The Effect of Triglycerides on Gluconeogenesis and Ketogenesis in the Isolated Perfused Rat Liver* (33726)

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Hepatic utilization of triglycerides is presumably initiated by hydrolysis to glycerol and free fatty acids mediated by the action of an hepatic lipase. Stein and Shapiro (1) demonstrated that when triglycerides labeled with ^{14}C glycerol and ^{3}H fatty acids were injected into rats, 60% of the injected radioactivity was recovered in the liver within 15 min. A similar study by Olivecrona (2) indicated that 90% of the injected radioactivity was found in the liver after 20 min, and ratios of ^{14}C:^{3}H in the hepatic triglyceride fraction indicated that little or no hydrolysis had taken place. However, with time a decline in the ^{14}C:^{3}H ratio indicated that appreciable hydrolysis and resynthesis had taken place. Bewsher and Ashmore (3) demonstrated that glucagon and cyclic 3′5′AMP increase the lipase activity of rat liver slices and homongenates. Claycomb and Kilsheimer (4) and Williamson *et al.* (5) also demonstrated that an increase in tissue free fatty acids and acyl-CoA esters occurs after addition of glucagon or 3′5′AMP.

That glucagon, 3′5′AMP and free fatty acids increase glucose production by isolated perfused rat livers has been demonstrated by the work of several laboratories (6–10). It has also been suggested that the primary action of glucagon and/or 3′5′AMP in stimulating gluconeogenesis involves the activation of an hepatic lipase and that the increase in glucose formation observed *in vitro* after such stimulation is the result of an increase in intracellular free fatty acid. On the other hand, Krebs (11) has presented evidence that the effects of fatty acids and glucagon are additive in glucose production by the perfused liver and therefore presumably act by different mechanisms.

The object of this study was to perfuse livers with triglycerides and to examine their role in ketogenesis and gluconeogenesis. In order to test the glucagon, 3′5′AMP hypothesis, effects of glucagon and theophylline on ketogenesis and gluconeogenesis were also examined. It was assumed that the action of theophylline on hepatic nucleotide phosphodiesterase would increase tissue cyclic 3′5′AMP levels (12), and that an increase in ketone body production would reflect an increase in the hydrolysis of triglycerides.

Materials and Methods. Livers from fasted 200–250 g rats of the Wistar strain were perfused as previously described (13). Tributyrin and L(+)-lactate were added to the medium 30 min after perfusion had begun and the initial concentrations were 1.5 and 20 m*M*, respectively. All perfusions were continued for 90 min after the 30-min preperfusion period. Ediol (Riker Laboratories, Northridge, Calif.) 0.16 ml/100 ml of media was added at the beginning of the perfusion. This amount of Ediol on hydrolysis yielded 0.5 mmoles of fatty acids, mainly by myristic, 18% and lauric, 51%. No measurable free fatty acids (FFA) were present in the media prior to hydrolysis, but 3.74 μmoles of free glycerol were present per 100 ml; DL-carnitine, 1m*M*, was present in the perfusing medium whenever Ediol was used. When theophylline was used, it was added to the medium prior to perfusion and the concentration was 10 m*M*. Glucagon (Lot No. 258-234B-167-1 supplied by Dr. W. N. Shaw, Lilly Research Laboratories, Indi-

* Supported in part by USPH grants AM 7211 and GM 953.

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TABLE I. Effects of Glucagon and Theophylline on Glucose and Ketone Body Production by Rat Livers Perfused in the Presence and Absence of Triglycerides.[a]

Substrates and drugs	Lactate utilized	Glucose produced	Ketone body production
None		4.4 ± 0.6	10 ± 0.7
Theophylline (10 m*M*)		9 ± 2	13 ± 1.4
Lactate (20 m*M*)	120 ± 10	32 ± 6	12 ± 1.8
+ theophylline (10 m*M*)	138 ± 9	44 ± 5	14 ± 1.1
+ tributyrin (1.5 m*M*)	110 ± 12	60 ± 5	23 ± 2.0
+ tributyrin + theophylline	168 ± 13	92 ± 9	30 ± 2.3
+ glucagon (2 μg/ml)	133 ± 12	68 ± 6	18 ± 1.5
+ tributyrin + glucagon	156 ± 20	72 ± 10	43 ± 4.1
+ Ediol	156 ± 13	66 ± 7	34 ± 4.3
+ Ediol + glucagon	160 ± 13	75 ± 8	45 ± 3.8
+ Ediol + theophylline	181 ± 4	92 ± 8	56 ± 6.2

[a] All values are expressed as μmoles/g of liver/90 min and represent the mean ± SE of four (or more) observations.

anapolis, Indiana) was added after the 30-min preperfusion.

Initial and final samples of the medium were analyzed for glucose (14), lactic acid (15), and ketone bodies (16). Samples of liver were obtained at the beginning and end of the perfusion and analyzed for glycogen (17). Fatty acids were measured by the method of Novack (18) and glycerol was estimated by the enzymatic method of Wieland (19).

