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The Immediate Effects of Insulin and Fructose on the Metabolism of the Perfused Liver

CHANGES IN LIPOPROTEIN SECRETION, FATTY ACID OXIDATION AND ESTERIFICATION, LIPOGENESIS AND CARBOHYDRATE METABOLISM

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1. When livers from fed rats were perfused with blood containing elevated concentrations of rat insulin or blood to which fructose was added, the oxidation of free fatty acids was depressed and their esterification was increased. 2. Raised concentrations of insulin or addition of fructose increased secretion of triglyceride in very-low-density lipoproteins, but only insulin caused more of the free fatty acids taken up by the liver to be incorporated into very-low-density lipoproteins. 3. When insulin and fructose were added together the combined effect on oxidation and esterification of free fatty acids and on secretion of very-low-density lipoproteins was equal to the sum of the effects of either alone. No statistically significant interaction between the effects of fructose and insulin was found for any of the parameters investigated. 4. Bovine insulin had similar effects, in most respects, to comparable studies with raised concentrations of rat insulin. 5. Lipogenesis was increased in the livers treated with fructose plus bovine insulin. 6. A significant proportion of the fatty acids in very-low-density lipoproteins were derived either from the liver triglyceride pool or from lipogenesis. This fraction was increased both by treatment with insulin or fructose, and was augmented further when both insulin and fructose were present together. 7. The uptake of fructose by the perfused liver was similar to that found *in vivo*. It was unaffected by the presence of insulin. 8. Addition of fructose to the perfused liver caused perfusate lactate concentrations to increase, as a result of diminished hepatic uptake of lactate. 9. The uptake of free fatty acids by the perfused liver was unaffected by the addition of either insulin or fructose. 10. The distribution among the various lipid classes in plasma lipoproteins of label arising from the hepatic uptake of [^{14}C]oleate was unaltered by the addition of either fructose or insulin. 11. It is suggested that the effects described are due principally to control of the balance between esterification of fatty acids and lipolysis of the ensuing triglyceride, fructose enhancing esterification and insulin inhibiting lipolysis.

The liver is the site of production of serum triglycerides present in VLD lipoproteins* derived from the esterification of plasma free fatty acids (Stein & Shapiro, 1959; Havel *et al.*, 1962; Mayes & Felts, 1967) and by synthesis *de novo* from carbohydrate (Windmueller & Spaeth, 1967). The intestines are also responsible for some formation of VLD lipoproteins from both exogenous and endogenous precursors (Ockner *et al.*, 1969*a*,*b*).

Correlations have been made between hypertriglyceridaemia, hyperinsulinism and coronary heart disease (Tzagournis *et al.*, 1968), and between hypertriglyceridaemia, hyperinsulinism and obesity (Ford *et al.*, 1968). Adipose tissue of hyperglyceridaemic patients releases higher quantities of free fatty acids (Kuo *et al.*, 1967), leading to slightly or moderately elevated concentrations of plasma free fatty acids (Nikkilä, 1969). It has been suggested as a result of experiments in humans that insulin may increase triglyceride secretion by the liver (Farquhar *et al.*, 1966; Reaven *et al.*, 1967). However, Nikkilä (1969) has concluded that none of the individual metabolic steps located on the pathway of flow of plasma free fatty acids to plasma triglyceride is specifically controlled by insulin. It was therefore decided to examine whether insulin has a direct role in the secretion of triglyceride by the liver.

In vitro, unequivocal direct metabolic effects of insulin have been shown on muscle (see, e.g., Park *et al.*, 1961) and on adipose tissue (see, e.g., Mahler

*Abbreviation: VLD lipoproteins, very-low-density lipoproteins (d<1.006).

et al., 1964), but similar effects have been rather more difficult to show on the liver. It was believed that *in vivo* the direct effects of insulin were extrahepatic (Levine & Fritz, 1956). Slice experiments failed to show any effect of insulin on hepatic glucose output (Renold *et al.*, 1955), and experiments with the isolated perfused liver showed small, inconsistent, responses (Haft & Miller, 1958; Haft, 1967, 1968). The clearest demonstration of a hepatic hypoglycaemic action of insulin was made on the perfused liver by Mortimore (1963). Insulin has also been shown to stimulate hepatic lipogenesis; this effect has been demonstrated in slices (Bloch & Kramer, 1948; Brady & Gurin, 1950; Brady *et al.*, 1951) and in the perfused liver (Altman *et al.*, 1951; Haft & Miller, 1958; Haft, 1967).

A correlation has also been made between fructose consumption and hypertriglyceridaemia (Kuo & Bassett, 1965; MacDonald, 1965), and a further correlation has been made between dietary sucrose, hyperinsulinism and coronary heart disease (Yudkin & Roddy, 1964; Yudkin *et al.*, 1969). Fructose is readily metabolized by the liver; a large proportion (30–60%) of that administered by intravenous infusion (Weichselbaum *et al.*, 1953; Muntz & Vanko, 1962) or absorbed from the intestine (Topping & Mayes, 1971) may be extracted by the liver *in vivo*.

In view of the central role of the liver in the metabolism of fructose and in the release of triglycerides in VLD lipoproteins, the isolated perfused liver was an obvious choice of technique with which to study the metabolic effects of both insulin and fructose on the secretion of VLD lipoproteins and associated metabolic pathways. This study was carried out with livers from fed animals at moderately raised concentrations of serum free fatty acids, characteristic of obese patients exhibiting hyperglyceridaemia, and in the presence of fructose concentrations attainable in the hepatic portal vein *in vivo*.

Preliminary results from this study have been reported (Mayes, 1970; Topping & Mayes, 1970).

Materials and Methods

Animals

Male Wistar albino rats of the M.R.C. strain were purchased from A. Tuck and Sons, Rayleigh, Essex, U.K. Liver donors weighed 340–360g and blood donors weighed 340–450g. The animals were kept for several weeks before use on a standard pellet diet, M.R.C. no. 41B (Bruce & Parkes, 1949).

Liver perfusions

All perfusions were carried out between 10.00 a.m. and 12 noon. The apparatus and the techniques for liver isolation and perfusion have been described by Mayes & Felts (1966). The preparation of the perfusion medium differed in the following respects. A total of 140ml of defibrinated whole rat blood was used, of which 80ml was dialysed for 48h at 0–4°C against three changes of 2 litres of bicarbonate buffer (Krebs & Henseleit, 1932) containing glucose (300mg/100ml) and amino acids (50mg/100ml). The remaining 60ml of blood was not dialysed but stored at 0–4°C. Dialysis is necessary to remove vasoconstrictor factors present in whole blood, which if not removed cause poor perfusions (Mayes & Felts, 1966). Simpson-Morgan (1967) has criticized the use of dialysed blood. To overcome this criticism partially, the preparation was perfused initially with the dialysed blood and, when perfusion was proceeding satisfactorily, the undialysed blood was added in two 30ml portions, 5min apart. Slight vasoconstriction was detected when the undialysed blood was added, but this rapidly diminished and did not prevent perfusion at a constant flow rate of 5ml of blood/min. The glucose content of the undialysed blood was adjusted so that when all the blood was mixed the glucose concentration was approx. 300mg/100ml, a concentration that the results of previous experiments had shown to prevent glycogenolysis. Under these conditions the appearance of the liver throughout the experimental period was similar to that *in vivo*. Electron microscopy did not indicate any abnormalities.

In those experiments where it was desired to have elevated physiological concentrations of rat insulin in the perfusate, the blood donors were given glucose [2ml of 10% (w/v) glucose in 0.9% NaCl] by intraperitoneal injection. Blood was drawn 30min after injection, at which time it was shown by radioimmunoassay, with a bovine insulin standard, that insulin concentrations were maximal and significantly elevated ($P = 0.01$–0.001) above those of animals not given glucose. Livers perfused with blood containing elevated concentrations of rat insulin are referred to as the 'rat insulin' group in the following account. Other experiments were also performed in which bovine insulin was added to the perfusate to give a concentration in the portal vein of 56ng/ml of serum. This concentration was based on the results of Howland & Nowell (1969) for the concentration of insulin in the blood of rats under glucose loading. It was assumed that up to 50% of the insulin in the blood was inactivated each time it passed through the liver (Mortimore *et al.*, 1959). Further additions of bovine insulin were made every 30min to compensate for this assumed rate of destruction. Crystalline bovine insulin (low in glucagon content) was a gift from Burroughs Wellcome and Co., Beckenham, Kent, U.K., and was dissolved in HCl (pH3) and diluted with 0.9% NaCl to pH6 before addition to the perfusate.

