Hormonal Control of Ketogenesis

RAPID ACTIVATION OF HEPATIC KETOGENIC CAPACITY IN FED RATS BY ANTI-INSULIN SERUM AND GLUCAGON

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ABSTRACT The enhanced capacity for long-chain fatty acid oxidation and ketogenesis that develops in the rat liver between 6 and 9 h after the onset of starvation was shown to be inducible much more rapidly by administration of anti-insulin serum or glucagon to fed rats. After only 1 h of treatment with either agent, the liver had clearly switched from a "nonketogenic" to a "ketogenic" profile, as determined by rates of acetoacetate and β-hydroxybutyrate production on perfusion with oleic acid. As was the case after starvation, the administration of insulin antibodies or glucagon resulted in depletion of hepatic glycogen stores and a proportional increase in the ability of the liver to oxidize long-chain fatty acids and (−)-octanoylcarnitine, suggesting that all three treatment schedules activated the carnitine acyltransferase system of enzymes. In contrast to anti-insulin serum, which produced marked elevations in plasma glucose, free fatty acid, and ketone body concentrations, glucagon treatment had little effect on any of these parameters, presumably due to enhanced insulin secretion after the initial stimulation of glycogenolysis. Thus, after treatment with glucagon alone, it was possible to obtain a "ketogenic" liver from a nonketotic animal. The results are consistent with the possibility that the activity of carnitine acyltransferase, and thus ketogenic capacity, is subject to bihormonal control through the relative blood concentrations of insulin and glucagon, as also appears to be the case with hepatic carbohydrate metabolism.

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INTRODUCTION

A prerequisite for the acceleration of ketone body production by the liver during fasting or in the diabetic state is an increased capacity for the oxidation of long-chain fatty acids (1). In the rat the activation of fatty acid oxidation and the enhanced ability to produce acetoacetic and β-hydroxybutyric acids does not appear until at least 6 h after the onset of starvation, but then develops rapidly between 6 and 9 h (2). Evidence has been obtained to suggest that the activation site resides at the carnitine acyltransferase[1] reaction, which catalyzes the transfer of long-chain fatty acids across the mitochondrial membrane (5). The nature of the "on-off" signal for this reaction, however, is completely unknown. Since it has long been suspected that the regulation of fatty acid oxidation in the liver is intimately related to some facet of carbohydrate metabolism (6–8), it seemed possible that alterations in the latter might be involved in the control of carnitine acyltransferase and thereby dictate ketogenic potential. Moreover, since hepatic carbohydrate metabolism, which plays a central role in glucose homeostasis, appears to be under bihormonal control by insulin and glucagon (9–13), the further possibility exists that fluctuation in the relative blood concentrations of these two hormones also represents the key determinant of hepatic ketogenic capacity.

In an attempt to dissect these interrelationships, we have treated nonketotic fed rats with anti-insulin serum and with glucagon. Insulin antibodies caused immediate

[1] Unless otherwise specified the term "carnitine acyltransferase" will be used to denote the coupled enzyme system comprising carnitine acyltransferases I and II (3, 4).

hyperglycemia, increased plasma free fatty acid concentrations, and ketosis, whereas glucagon, even in very high doses, had little effect on any of these parameters. However, after only 1 h of either treatment, livers from both groups of animals had been switched from a "nonketogenic" to a "ketogenic" metabolic state.[2] Thus, for the first time, a liver with a demonstrably high ketogenic capacity has been obtained from a nonketotic animal. The mechanism of the enhanced capacity for ketone body production in both cases appears to be an activation of the carnitine acyltransferase step.

METHODS

Animals. Male Sprague-Dawley rats weighing approximately 100 g were used in all experiments. Animals were fed a diet containing 58.5% sucrose, 21% casein, and less than 1% fat, together with all necessary vitamins and minerals (General Biochemicals, Chagrin Falls, Ohio). They were used for experiments between 7:00 and 8:00 a.m. Fasted rats were deprived of food for 24 h before use.