Results. No free fatty acids were present in the initial medium and only a small amount of free glycerol (3.74 μmoles/100 ml). Since heparin was used in the perfusion, the possibility existed of activation of a lipase present in the small amount of serum which was left in the liver. To study this possibility a liver was placed in the perfusion apparatus, perfused for 10 min with Ediol, and then removed. The perfusion, without a liver present, was continued for 120 min. Samples were taken each 30 min and assayed for FFA, acetoacetate, and beta-hydroxybutyrate. No detectable amount of FFA or ketones were observed.

Glycogen determinations confirmed the fasting state of the livers. Initial glycogen values ranged from 8 to 13 μmoles of glucose/g and tended to increase slightly during the course of the perfusion except when glucagon or theophylline was added in which cases a decrease of 5–8 μmoles was observed. Total ketone body production was obtained by adding the values for acetoacetate and beta-hydroxybutyrate. At least four livers were perfused for each result presented (Table I). When lactate alone was used as the substrate, 32 ± 6μmoles/g/90 min of new glucose were produced, and 12 ± 1.8 μmoles/g/90 minutes of ketone bodies were formed (Table I). Addition of tributyrin to the medium using the same lactate concentration increased glucose production to 60 ± 7 μmoles/g/90 minutes and ketone bodies to 23 ± 2μmoles/g/90 min. When both theophylline and tributyrin were added to the medium, glucose production increased to 92 ± 9μmoles/g/90 min and ketone production increased to 30 ± 2.3 μmoles/g/90 min. With theophylline in the medium, but no lactate, glucose production fell to 9 ± 2 μmoles/g/90 min, and there was no change in ketone body production as compared to lactate alone. When glucagon, 2μg/ml, was added to the medium, with lactate alone, glucose production increased to 68 ± 7 μmoles/g/90 min, and there was a small rise in ketone body production to 18 ± 1.5 μmoles/g/90 min. When livers were perfused with both glucagon and tributyrin, 72 ± 10 μmoles/g/90 min of new glucose were formed which represents no change when compared with glucagon and lactate. However, there

TABLE II. Glucose Production by Rat Livers Perfused with Glycerol and Lactate.[a]

Substrates	Drugs	Glucose produced
Glycerol (20 m*M*)	—	40 ± 2
	Glucagon (2 μg/ml)	55 ± 4
	Octanoate (4 m*M*)	38 ± 3
Lactate (20 m*M*)	—	32 ± 6
+ glycerol (20 m*M*)	—	86 ± 9

[a] All values are expressed as μmoles/g of liver/90 min and represent the mean ± SE of four observations.

was an increase in ketone body production to 43 ± 4.1 μmoles/g/90 min.

When Ediol was perfused with 20 m*M* lactate, glucose production increased to 66 ± 7 μmoles/g/90 min and ketone body production increased to 34 ± 4.3 μmoles/g/90 min. When glucagon 2 μg/ml was added to Ediol and lactate, glucose production was 75 ± 8 μmoles/g/90 min which represents no significant change over that seen with Ediol alone; however, ketone body production increased to 45 ± 3.8 μmoles/g/90 min. Theophylline in the presence of Ediol and lactate increased glucose production to 92 ± 8μmoles/g/90 min and ketone body production to 56 ± 6.2 μmoles/g/90 min.

Glycerol, 20 m*M*, used alone as a substrate resulted in 40 ± 2 μmoles/g/90 min of glucose produced (Table II). Glucagon, 2 μg/ml, and glycerol increased glucose production to 55 ± 4 μmoles/g/90 min. When glycerol and octanoate (4 m*M*) were added to the medium, there was no significant change in glucose production as compared to glycerol alone. When both glycerol and lactate were used as substrates, glucose production was 86 ± 9μmoles/g/90 min, and their effect on gluconeogenesis was additive.

Table I summarizes the results of lactate utilization. When lactate alone was used as the substrate 120 ± 10 μmoles/g/90 min was utilized, and 32 ± 6μmoles/g/90 min of glucose was formed. Since it takes 2 moles of lactate to make 1 mole of glucose, the maximum amount of lactate which could have been converted to glucose would be 64 μmoles/g/90 min and this could account for only 53% of the lactate which was utilized. When tributyrin alone and glucagon alone were added to the medium, there was no significant change in the absolute rate of lactate utilization, but the percentage of lactate used which could have gone to form glucose increased to 109% and 113%, respectively. Lactate utilization was apparently increased in the presence of either Ediol or theophylline.

Discussion. It has been suggested that glucagon activates an hepatic lipase via the adenyl cyclase system (3). Theophylline, or other methylxanthines would be expected to have a similar effect on lipase due to their inhibition of 3′5′AMP metabolism by phosphodiesterase (12). Activation of hepatic lipase results in the breakdown of triglycerides to glycerol and fatty acids. Williamson *et al* (5) demonstrated that glucagon will increase levels of both acyl and acetyl CoA esters in liver. Fatty acids and their CoA esters were shown to inhibit numerous hepatic enzymes (20–22). The pattern of inhibition produced would tend to reduce glucose metabolism by glycolysis and the pentose pathway, and acetate oxidation via the TCA cycle, but would favor the conversion of lactate to glucose. In addition, increased levels of acetyl CoA could stimulate the conversion of pyruvate to oxaloacetate by pyruvate carboxylase (23). Although fatty acids cannot directly contribute to glucose formation, the glycerol released by hydrolysis of triglycerides provides an excellent substrate for gluconeogenesis. Much of the extra glucose formed in the presence of triglycerides is most likely derived from glyceride glycerol.