Infusion of substrates

As soon as the flow rate of the blood perfusate through the liver was 5ml/min, the P_{O_2} was 90–100mmHg and the temperature was constant at 37°C, substrate infusion was started ('zero time'). A constant infusion into the main blood reservoir preceded by a rapid priming infusion was used to maintain steady concentrations of substrates in the perfusate. Oleic acid [Sigma (London) Chemical Co. Ltd., London S.W.1, U.K.] labelled with 5μCi of [1-^{14}C]-oleic acid (50mCi/mmol, purchased from The Radiochemical Centre, Amersham, Bucks., U.K., or 40mCi/mmol, purchased from New England Nuclear Corp., Boston, Mass., U.S.A.) was made into a complex with crystalline bovine serum albumin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.). The [1-^{14}C]oleic acid, shown by t.l.c. to be radiochemically pure and to migrate with a similar R_F to pure oleic acid, was dissolved in benzene, added to 78.0mg of oleic acid and the solvent evaporated under a stream of N_2. This material was left in contact with 0.5ml of 1M-KOH plus 1.0ml of water overnight at room temperature, followed by gentle warming until it was dissolved. Albumin (1.0g) was dissolved in 12.0ml of 0.9% NaCl. The two solutions were warmed separately to 40°C, and the albumin was added to the soap with rapid mixing. The final pH of the albumin–free fatty acid complex was 9.5 and, after preparation, it was kept at approx. 40°C to prevent precipitation. It was infused to maintain a concentration in the perfusate of approx. 1.0μmol of free fatty acid/ml of serum. Similarly, 150μCi of sodium [*Me*-^{3}H]acetate (specific radioactivity >2000mCi/mmol; The Radiochemical Centre) dissolved in 0.9% NaCl and adjusted to pH9 with NaOH was infused without added carrier. Fructose (Bacteriological Grade; British Drug Houses Ltd., Poole, Dorset, U.K.) was dissolved in 0.9% NaCl and infused to maintain a concentration between 25 and 45mg/100ml of blood, maximal concentrations of this magnitude having been recorded in the portal vein of rats absorbing fructose (Topping & Mayes, 1971).

Sampling

Blood samples were withdrawn by syringe from the cannulae entering and leaving the liver at zero time and at 30min intervals over a period of 2h, care being taken to prevent alterations in blood flow through the liver. Samples were chilled in ice immediately. The serum volume of each blood sample and of the perfusate was calculated from the haematocrit, which was determined by using MSE micro haematocrit tubes. Where appropriate, calculations involving total perfusate volumes were corrected for the volume of the samples removed, for bile production and for the volume of fluid added by the infusions.

Analytical techniques

Liver glycogen was determined after the method of Good *et al.* (1933) and the liberated glucose was measured by a glucose oxidase method (Worthington Biochemical Corp., Freehold, N.J., U.S.A.); this method was also used to measure glucose in the perfusate. L(+)-Lactate was determined with lactate dehydrogenase (Hohorst, 1963) supplied by C. F. Boehringer Corp. (London) Ltd., London W.5, U.K. Fructose was determined by the Long (1957) modification of the Roe (1934) procedure with ferric chloride–resorcinol reagent.

Serum was obtained by centrifugation of the blood samples at 4°C for 20min at 2500rev./min (MSE Minor centrifuge). Serum (2ml) was layered under 0.9% NaCl in cellulose nitrate tubes (Beckman–Spinco, Palo Alto, Calif., U.S.A.) and centrifuged at 110000*g* for 18h at 4°C (MSE Superspeed 50 centrifuge, rotor 2408). VLD lipoproteins were harvested from the top of the centrifuge tubes with the aid of a tube cutter, and the lipids were extracted with a minimum of 20vol. of chloroform–methanol (2:1, v/v). After the middle fraction had been discarded, the infranatant (approx. 2ml), containing lipoproteins of $d>1.006$, was extracted similarly. Liver samples were homogenized and extracted with the chloroform–methanol mixture. After standing at room temperature overnight, all extracts were filtered and shaken with one-fifth of their volume of 0.03M-HCl. After the clearing of any emulsion the chloroform extracts were separated and stored at 4°C. Portions of the chloroform extracts were evaporated under N_2 and separated into lipid classes by silicic acid column chromatography, the methods described by Havel *et al.* (1962) being used. The procedures removed virtually all of the ^{3}H present in the samples as [^{3}H]acetate or ^3H_2O.

Triglyceride was determined by the method of Carlson (1959), and serum free fatty acids were determined by the Trout *et al.* (1960) modification of the Dole procedure. $^{14}CO_2$ in respiratory gases was trapped and measured as described by Mayes & Felts (1966). The extra step was introduced of drying the samples of NaOH containing trapped $^{14}CO_2$ to remove contamination by ^3H_2O. ^{14}C-labelled ketone bodies in blood were determined by methods described previously (Mayes & Felts, 1967*a*). All radioactivity was counted in a Tri-Carb model 3003 liquid-scintillation spectrometer (Packard Instruments, LaGrange, Ill., U.S.A.). Lipid fractions were assayed for radioactivity by using a double-isotope setting. The scintillation mixture comprised 12ml of toluene containing 2,5-diphenyloxazole (0.5%) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene

(0.025%) plus 6ml of additional toluene. $^{14}CO_2$ and ^{14}C-labelled ketone bodies were assayed in a similar scintillation mixture except that the 6ml of toluene was replaced with 6ml of ethanol. The radioactivity of ^{14}C-labelled ketone bodies was counted with a setting modified to exclude ^{3}H. Quenching was corrected for by the external standard method, and all radioactivity was corrected for efficiency by a previously published method using a computer program (Felts & Mayes, 1967).

Statistical methods

Statistical significance of differences between individual groups of perfused livers was determined by the analysis of variance. The experimental design of four main groups of experiments consisted of one control group, one group with higher concentrations of rat insulin, one group with added fructose and a fourth group with higher concentrations of rat insulin plus added fructose. The results were analysed by the method of two-factor analysis with replication (Brownlee, 1949). This allowed the detection of any co-operative effects. A fifth group of livers with added bovine insulin plus fructose in the perfusate was a control against the group with rat insulin plus fructose.

Results

A summary of the analysis of variance of the major effects of rat insulin and fructose is given in Table 1.

The blood flow was held constant in all experimental groups. As judged from the P_{O_2} of the perfusate, which was monitored continuously, O_2 consumption remained constant and did not differ significantly between any of the experimental groups. The mean rate of bile production was 1.0ml/h. The average weight of the livers was 12.65g. Neither of these measurements differed significantly between the experimental groups.

Carbohydrate metabolism

Blood glucose and liver glycogen. After an initial period of 30min the blood glucose concentration

Table 1. *Summary of the analysis of variance of a 2×2 factorial experiment on the effects of insulin and fructose on the metabolism of perfused rat liver*

The livers were from fed rats. The four experimental groups consisted of one control, one with added fructose, one with a raised concentration of rat insulin and one with added fructose plus a raised concentration of rat insulin. A minimum of three independent results was present in each group. The livers were infused with [1-^{14}C]oleate and [*Me*-^{3}H]acetate. $*P<0.05$; $**P<0.01$; $***P<0.001$; N.S., $P>0.05$.

