpublished work). Under the present experimental conditions the microsomal activities are stimulated by less than 50% by Mg^{2+} . By contrast, the soluble phosphatidate phosphohydrolase was stimulated by about 4.5-fold by 2–5 mM- $MgCl_2$ (Bowley *et al.*, 1977). This enzyme does not have an absolute requirement for Mg^{2+} , but appears to require a more positive charge on the phosphatidate. These observations support the hypothesis that the liver contains different enzymes that are capable of hydrolysing phosphatidate and that probably have different physiological functions (Brindley, 1974).

It has been argued that most of the phosphatidate phosphohydrolase that is involved in triacylglycerol synthesis is isolated in the soluble fraction after cell fractionation (Hübscher *et al.*, 1967). Although the activity of the total microsomal phosphatidate phosphohydrolase activity (measured with a phosphatidate emulsion) is 4–5-fold greater than that of the soluble enzyme, only about 6–8% of the glycerol phosphate incorporated into phosphatidate is hydrolysed to neutral lipids (Table 2). This type of result is normally obtained with cell-free systems and it is necessary to add the soluble phosphatidate phosphohydrolase back to the microsomal fraction to convert the major portion of the microsomally bound phosphatidate into triacylglycerol (Hübscher *et al.*, 1967). It is also the soluble phosphohydrolase that shows the greatest response

to stimuli that affect the synthesis of triacylglycerols.

This is seen in the present work and in other conditions where the concentration of serum unesterified fatty acids is raised and where their flux to the liver is increased. These conditions include starvation (Vavrečka *et al.*, 1969; Mangiapane *et al.*, 1973) and the stress produced by laparotomy or subtotal hepatectomy (Mangiapane *et al.*, 1973). It is expected that a large proportion of the fatty acids that enter the liver in these conditions will be oxidized. The preference for fatty acid oxidation versus esterification to triacylglycerol is especially well observed in starvation (Mayes & Felts, 1967; Imazumi *et al.*, 1973; Mayes, 1976; Iritani *et al.*, 1976; Groener & van Golde, 1977). A decreased rate of triacylglycerol synthesis has been reported with cell-free preparations obtained from the livers of starved rats (Rubenstein & Rubenstein, 1966; Fallon & Kemp, 1968). The hepatic activity of glycerol phosphate acyltransferase is also decreased in this condition (Vavrečka *et al.*, 1969; Aas & Daae, 1971; Mangiapane *et al.*, 1973; van Tol, 1974) or shows no change (Fallon & Kemp, 1968; Wiegand *et al.*, 1973). A lower activity of glycerol phosphate acyltransferase may be partly responsible for enhancing the rate of fatty acid oxidation as opposed to their esterification. However, this enzyme does not appear to be rate-limiting in triacylglycerol synthesis *in vitro* and its activity correlates poorly with the observed rates of triacylglycerol synthesis (Hübscher *et al.*, 1967; Fallon & Kemp, 1968; Vavrečka *et al.*, 1969; Mangiapane *et al.*, 1973; Fallon *et al.*, 1977; Pritchard *et al.*, 1977). The enzymic capacity to synthesize triacylglycerols (as opposed to phosphatidate) is maintained, or even increased, in starvation provided that the assays are performed in the presence of optimum concentrations of cofactors (Rubenstein & Rubenstein, 1966; Vavrečka *et al.*, 1969; Mangiapane *et al.*, 1973). The retention of this capacity appears to depend on the activity of the soluble phosphatidate phosphohydrolase. It probably enables the liver to remove potentially toxic fatty acids and to recycle CoA should the influx of fatty acids exceed the rate of β -oxidation. This probably contributes to the accumulation of hepatic triacylglycerols that is observed in starvation (Harrison, 1953; Mayes, 1962; Gillman *et al.*, 1962). Phosphatidate phosphohydrolase is also required for synthesizing phosphatidylcholine and phosphatidylethanolamine. The synthesis of these lipids is much less affected by food deprivation than that of triacylglycerol (Iritani *et al.*, 1976; Groener & van Golde, 1977).

The soluble phosphatidate phosphohydrolase is also the enzyme of glycerolipid synthesis that shows the greatest increase when hepatic triacylglycerol synthesis is stimulated. This is seen after feeding fructose (Lamb & Fallon, 1974; Fallon *et al.*, 1977) and ethanol (Lamb & Fallon, 1977; Pritchard &

Brindley, 1977; Pritchard *et al.*, 1977) and in genetic obesity (Jamdar *et al.*, 1976; Fallon *et al.*, 1977).