A diabetogenic effect of glucagon, characterized by increased blood sugar, urea production, triglyceride breakdown and ketone

body production, and associated with a decrease in liver glycogen and fatty acid synthesis and negative nitrogen balance was described (24). Schimussek and Mitzkut (25) observed increased glucose production by the isolated perfused liver upon *in vitro* addition of glucagon and these observations were extended by Garcia *et al.* (6) and Struck *et al.* (7). That the gluconeogenic effect of glucagon is mediated by cyclic 3′5′AMP is suggested by the work of Exton *et al.* (8). In addition to glucagon, epinephrine and cyclic 3′5′AMP produce similar effects on glucose production by the isolated perfused liver.

Addition of fatty acids to the liver perfusion system produces increases in glucose production comparable to the addition of glucagon (7, 9, 10). Krebs reported that the combination of glucagon plus fatty acids results in a further increase in glucose production over that observed with either stimulant alone (11); however, such an effect was not observed in the initial study by Struck *et al.* (7).

In the present study, addition of triglycerides to the perfusion medium produced a significant increase in glucose production, but glucagon plus triglyceride did not further increase glucose production. This suggests that glucagon can result in sufficient acetyl CoA production from breakdown of endogenous triglycerides for maximal stimulation of the conversion of pyruvate to oxaloacetate. Ketone body production, on the other hand, would be increased in proportion to the amount of acetyl CoA formed. Thus, added triglyceride would increase ketogenesis, but not gluconeogenesis above that seen with glucagon alone. The combined effect of glucagon and triglyceride would result in a further increase in ketone body production. On the other hand, theophylline produced a more marked increase in glucose and ketone body production than did glucagon. Furthermore, the combined effect of theophylline plus triglyceride was greater than either alone on both glucose and ketone body production. This may be related to the rapid destruction of glucagon by the liver, and would support the observation of Hynie *et al.* (26) that theophylline produces maximal activation of the cyclic 3′5′AMP system.

Since hydrolysis of triglycerides results in the production of glycerol as well as fatty acids, it was of interest to determine glucose production with glycerol as added substrate. Glucose formation from glycerol was not stimulated by octanoate, indicating that fatty acids, and presumably acetyl CoA as well, do not increase gluconeogenesis from glycerol. Glucagon, however, did cause a small increase in glucose production. On this point there is no uniform agreement. Ross *et al.* (27) did not find an increase in glucose production in the presence of glucagon and glycerol. Both fructose and glycerol enter the glycolytic cycle at the triose phosphate level and Garcia *et al.* (6) reported glucagon stimulation of glucose formation from fructose, but this observation was not confirmed by Exton *et al.* (8). Therefore, the significance of an apparent glucagon stimulation of glucose formation from glycerol may be questioned.

Some question may also be raised as to whether or not tributyrin is an adequate substrate to test for lipase activation since this triglyceride may be hydrolyzed by esterases and nonspecific lipases. A similar criticism can also be made for using Ediol since it is a mixture of lipids. There may well be several lipases and/or esterases involved in the hydrolysis of the substrates used in these experiments. However, evidence has been presented that the hydrolysis occurred intracellularly and that the lipase (lipases or esterases) responded to glucagon and theophylline. Triglycerides when given in $\frac{1}{3}$ equimolar amounts are just as effective as fatty acids in stimulating gluconeogenesis and ketone body production. Although the levels of triglycerides in liver are low, 3–4 mg/g liver the serum level in fasting is quite appreciable and ranges from 85 to 125 mg/100 ml. Calculated as tripalmitin, this gives a concentration of 1 to 1.5 m*M*, which was the level used in these experiments. Serum triglyceride levels would be much greater than this postprendially and in disease states associated with hyperlipemia. Thus, they could exert a profound

effect on carbohydrate metabolism and ketogenesis.

Summary. Glucose and ketone body production was measured in livers from 24 hr starved rats perfused with 20 m*M* lactate. Addition of either tributyrin (1.5 m*M*) or Ediol (5 m*M*) to the perfusate markedly increased both glucose and ketone body production. Addition of theophylline (10 m*M*) further increased glucose and ketone body production in the presence of triglyceride. Glucagon (1 μg/ml) resulted in an increase in ketone body production with either triglyceride present, but glucose production was not increased over that observed with glucagon alone. Glucose production, when both glycerol (20 m*M*) and lactate were present, was greater than that observed with either substrate alone. Intracellular hydrolysis of triglycerides may contribute to glucose formation directly by the conversion of glycerol to glucose and indirectly stimulate gluconeogenesis by the effects of fatty acids and their metabolites on glucose formation from lactate or pyruvate.

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Received Oct. 24, 1968. P.S.E.B.M., 1969, Vol. 130.