Parameter	Effect		
	Insulin (I)	Fructose (F)	I×F interaction
Liver weight	N.S.	N.S.	N.S.
Bile production	N.S.	N.S.	N.S.
Blood glucose (lowering)	*	N.S.	N.S.
Liver glycogen	N.S.	N.S.	N.S.
Fructose uptake	N.S.	***	N.S.
Blood lactate (rise)	N.S.	***	N.S.
Free fatty acid uptake	N.S.	N.S.	N.S.
VLD-lipoprotein-triglyceride production	***	***	N.S.
^{14}C incorporation into VLD lipoproteins	**	N.S.	N.S.
^{14}C incorporation into total liver lipids	N.S.	*	N.S.
Total ^{14}C esterification	**	**	N.S.
^{14}C incorporation into liver phospholipids	N.S.	*	N.S.
^{14}C incorporation into liver triglycerides	*	*	N.S.
^{14}C incorporation into liver cholesteryl esters	N.S.	N.S.	N.S.
^{14}C oxidation to CO_2+ketone bodies	**	*	N.S.
^{14}C oxidation to CO_2	***	***	N.S.
^{14}C oxidation to ketone bodies	N.S.	N.S.	N.S.
^{3}H incorporation into VLD lipoproteins	*	N.S.	N.S.
^{3}H incorporation into liver+VLD-lipoprotein lipids	N.S.	N.S.	N.S.
^{14}C incorporation into $d>1.006$ lipoproteins	N.S.	N.S.	N.S.
^{3}H incorporation into $d>1.006$ lipoproteins	N.S.	N.S.	N.S.

became stabilized (Fig. 1). All groups treated with rat insulin or fructose maintained concentrations that were lower than those in the control group. Analysis of the average values of the glucose concentration between 30min and 120min showed that only the lowering effect of rat insulin attained statistical significance. The mean value for the liver glycogen content of all groups at the termination of the experiments was 1.6% of the wet weight (Fig. 2). This was within the range expected for rats in the fed but post-absorptive state (Mayes, 1962), and demonstrated that glycogenolysis was minimal during the perfusion. No statistical differences in liver glycogen content were observed between the four main groups studied, but when the results from these groups were combined the mean was significantly lower ($P = 0.05$–0.01) than that for the group of livers treated with fructose plus bovine insulin.

Fructose. The priming infusion of fructose caused its concentration in the perfusate to rise rapidly. It rose more slowly between the 30min and 120min time-intervals (Fig. 3). Thus the concentrations were kept within the range 25–45mg/100ml. No statistical differences were found between any of the groups, demonstrating a lack of any effect of insulin on fructose uptake by the liver. The average fractional uptake of fructose, as measured by difference between the concentrations in the portal vein and the hepatic vein, was 58.5%. The total uptake during the 2h perfusion was approx. 140mg.

Lactate. In the control perfusions (Fig. 4) the lactate in the perfusate stabilized at about 9μmol/ml, at which concentration it may be deduced that the liver has a net uptake of lactate equivalent to the total lactate production by the blood. The presence of rat insulin did not alter the uptake of lactate by the liver. However, in all groups in which fructose was infused the lactate concentration rose above the control value, the effect being highly significant. Previous experiments (P. A. Mayes, unpublished work) showed that the rate of formation of lactate from glucose by rat blood under the conditions of the present experiments was approx. 3.7μmol of lactate/ml per h. In the current experiments the mean net increase in lactate production during the first 30min in the presence of fructose was 4.6μmol/ml per h, indicating that under these conditions the liver may possibly be a net producer of lactate from fructose. Nevertheless the net lactate production in the perfusate from 30 to 90min could more than be accounted for by glycolysis in the blood. Thus for this period, when the liver can be regarded as being more in metabolic equilibrium with the fructose infusion, the rise in lactate concentration in the presence of fructose can be ascribed to diminished uptake of lactate rather than to net production of lactate by the liver.

Lipid metabolism

Free fatty acids. The infusion of [^{14}C]oleate caused the concentration of serum free fatty acids to rise rapidly and stabilize at approx. 1μmol/ml (Fig. 5). There were no significant differences between any of the groups tested, indicating that neither insulin nor fructose, either alone or in combination, had any

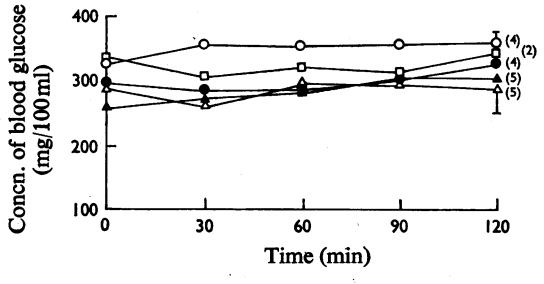


Fig. 1. *Blood glucose concentrations in perfused livers from fed rats*

The livers were infused continuously with free fatty acids (oleate) to maintain a concentration of approx. 1μmol/ml of serum. The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of experiments in each group being given in parentheses.

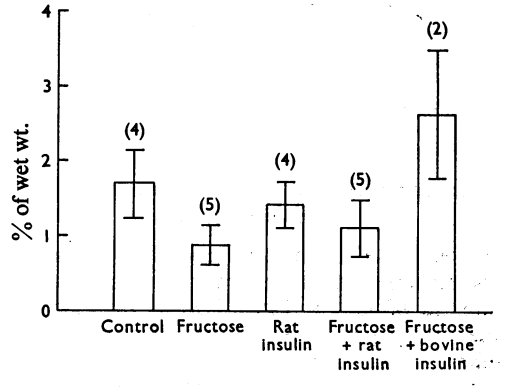


Fig. 2. *Liver glycogen content of perfused livers from fed rats*

The livers were infused continuously with free fatty acids (oleate) to maintain a concentration of approx. 1μmol/ml of serum. Additions to the perfusate are indicated. Mean values are shown and the bars indicate the S.E.M., the numbers of experiments in each group being given in parentheses.

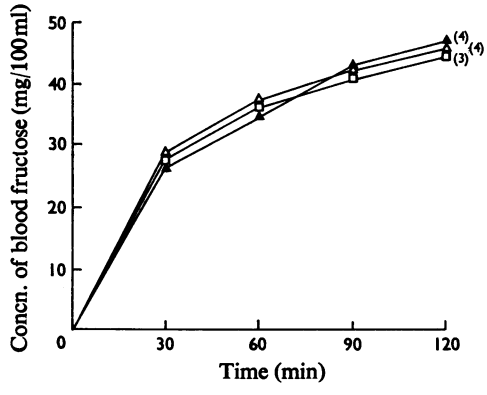


Fig. 3. *Blood fructose concentrations in perfused livers from fed rats*

The livers were infused continuously with free fatty acids (oleate) to maintain a concentration of approx. 1 μmol/ml of serum. The following additions were made to the perfusate: △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown, the numbers of experiments being given in parentheses.

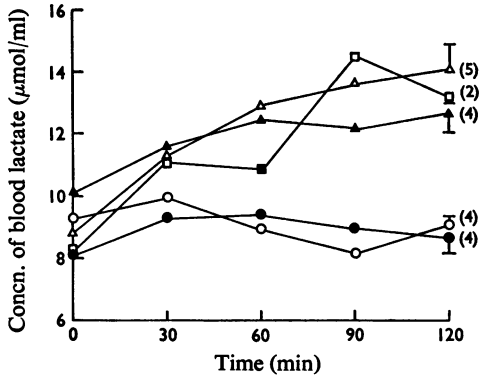


Fig. 4. *Concentrations of blood lactate in perfused livers from fed rats*

The livers were infused continuously with free fatty acids (oleate) to maintain a concentration of approx. 1 μmol/ml of serum. The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

effect on the uptake of free fatty acids by the liver. Similar conclusions may be made with respect to the ^{14}C-labelled free fatty acids in the serum, the specific

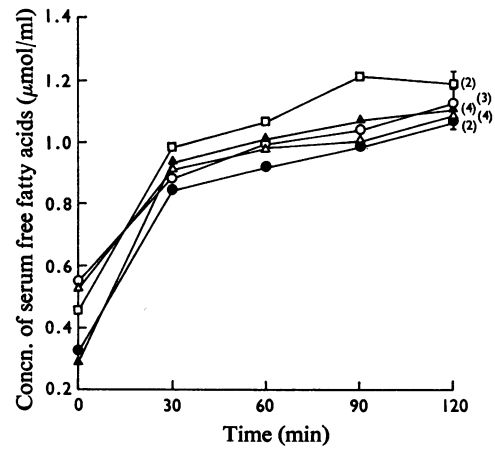


Fig. 5. *Concentrations of serum free fatty acids in perfused livers from fed rats*

The livers were infused continuously with oleate in complex with serum albumin. The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

radioactivity of which rose just perceptibly throughout, indicating that no significant quantities of non-radioactive free fatty acids were added to the perfusate from the liver. Owing to sampling, the perfusate volume diminished from 140 to 108ml during the perfusion, accounting for the slight rise in the concentration of free fatty acids between 30 and 120min (Fig. 5). However, when the total radioactivity of free fatty acids present in the perfusate was plotted (Fig. 6), it was seen to remain constant, indicating that the liver removed free fatty acids from the perfusate at the same rate as it was added by the constant infusion. The mean fractional removal of the free fatty acids flowing through the liver was 43%.