Treatment of animals. Animals were lightly anesthetized with ether for the placement of catheters in the femoral artery on one side and the inferior vena cava through the femoral vein on the other (14). They were then placed in individual restraining cages and allowed to awaken from the anesthesia before experiments were begun. After the infusion of 100 U of heparin intravenously and the collection of a zero-time blood sample (0.2 ml) into iced heparinized tubes, an infusion of guinea pig serum containing insulin antibodies, glucagon, or neither agent was given through the venous catheter, and further arterial blood samples were taken for analysis throughout the experimental period. After collection of each sample, 0.9% sodium chloride, equivalent to the volume of blood removed, was infused into the animal. At the indicated times the rats were given an intraperitoneal injection of pentobarbital after which livers were either used for perfusion to determine their ketogenic capacity or were rapidly frozen in liquid N_2 and analyzed for their glycogen content. In certain instances a sample of blood was taken from the abdominal aorta for analysis of plasma free fatty acid, insulin, and glucagon concentrations.

Liver perfusion studies. Livers were perfused with recirculating medium using the apparatus and techniques described previously (15, 16). The perfusion medium consisted of aged human erythrocytes suspended to an hematocrit of 20% in Krebs bicarbonate buffer, pH 7.4, containing 5% bovine albumin (fraction V, Armour Pharmaceutical Company, Chicago, Ill.). The red cells were dialyzed for 24 h against 0.9% sodium chloride at 4°C before use to remove lactate (2). When used, sufficient oleic acid was bound to the albumin to provide an initial fatty acid concentration of approximately 0.7 mM in the cell-free fluid (2, 16). In studies with (−)-octanoylcarnitine, oleic acid was omitted from the medium, and the (−)-carnitine ester was added to the system after 15 min of perfusion (5).

Analytical procedures. All samples of blood and perfusion media were centrifuged, and analyses were carried out on plasma or the erythrocyte-free supernatant fluid as described in previous reports (2, 15–17). In the text the term "ketone bodies" will always refer to the sum of acetoacetate and β-hydroxybutyrate. Liver glycogen was measured by the method of Good, Kramer, and Somogyi (18).

Materials. (−)-[1-^{14}C]Octanoylcarnitine was synthesized from [1-^{14}C]octanoyl chloride (New England Nuclear, Boston, Mass.) and (−)-carnitine (General Biochemicals) by the method of Bremer (19). Guinea pig serum containing antibody to pork or beef insulin was prepared as described by Wright, Makulu, and Posey (20). Neutralizing capacity was 1.6–2.1 U of hormone per ml. Control guinea pig serum was purchased from Pel-Freez Biologicals, Inc., Rogers, Ark. Glucagon was from Sigma Chemical Co., St. Louis, Mo. Intralipid, as a 10% fat emulsion, was obtained from Vitrum, Stockholm, Sweden and was infused together with heparin into one group of rats to cause an acute elevation of plasma free fatty acid levels, as described by Meng and Edgren (21).

RESULTS

Effects of anti-insulin serum and glucagon administration in fed rats. Consistent with earlier findings (22–24), treatment of fed rats with anti-insulin serum resulted in acute and profound hyperglycemia (Fig. 1A). Simultaneously, there was a prompt elevation in plasma ketone body concentration (Fig. 1B) which after 2 h had reached a level close to that seen in rats fasted for 24 h (2). In contrast, glucagon, used here in high dosage to study maximal responses, caused only a small and transient rise in plasma glucose concentration and was essentially without effect on plasma ketone levels (Fig. 1). As expected, neither parameter was affected by control serum.

Table I shows the effects of the various treatments on plasma free fatty acid concentrations and liver glycogen content in fed rats. Although the latter was reduced rapidly by both insulin antibodies and glucagon, the characteristic elevation in plasma free fatty acid levels caused by anti-insulin treatment (23–26) did not occur after glucagon infusion. The mild elevation of plasma free fatty acids and the partial depletion of liver glycogen that occurred in animals receiving the control serum were likely due to the trauma associated with the initial surgery and the restraint imposed upon the animals during the experimental period.