A metabolic pathway is normally regulated at an early stage when the substrates first become committed to that route of metabolism. The activities of the total microsomal acyl-CoA synthetase (EC 6.2.1.3) and of glycerol phosphate acyltransferase do respond to some physiological stimuli, but the activities do not always correlate well with the overall rates of triacylglycerol synthesis, and the changes, when observed at all, are usually smaller than those for the soluble phosphatidate phosphohydrolase (Fallon & Kemp, 1968; Vavrečka *et al.*, 1969; Aas & Daae, 1971; Mangiapane *et al.*, 1973; Wiegand *et al.*, 1973; Brandes *et al.*, 1973; Lamb & Fallon, 1974; Fallon *et al.*, 1977; Pritchard *et al.*, 1977; Table 2). The activity of the soluble phosphatidate phosphohydrolase can change rapidly in response to physiological stimuli and parallels the altered rate of triacylglycerol synthesis (Mangiapane *et al.*, 1973; Lamb & Fallon, 1974; Fallon *et al.*, 1977; Pritchard *et al.*, 1977). This enzyme is in the middle of the pathway of glycerolipid synthesis and could be involved in controlling the branch point leading to the synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine on the one hand, and the acidic lipids such as phosphatidylinositol on the other. Some evidence for an acute reciprocal control of phosphatidate phosphohydrolase and phosphatidate cytidyltransferase has been obtained (Sturton & Brindley, 1977), and their activities do change in opposite directions after fructose feeding (Fallon *et al.*, 1975). This relationship was not observed in the present work and the activity of phosphatidate cytidyltransferase was positively correlated with the activities of the microsomal phosphatidate phosphohydrolase ($r = 0.78$; $P < 0.001$) and with the soluble phosphohydrolase ($r = 0.51$; $P < 0.05$). However, the increased activity of the soluble phosphohydrolase in diabetes was accompanied by a decrease in the activity of CDP-diacylglycerol-inositol phosphatidyltransferase (Table 2). This enzyme was also less active in brain and sciatic nerve of such diabetic rats (P. H. Whiting, K. P. Palmano & J. N. Hawthorne, unpublished work), and this change may contribute to the lower concentration of inositol lipids in the brain tissue (Palmano *et al.*, 1977). It is possible that defects in the metabolism of phosphatidylinositol contribute to human diabetic neuropathy, since this lipid and its phosphorylated derivatives are important in excitation processes. Phosphatidylinositol turnover, as measured by ^{32}P incorporation, is not particularly high in liver, however, as compared with brain (Hawthorne, 1960). The inositol lipid concentration in diabetic-rat liver was not significantly different from normal (Palmano *et al.*, 1977), despite the lower activity of CDP-diacylglycerol-inositol phosphatidyltransferase.

A further route for the metabolism of phosphatidate is its degradation to lysophosphatidate and glycerol phosphate, and this is known to account for a large proportion of phosphatidate turnover (Tzur & Shapiro, 1976). The reactions involved in phosphatidate synthesis and degradation could constitute substrate cycling and thus facilitate metabolic control. The continuous release of fatty acids into the unesterified fatty acid pool by phospholipases of the A type (Tzur & Shapiro, 1976) could enable them to enter other pathways such as β -oxidation. The limitation of diacylglycerol synthesis through the activity of phosphatidate phosphohydrolase and the recycling of phosphatidate could provide a mechanism whereby the phosphohydrolase could regulate glycerolipid synthesis.

The present work shows that, of the enzymes of triacylglycerol synthesis in the liver, it is the soluble phosphatidate phosphohydrolase that is specifically increased in these mildly diabetic rats (Table 2). These results support the hypothesis that changes in the activity of this enzyme contribute to the overall control of hepatic triacylglycerol synthesis (Mangiapane *et al.*, 1973; Lamb & Fallon, 1974; Fallon *et al.*, 1977; Pritchard *et al.*, 1977). The hypothesis is also based on the observation that changes in the activity of the phosphohydrolase generally parallel those in the rate of hepatic triacylglycerol synthesis. In the present work the increased activity of the soluble phosphohydrolase could contribute to the enhanced synthesis of hepatic triacylglycerols that is commonly observed in diabetes (Nikkilä & Kekki, 1973; Nikkilä, 1974). This in turn could be one of the factors leading to the hypertriglyceridaemia reported in Table 1.

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