Balance between oxidation and esterification of free fatty acids. A reciprocal relationship between oxidation and esterification of free fatty acids taken up by the liver was well demonstrated by the current experiments (Fig. 7). The balance between oxidation and esterification was significantly affected by both insulin and fructose. Total oxidation was measured as the sum of ^{14}C incorporated into CO_2 and total ketone bodies. Total esterification was taken to be the sum of ^{14}C incorporated into triglycerides, phospholipids and cholesteryl esters in liver plus serum lipoproteins.

Total esterification was least in the control group and increased significantly as a result of the presence

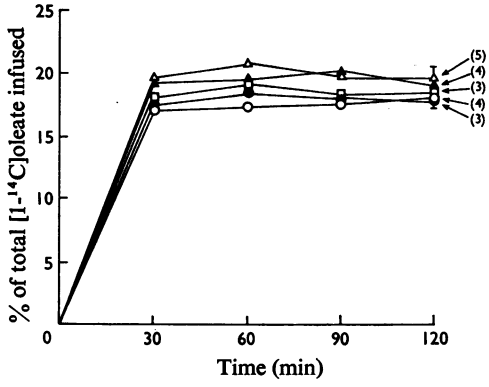


Fig. 6. *^{14}C in lipoproteins of $d>1.006$ present in the perfusate of livers infused continuously with [1-^{14}C]-oleate in complex with albumin*

The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. The values, which represent mainly ^{14}C-labelled free fatty acids (Table 4), are the means and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

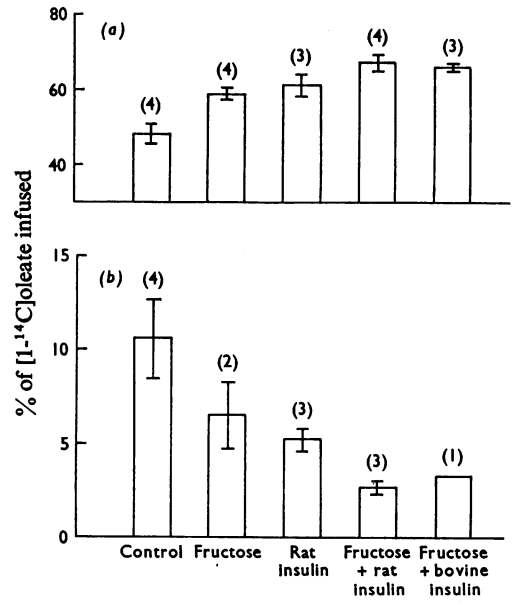


Fig. 7. *Incorporation of ^{14}C into products of (a) esterification and (b) oxidation resulting from the infusion of [1-^{14}C]oleate in perfused livers of fed rats*

Products of oxidation comprised CO_2 plus ketone bodies and products of esterification comprised liver and perfusate triglycerides, phospholipids and cholesteryl esters. Additions to the perfusate are indicated. The perfusions were carried out for 120 min at a free fatty acid concentration of approx. 1 μmol/ml of serum. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

of either insulin or fructose alone (Table 1). When insulin and fructose were present together, incorporation of ^{14}C was increased by an amount equal to the sum of the effects when either insulin or fructose was present alone (Fig. 7*a*).

Total oxidation was greatest in the control group (Fig. 7*b*). It was decreased significantly by the addition of either rat insulin or fructose alone, and was further decreased when fructose was present together with either rat insulin or bovine insulin. When insulin and fructose were present together their effects were additive, there being no significant interaction (Table 1). Although there was some diminution in formation of ^{14}C-labelled ketone bodies, this was not significant, most of the effect of either insulin or fructose in decreasing total oxidation being due to the decrease in oxidation of ^{14}C-labelled free fatty acids to $^{14}CO_2$ (Fig. 8), which was statistically highly significant. It was noted that incorporation into CO_2 did not assume a steady state until after 30–60 min from the start of the infusion of [^{14}C]oleate.

Incorporation of free fatty acids into liver lipids. Most of the radioactivity incorporated from [^{14}C]-oleate into liver lipids was found in triglycerides or phospholipids, with less than 1% of the total ^{14}C infused found in cholesteryl esters or free fatty acids (Table 2). The quantity of [^{14}C]oleate incorporated into cholesteryl esters or free fatty acids was not significantly different in any of the groups studied.

Both insulin and fructose raised significantly the incorporation of [^{14}C]oleate into triglycerides. Although both insulin and fructose increased the incorporation into phospholipids, only the effect due to fructose reached statistical significance (Table 1). The livers treated with fructose plus bovine insulin showed similar effects to the livers treated with fructose plus rat insulin.

Lipoprotein secretion. The output of triglyceride in VLD lipoproteins is shown in Fig. 9. Production was linear during the whole of the experimental period. Control livers secreted 41 μmol of triglyceride fatty acid/liver per h. The separate infusion of fructose or elevation of the concentration of rat insulin in the perfusate raised the rate of secretion to 52 and 59 μmol of triglyceride fatty acid/liver per h respectively. The presence of fructose and insulin together raised the rate of secretion to 71 μmol of triglyceride fatty acid/liver per h. The effects of

insulin and fructose were highly significant (Table 1), and there was no significant interaction between the effects when both were present together. The addition of bovine insulin to fructose-infused livers gave a rate of triglyceride secretion that was not statistically different from that of the livers treated with rat insulin plus fructose. The linear plots in Fig. 9 clearly pass through the origin, indicating that the effects of insulin and fructose on the secretion of VLD lipoproteins are immediate and maximal as soon as the stimuli are present.

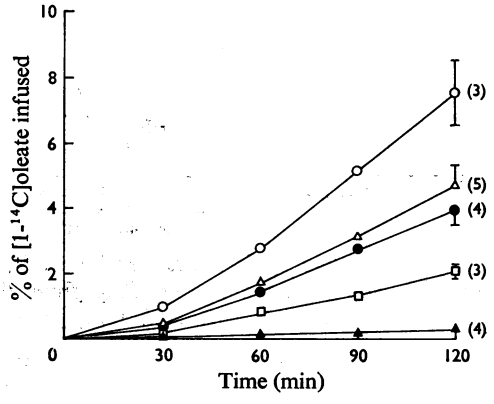


Fig. 8. *Course of $^{14}CO_2$ production in perfused livers from fed rats*

The livers were infused continuously with [1-^{14}C]-oleate to maintain a free fatty acid concentration of approx. 1 μmol/ml of serum. The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

Incorporation of [^{14}C]oleate into the VLD-lipoprotein fraction (mainly into triglycerides) was linear after the labelled fatty acid had been infused for 30–60 min (Fig. 10). Control and fructose-treated livers incorporated approx. 12% of the infused ^{14}C into VLD lipoproteins. Treatment with rat insulin, with and without fructose, raised the amount incorporated significantly, to about 18%. In this particular case bovine insulin was not as effective in mimicking the effect of the rat insulin. Of the [^{14}C]-oleate incorporated into VLD lipoproteins, by far the greatest proportion appeared in triglycerides, with very small incorporations into cholesteryl esters, phospholipids or free fatty acids (Table 3). None of the treatments was effective in altering the incorporation into these minor lipid components of the VLD-lipoprotein fraction.

Almost all the ^{14}C in lipoprotein found in the $d>1.006$ infranatant was in free fatty acids derived from the infusion of [^{14}C]oleate (Table 4). About 1% of the administered radioactivity was present as phospholipids, with less than 1% in cholesteryl esters and triglycerides. Although the mean incorporation into cholesteryl esters was considerably raised in the presence of insulin or fructose alone, the rise was not statistically significant owing to too great a variability. When insulin and fructose were present together the incorporation into cholesteryl esters was not different from the control value.

Lipogenesis. Incorporation of [^{3}H]acetate into lipids present in liver and perfusate lipoproteins was used as an index of the rate of synthesis of fatty acids. Radioactivity in the blood due to the [^{3}H]acetate infused was maintained at a constant concentration throughout the perfusion. Incorporation of ^{3}H into total liver and serum lipids was approx. 4% of the dose infused and was not significantly different between the main groups tested (Fig. 11). However, an incorporation of 7.3% was found in the group treated with fructose plus bovine insulin, this being

Table 2. *Incorporation of [1-^{14}C]oleate into lipids of perfused rat liver*

Perfused livers from fed rats were infused for 120 min with [1-^{14}C]oleate at serum free fatty acid concentrations of approx. 1 μmol/ml. The separation of liver lipid extracts and measurement of radioactivity are described in the text. Values shown are the means ± S.E.M. with the numbers of observations in each group in parentheses.

Additions to perfusate		% of [1-^{14}C]oleate infused in: Cholesteryl esters	Triglycerides	Free fatty acids	Phospholipids	Total (before separation)
None (controls)	(3)	0.30 ± 0.05	23.66 ± 4.25	0.47 ± 0.13	9.99 ± 0.74	37.08 ± 5.56
Fructose	(4)	0.47 ± 0.17	33.46 ± 0.84	0.38 ± 0.21	11.89 ± 0.92	47.60 ± 0.60
Rat insulin	(3)	0.24 ± 0.09	32.10 ± 0.52	0.47 ± 0.12	11.08 ± 1.32	44.00 ± 2.01
Fructose + rat insulin	(4)	0.35 ± 0.10	35.36 ± 1.76	0.30 ± 0.07	14.65 ± 1.21	51.01 ± 1.86
Fructose + bovine insulin	(3)	0.35 ± 0.00	40.59 ± 2.66	—	15.20 ± 1.52	54.56 ± 1.52

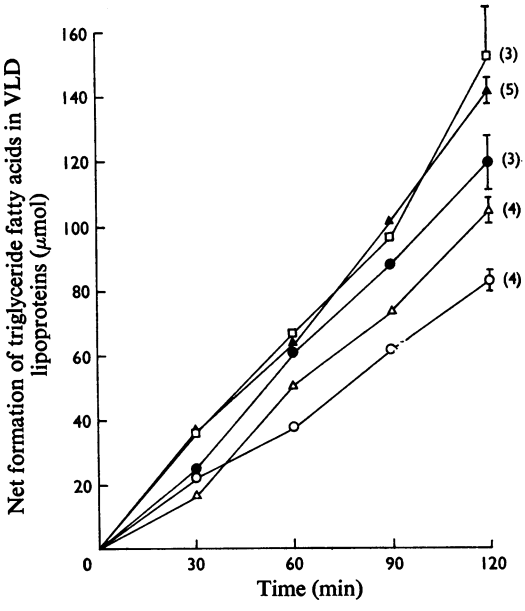


Fig. 9. *Net production of triglyceride in VLD lipoproteins in perfused livers from fed rats*

The livers were infused with free fatty acids (oleate) to maintain a concentration of approx. 1 μmol/ml of serum. The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

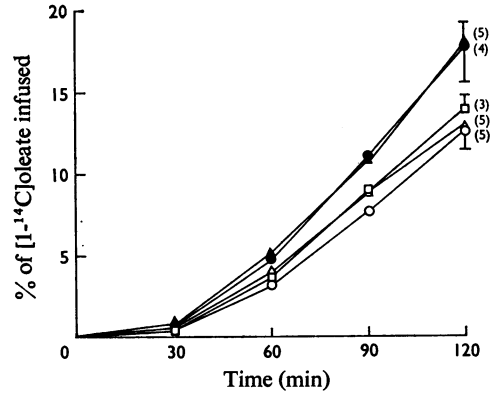


Fig. 10. *Course of incorporation of ^{14}C into VLD lipoproteins in perfused livers from fed rats infused continuously with [1-^{14}C]oleate to maintain a free fatty acid concentration of approx. 1 μmol/ml of serum*

The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

significantly higher ($P<0.001$) than those in the other groups combined. This rise could be attributed to an increased incorporation of ^3H into all major lipid classes in the liver (Table 5), where the incorporation was seen to be mainly in the triglyceride and phospholipid fractions.

There was a significant effect of rat insulin, but not of fructose, in enhancing incorporation of [^3H]-acetate into the lipids of the VLD-lipoprotein fraction (Fig. 12). As with [^{14}C]oleate, incorporation of [^3H]acetate into VLD lipoproteins did not achieve a steady state before 30–60 min had elapsed from the start of the [^3H]acetate infusion. Details of the distribution of the isotope in the different lipid classes are shown in Table 6. Most of the incorporation of label was in triglycerides.

^3H was also recovered in $d>1.006$ lipoproteins, although the total incorporation was less than 0.5% of the infused dose. Details of the distribution of ^3H in lipid classes is shown in Table 7. There were no significant differences between the experimental groups. Unlike the incorporation of label into VLD lipoproteins, [^3H]acetate incorporation into $d>1.006$ lipoproteins was linear throughout the experimental period (Fig. 13).

Calculations have been made of the contribution of the ^{14}C-labelled free fatty acids to the total triglyceride in the VLD-lipoprotein fraction (Table 8). Similar information may be gained from a calculation of the specific radioactivity of the fatty acids in VLD lipoproteins compared with the specific radioactivity of serum free fatty acids (Table 9). On combination of the information from these tables, which show similar trends between the experimental groups, it would appear that in the control group about half of the fatty acids of the VLD lipoproteins were derived from serum free fatty acids. In the presence of fructose the augmentation in output of VLD lipoproteins was accounted for entirely by increased output of unlabelled triglyceride molecules, but when the concentration of rat insulin was elevated the increased output of triglyceride of the VLD-lipoprotein fraction was derived from both free fatty acids and an unlabelled source. When fructose and rat insulin were present together, increased output of VLD lipoproteins was due to increased contributions from both serum free fatty acids and an unlabelled source of glycerides. In the presence of fructose plus bovine insulin the increased output was due more to an increased output of unlabelled

Table 3. *Incorporation of* [1-^{14}C]*oleate into lipids of serum lipoproteins of* $d<1.006$ (*VLD lipoproteins*) *by perfused rat liver*

Perfused livers from fed rats were infused for 120min with [1-^{14}C]oleate at serum free fatty acid concentrations of approx. 1 μmol/ml. The separation of lipid extracts of ultracentrifugally prepared lipoproteins and measurement of radioactivity are described in the text. The results are corrected for withdrawal of samples during the perfusion. Values shown are the means ± S.E.M. with the numbers of observations in each group in parentheses.

Additions to perfusate		% of [1-^{14}C]oleate infused in: Cholesteryl esters	Triglycerides	Free fatty acids	Phospholipids	Total (before separation)
None (controls)	(3)	0.04 ± 0.00	10.63 ± 1.59	0.24 ± 0.01	0.25 ± 0.01	11.68 ± 1.47
Fructose	(5)	0.04 ± 0.01	10.86 ± 1.26	0.37 ± 0.18	0.18 ± 0.04	12.63 ± 1.51
Rat insulin	(4)	0.06 ± 0.01	16.89 ± 2.28	0.65 ± 0.10	0.53 ± 0.10	17.64 ± 2.22
Fructose + rat insulin	(5)	0.05 ± 0.01	16.34 ± 1.17	0.20 ± 0.04	0.40 ± 0.07	17.73 ± 1.35
Fructose + bovine insulin	(3)	—	—	—	—	13.84 ± 0.73

Table 4. *Incorporation of* [1-^{14}C]*oleate into lipids of lipoproteins of* $d>1.006$ *by perfused rat liver*

Perfused livers from fed rats were infused for 120min with [1-^{14}C]oleate at serum free fatty acid concentrations of approx. 1 μmol/ml. The separation of lipid extracts of ultracentrifugally prepared lipoproteins and measurement of radioactivity are described in the text. Apart from free fatty acids, which were utilized, the results are corrected for withdrawal of samples during the perfusion. It follows that this correction could not be applied to the total lipids before separation. Values shown are the means ± S.E.M. with the numbers of observations in each group in parentheses (* indicates one result only).

Additions to perfusate		% of [^{14}C]oleate infused in: Cholesteryl esters	Triglycerides	Free fatty acids	Phospholipids
None (controls)	(4)	0.06 ± 0.00	0.00 ± 0.00	18.10 ± 0.23	1.12 ± 0.12
Fructose	(5)	0.17 ± 0.16	0.34*	18.82 ± 0.91	1.17 ± 0.23
Rat insulin	(3)	0.29 ± 0.19	0.81 ± 0.10	16.71 ± 0.27	1.23 ± 0.10
Fructose + rat insulin	(4)	0.10 ± 0.02	0.01 ± 0.00	19.09 ± 0.99	1.26 ± 0.15
Fructose + bovine insulin	(3)	0.09 ± 0.00	0.50 ± 0.10	18.18 ± 0.52	1.43 ± 0.04

material and less to the contribution from free fatty acids.

Discussion

The action of substrates and hormones in altering hepatic metabolism may involve relatively slow changes in the rate of synthesis of adaptive enzymes, or rapid effects involving other mechanisms. The present work is concerned with the immediate effects of insulin and fructose, and these are therefore not regarded as being due to significant changes in total enzyme protein.

Effects of fructose

Fructose is known to be actively metabolized by the liver, and the present results indicate a substantial uptake by the perfused liver at blood concentrations attainable after oral administration of fructose in the intact animal. Knowledge of its metabolism has been summarized by Sillero *et al.* (1969), who confirm that the Hers pathway is the principal route for fructose metabolism in rat liver. Fructose is phosphorylated to fructose 1-phosphate, the reaction being catalysed by fructokinase (EC 2.7.1.3). Fructose 1-phosphate is split by fructose 1-phosphate aldolase (EC 4.1.2.7) to dihydroxyacetone phosphate and D-glyceraldehyde. The latter is converted into glyceraldehyde 3-phosphate by triokinase (EC 2.7.1.28). Thus a quantitative conversion occurs of fructose into triose phosphates, which are in turn the direct precursors of glycerol 3-phosphate. It has been demonstrated that addition of fructose to the perfused liver leads to an increase in the concentration of glycerol 3-phosphate (Wieland & Matschinsky,

1962; Woods *et al.*, 1970), which is a co-substrate in the esterification of acyl-CoA to glycerides. It is therefore reasonable to suggest that, in the present work, fructose enhanced the esterification of free fatty acids by virtue of its conversion into glycerol 3-phosphate. Pereira & Jangaard (1971) have shown substantial incorporation of labelled fructose into hepatic glyceride glycerol. As previous studies have shown that oxidation of free fatty acids is inversely related to its esterification (Mayes & Felts, 1966, 1967*b*), the decreased oxidation of free fatty acids, as a result of the presence of fructose, may be ascribed to its increased esterification. Alternatively, it cannot be ruled out that the oxidation of acetyl-CoA, formed from fructose via pyruvate, spares the oxidation of fatty acids, allowing more of the free fatty acids to be esterified in glycerides. Administration of fructose *in vivo* (Heinz & Junghänel, 1969) and in the perfused liver (Woods *et al.*, 1970) leads to a rise in concentration of intermediates in glycolysis, including lactate and pyruvate. These findings help to explain the increase in perfusate lactate in the presence of fructose. The present findings show that, at the physiological concentrations of fructose used in the study, the increase in perfusate lactate is due to decreased uptake of lactate rather than to its actual production by the liver. It would appear that in the presence of fructose a new steady state between blood lactate and hepatic lactate uptake is attained at a higher concentration of lactate in the blood.

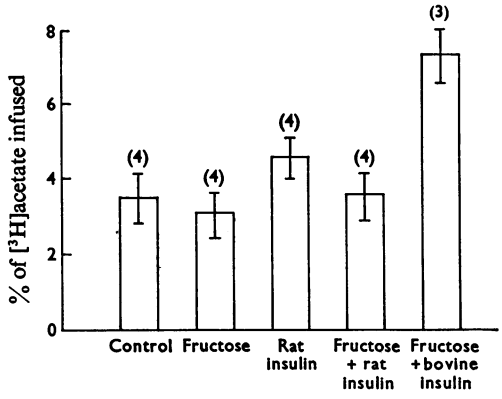


Fig. 11. *Incorporation of [Me-³H]acetate into liver lipids plus VLD-lipoprotein lipids*

Perfused livers from fed rats were infused continuously for 120min with trace amounts of [*Me*-³H]acetate. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

Effects of insulin

Few unequivocal direct effects of insulin on hepatic metabolism have been established. The better documented effects include a decrease in glucose and urea production (Mortimore, 1963) and several reports (e.g. Haft & Miller, 1958) demonstrating increased lipogenesis. Poledne & Mayes (1970) have reported that addition of insulin to perfused livers of starved rats increases the output of VLD lipoproteins and decreases the formation of ketone bodies derived from endogenous hepatic lipids. The current work has augmented this information by demonstrating that a raised concentration of rat insulin or addition of bovine insulin at physiological concentrations to perfused livers of normal fed rats causes a very significant enhancement in the secretion of VLD lipoproteins. In addition, the fate of plasma free fatty acids taken up by the liver is altered significantly. Thus insulin decreased the oxidation of labelled oleate but enhanced its esterification to glycerides together

Table 5. *Incorporation of [Me-³H]acetate into lipids of perfused rat liver*

Perfused livers from fed rats were infused for 120min with trace amounts of sodium [*Me*-³H]acetate at serum free fatty acid concentrations of approx. 1 μmol/ml. The separation of lipid extracts and measurement of radioactivity are described in the text. Values shown are the means ± S.E.M. with the numbers of observations in each group in parentheses.

Additions to perfusate		% of [³H]acetate infused in: Cholesteryl esters	Triglycerides	Free fatty acids	Phospholipids	Total (before separation)
None (controls)	(3)	0.19 ± 0.07	1.53 ± 0.30	0.01 ± 0.00	0.49 ± 0.11	2.42 ± 0.40
Fructose	(5)	0.23 ± 0.07	1.45 ± 0.28	0.02 ± 0.00	0.55 ± 0.18	2.42 ± 0.47
Rat insulin	(4)	0.14 ± 0.04	2.17 ± 0.22	0.05 ± 0.00	1.05 ± 0.16	3.60 ± 0.31
Fructose + rat insulin	(4)	0.13 ± 0.04	1.77 ± 0.37	0.02 ± 0.00	0.92 ± 0.22	2.82 ± 0.49
Fructose + bovine insulin	(3)	0.44 ± 0.09	3.81 ± 0.34	0.09 ± 0.01	1.69 ± 0.20	6.28 ± 0.59

Table 6. *Incorporation of [Me-^{3}H]acetate into lipids of serum lipoproteins of d<1.006 (VLD lipoproteins) by perfused rat liver*

Perfused livers from fed rats were infused for 120min with trace amounts of sodium [*Me*-^{3}H]acetate at serum free fatty acid concentrations of approx. 1 μmol/ml. The separation of lipid extracts of ultracentrifugally prepared lipoproteins and measurement of radioactivity are described in the text. The results are corrected for withdrawal of samples during the perfusion. Values shown are the means ± S.E.M. with the numbers of observations in each group in parentheses.

Additions to perfusate		% of [^{3}H]acetate infused in: Cholesteryl esters	Triglycerides	Free fatty acids	Phospholipids	Total (before separation)
None (controls)	(2)	0.01 ± 0.00	0.27 ± 0.07	0.00 ± 0.00	0.01 ± 0.00	0.32 ± 0.09
Fructose	(5)	0.03 ± 0.01	0.33 ± 0.08	0.01 ± 0.00	0.02 ± 0.00	0.40 ± 0.09
Rat insulin	(3)	0.03 ± 0.01	1.04 ± 0.22	0.01 ± 0.00	0.01 ± 0.01	1.20 ± 0.24
Fructose + rat insulin	(3)	0.01 ± 0.00	0.53 ± 0.1	0.01 ± 0.00	0.02 ± 0.00	0.57 ± 0.09
Fructose + bovine insulin	(3)	—	—	—	—	1.05 ± 0.14

Table 7. *Incorporation of [Me-^{3}H]acetate into lipids of serum lipoproteins of d>1.006 by perfused rat liver*

Perfused livers from fed rats were infused for 120min with trace amounts of sodium [*Me*-^{3}H]acetate at serum free fatty acid concentrations of approx. 1 μmol/ml. The separation of lipid extracts of ultracentrifugally prepared lipoproteins and measurement of radioactivity are described in the text. The results are corrected for withdrawal of samples during the perfusion. Values shown are the means ± S.E.M. with the numbers of observations in each group in parentheses.

Additions to perfusate		% of [^{3}H]acetate infused in: Cholesteryl esters	Triglycerides	Free fatty acids	Phospholipids	Total (before separation)
None (controls)	(1)	0.00	0.05	0.06	0.06	0.19
Fructose	(3)	0.01 ± 0.00	0.13 ± 0.05	0.05 ± 0.01	0.10 ± 0.03	0.20 ± 0.03
Rat insulin	(2)	0.02 ± 0.01	0.17 ± 0.07	0.07 ± 0.01	0.11 ± 0.02	0.45 ± 0.03
Fructose + rat insulin	(3)	0.01 ± 0.00	0.16 ± 0.02		0.07 ± 0.01	0.30 ± 0.08
Fructose + bovine insulin	(3)	0.02 ± 0.00	0.14 ± 0.02	0.13 ± 0.03	0.09 ± 0.03	0.37 ± 0.03

with its transport out of the livers in VLD lipoproteins. The present results also confirm the effects of insulin in decreasing hepatic glucose production and in causing an enhancement of lipogenesis.

We believe that the effect of insulin in decreasing the concentration of hepatic cyclic AMP (adenosine 3′:5′-cyclic monophosphate) (Jefferson *et al.*, 1968) can account for most of the present results. The details of its mechanism of action in this respect are still in dispute. One line of evidence (Hepp, 1971) suggests that insulin decreases the formation of cyclic AMP by inhibition of adenylate cyclase; the other (Senft *et al.*, 1968) suggests that insulin enhances the breakdown of cyclic AMP by activating 3′:5′-cyclic nucleotide phosphodiesterase (EC 3.1.4.C). Jefferson *et al.* (1968) and Menahan & Wieland (1969) have provided evidence to show that the antagonism between insulin and glucagon in the perfused liver is mediated via changes in the concentration of cyclic AMP, glucagon stimulating adenylate cyclase activity with formation of cyclic AMP and insulin antagonizing this effect.

A hormone-sensitive lipase, similar in many respects to the better-documented lipase in adipose tissue, has been described in liver. This enzyme is activated by glucagon and cyclic AMP (Bewsher & Ashmore, 1966; Guder *et al.*, 1970), and the reaction is accompanied by increases in acyl-CoA concentration and in ketogenesis (Claycomb *et al.*, 1969). Thus glucagon increases lipolysis in liver

Table 8. *Steady-state production of VLD lipoproteins by perfused livers from fed rats infused with [1-^{14}C]oleate*

[^{14}C]Oleate was infused at a constant rate. Production rates of triglyceride in VLD lipoproteins were calculated for the period 0–120min of perfusion. The contribution made by ^{14}C-labelled free fatty acids was calculated from the rate infused and from the steady-state rate of incorporation of ^{14}C into VLD lipoproteins.

Additions to perfusate	Production of triglyceride (μmol of fatty acid/h)		
	Total	Arising from ^{14}C-labelled free fatty acid	Arising from an unlabelled source
None (control)	41.1	19.4	21.7
Fructose	52.2	18.5	33.7
Rat insulin	59.4	27.2	32.2
Fructose+rat insulin	70.8	27.2	43.6
Fructose+bovine insulin	76.3	22.3	54.0

Table 9. *Relative specific radioactivities of free fatty acids, lipoproteins and liver triglycerides in perfused rat livers infused with [1-^{14}C]oleate*

[^{14}C]Oleate was infused at a constant rate into perfused livers from fed rats. Specific radioactivities are expressed relative to that of serum free fatty acids (= 1.0). The calculations of the specific radioactivities of free fatty acids and VLD lipoproteins are based on results obtained in the steady state, 60–120min after the start of the [^{14}C]oleate infusion.

Additions to perfusate	Relative specific radioactivity in	
	VLD-lipoprotein triglyceride fatty acids	Liver triglyceride fatty acids
None (control)	0.68	0.12
Fructose	0.60	0.12
Rat insulin	0.67	0.11
Fructose+rat insulin	0.61	0.13
Fructose+bovine insulin	0.59	0.17

probably by increasing the concentration of cyclic AMP. It would seem that insulin, by virtue of the fact that it lowers the concentration of hepatic cyclic AMP, is anti-lipolytic in the liver, acting on the same lipolytic enzyme system as glucagon. It is noteworthy that insulin has an analogous and marked anti-lipolytic effect in adipose tissue, apparently by lowering the concentration of cyclic AMP (Butcher *et al.*, 1966).

With respect to the current work it may be asked: how does the anti-lipolytic effect of insulin decrease the oxidation and enhance esterification of exogenous free fatty acids and enhance the secretion of VLD lipoproteins? Essentially the same problem is posed by the results of Heimberg *et al.* (1969), who showed that addition of glucagon to perfused livers caused an increase in the oxidation of free fatty acids to ketone bodies and a decrease in the secretion of triglyceride. To evaluate the mechanisms underlying both our results and those of Heimberg *et al.* (1969), it is necessary to consider the nature of the hepatic triglyceride pools and their relationship with the secretion of VLD lipoproteins on the one hand and the tissue free fatty acid pool and oxidative pathways on the other.

Hepatic triglyceride fatty acids are the immediate precursors of triglyceride fatty acids secreted in VLD lipoproteins (Havel *et al.*, 1962). Present results obtained in the steady state in isotopic equilibrium (Tables 8 and 9) show that the specific radioactivity of the triglyceride fatty acid in VLD lipoproteins of control perfusions was about half that of the infused free fatty acids, whereas the specific radioactivity of the total liver triglyceride remained considerably lower (Table 9). These results demonstrate clearly that the VLD-lipoprotein triglyceride precursor pool in the liver (pool 1 in Scheme 1) must be in close metabolic proximity not only to the secretion of

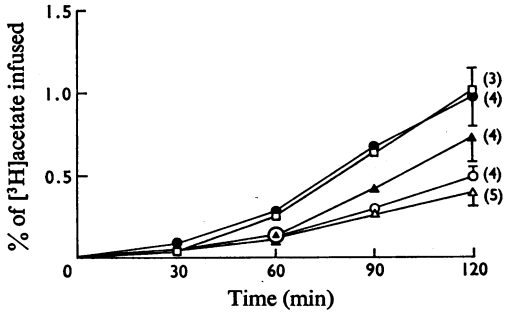


Fig. 12. *Course of incorporation of ^3H into lipids of VLD lipoproteins by perfused livers from fed rats infused continuously with trace amounts of [Me-^3H]-acetate and free fatty acids (oleate) to maintain a concentration of approx. 1 μmol/ml of serum*

The following additions were made to the perfusate: ○, none (control); ●, rat insulin; △, fructose; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

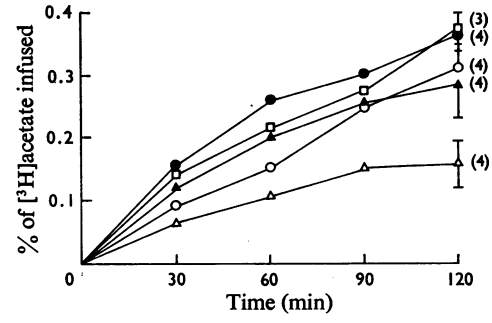


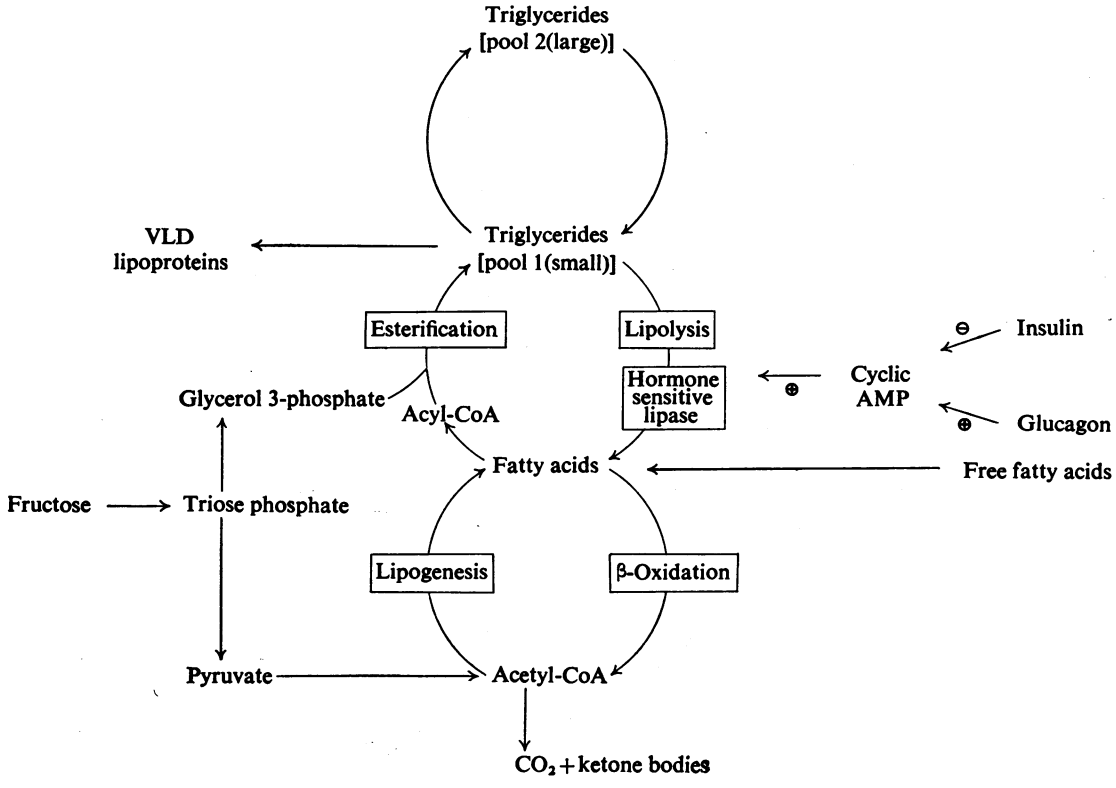
Fig. 13. *Course of incorporation of ^3H into lipids of lipoproteins of $d>1.006$ by perfused livers from fed rats infused continuously with trace amounts of [Me-^3H]acetate and with free fatty acids (oleate) to maintain a concentration of approx. 1 μmol/ml of serum*

The following additions were made to the perfusate: ○, none (control); ●, rat insulin; ▲, fructose plus rat insulin; □, fructose plus bovine insulin. Mean values are shown and the bars indicate the S.E.M., the numbers of observations being given in parentheses.

VLD lipoproteins but also to the liver free fatty acid pool. It follows that the main mass of hepatic triglyceride (pool 2 in Scheme 1) is probably a side branch from the smaller pool 1, into which label from pool 1 travels but from which it is very slow to return, owing presumably to dilution and to slow turnover of the larger pool. That more than one metabolic pool of triglyceride exists in liver has been suggested by previous investigators (Stein & Shapiro, 1959; Baker & Schotz, 1964; Jones *et al.*, 1967; Richards *et al.*, 1968). To account for the ability of insulin to control the balance between esterification of plasma free fatty acids and their oxidation, it is suggested that insulin inhibits lipolysis of pool 1. This would effectively cause the concentration of the free fatty acid pool to fall, thereby diminishing oxidation, and the triglyceride in pool 1 to increase, thereby increasing the secretion of VLD lipoproteins. If glucagon stimulates the lipase that insulin inhibits, the postulated mechanism would also explain the results of Heimberg *et al.* (1969). The fact that these authors found that glucagon had no detectable effect on lipolysis of the main mass of triglyceride in the liver is additional information in support of our suggestion that the hormone-sensitive lipase probably hydrolyses the triglyceride in pool 1 rather than that in pool 2. Scheme 1 shows clearly that, because net lipolysis of triglyceride is inversely related to net esterification of free fatty acids, the esterification of free fatty acids in the liver could well be controlled by the activity of lipolytic enzymes, as well as by factors directly influencing esterification.

Secretion of VLD lipoproteins

Previous results (Mayes & Felts, 1966; Mayes, 1970) have shown that increasing the concentration of free fatty acids in the perfusate of the isolated liver leads to an increase in the rate of secretion of VLD lipoproteins and that the effect is immediately maximal. The same effect was seen in the current work when the secretion of VLD lipoproteins was stimulated immediately and maximally on the addition of fructose or insulin to the perfusate. The common feature in all these experiments is increased synthesis of triglyceride, suggesting that the secretion of VLD lipoproteins is immediately responsive to an increase in triglyceride mass in pool 1 (Scheme 1). The initial delay in the secretion of ^{14}C-labelled VLD lipoproteins under all present conditions (Fig. 10) has also been observed in previous experiments (Mayes & Felts, 1966), and may be attributed to initial dilution of the label in pool 1 until it attains isotopic equilibrium with the infused ^{14}C-labelled free fatty acids. It would appear that a considerable proportion of the triglyceride fatty acid secreted initially in response to the stimulus of a raised concentration of free fatty acids or due to insulin or fructose administration does not originate from the free fatty acids taken up by the liver at the time of the stimulus.



Scheme 1. *Proposed scheme to account for the effects of insulin and fructose in promoting the esterification and decreasing the oxidation of free fatty acids by the liver and in promoting the secretion of VLD lipoproteins*

Although raised concentrations of insulin or addition of fructose increased net esterification of ^{14}C-labelled free fatty acids (Fig. 7*a*) and both factors enhanced secretion of VLD-lipoprotein triglyceride (Fig. 9), only insulin caused a significant increase in transport of labelled fatty acids out of the liver in VLD lipoproteins. It might be suggested that insulin selectively enhanced the formation of VLD lipoproteins from pool 1, but if this were so it would be expected that, when fructose was present in addition to insulin, there would be more than an additive effect on the secretion of VLD lipoproteins. Table 1 shows clearly that no interaction between the insulin and the fructose effects was detected in any parameter investigated. More than one precursor pool of VLD-lipoprotein triglyceride may have to be invoked to explain the results fully.

Lipogenesis and the secretion of VLD lipoproteins

Windmueller & Spaeth (1967) showed that synthesis *de novo* of fatty acids caused the perfused liver to secrete greater quantities of VLD lipoproteins. In the present investigations the contribution of unlabelled fatty acids to the VLD-lipoprotein triglyceride (Table 8) could be derived either from the larger hepatic triglyceride pool of low specific radioactivity (pool 2) or from synthesis *de novo*. In the presence of fructose plus bovine insulin incorporation of [^{3}H]acetate into total liver plus VLD-lipoprotein lipids (Fig. 11) and into the latter lipids alone (Fig. 12) was doubled compared with the values for the control group. These results suggest that the increase of the contribution of unlabelled fatty acids to VLD lipoproteins recorded in this particular group (Table 8) could be due, in part, to increased lipogenesis. However, the other results in Fig. 11, which show no changes, are inconsistent with the results in Tables 8 and 9. The failure to detect lipogenesis in these groups may be due to the measurement of the incorporation of [^{3}H]acetate being insensitive to the small changes involved because of the variation in results within each group. It is also possible that an increase in the size of the acetyl-CoA pool or a decrease in the activity of acetyl-CoA synthetase might decrease the incorporation of [^{3}H]acetate into fatty acids.

It must be pointed out that some differences may

be expected between the results obtained with fructose plus higher concentrations of rat insulin and those obtained with fructose plus bovine insulin. The administration of glucose to the blood donors to promote a higher blood concentration of rat insulin may also lower the glucagon concentration. Although this effect would be expected to be small, as the donor animals were in the fed state, it may account for the higher rate of $^{14}CO_2$ production and lower ^{14}C radioactivity in VLD-lipoprotein triglyceride in the group treated with bovine insulin.

It is well established that insulin may affect hepatic metabolism indirectly by regulating the availability of free fatty acids from adipose tissue. The present results show that insulin also plays a substantial and direct role in regulating hepatic lipid metabolism. Both insulin and fructose have immediate effects on the liver leading to enhanced secretion of VLD lipoproteins. These investigations therefore supply a biochemical basis for clinical correlations that have been made between hyperinsulinism, hypertriglyceridaemia and coronary heart disease, between hyperinsulinism, hypertriglyceridaemia and obesity, and between dietary sucrose (i.e. fructose), hyperinsulinism and coronary heart disease.

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