Effects of anti-insulin serum and glucagon treatment in vivo on hepatic ketogenesis from oleate. To assess the effects of the various in vivo manipulations on hepatic ketogenic capacity,[3] livers were subsequently perfused with oleic acid and rates of ketone production

[2] The terms "ketogenic" and "nonketogenic" are used here in a relative sense. Although livers from normal animals can synthesize ketone bodies, this capacity is very low even in the face of high fatty acid loads when compared with rates obtained in livers from ketotic animals. For a detailed discussion of this phenomenon, see references 1 and 2.

[3] Under the perfusion conditions used here, the major products of fatty acid oxidation are acetoacetic and β-hydroxybutyric acids (15, 16). Thus, throughout the text, rates of ketone production will be taken as a reflection of the activity of the β-oxidation process and will be referred to as the "ketogenic capacity" of the liver.

were measured. As seen in Fig. 2, after only 1 h of infusion of the donor animals with anti-insulin serum, the ketogenic capacity of the liver had increased 2.5-fold, and after 3 h of such treatment, ketone production had attained 75% of the rate given by livers from 24-h fasted rats. Of particular interest was the finding that glucagon infusion for either 1 or 3 h induced essentially the same degree of enhancement in ketogenesis as did anti-insulin serum. As expected on the basis of earlier starvation-refeeding experiments (2), the administration of control serum for a period of 3 h had no stimulatory effect on ketone production. Also, in experiments not shown, livers from untreated rats were perfused with oleate plus anti-insulin serum or glucagon added directly to the perfusion fluid in amounts identical with those used in vivo; in neither case was there a significant stimulation of ketogenesis over the 1-h perfusion

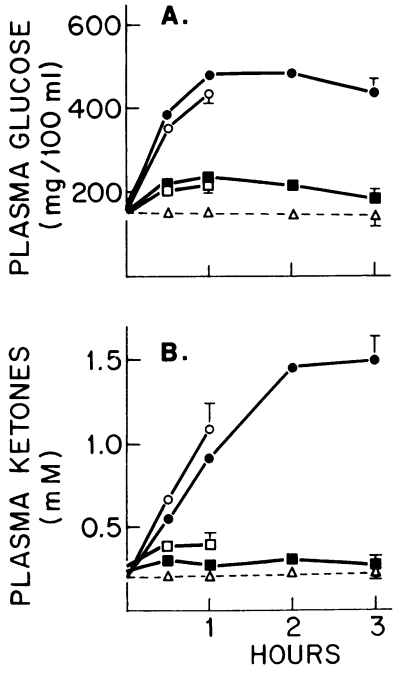

FIGURE 1 Effects of anti-insulin serum and glucagon on plasma glucose and ketone levels in fed rats. Animals were fitted with arterial and venous catheters and placed in restraining cages as described under Methods. After the injection of 100 U of heparin and collection of a zero-time arterial blood sample (0.2 ml), an infusion of guinea pig serum containing 1.6–2.1 U of insulin antibody per ml (○, 1 h, ●, 3 h), 100 μg of glucagon per ml (□, 1 h, ■, 3 h) or neither agent (△) was given through the venous catheter at a rate of 200 μl per min for 5 min, followed by 10 μl per min for the remainder of the experiment. Further arterial blood samples were taken at the indicated times for analysis of plasma glucose and ketone concentrations. Values represent means for eight animals in each group. For purposes of clarity standard errors of the means are shown only for the final time point but in every case were smaller than this at earlier times.

TABLE I

Effects of Anti-Insulin Serum and Glucagon on Plasma Free Fatty Acid Concentration and Liver Glycogen Content in Fed Rats*

Treatment	Plasma free fatty acids	Liver glycogen
	mM	*mg/g wet wt*
None	0.25±0.01 (4)	48.3±2.5 (4)
Control serum, 3 h	0.54±0.10 (4)	31.6±6.0 (4)
AIS, 1 h	1.11±0.04 (5)	19.5±4.2 (6)
AIS, 3 h	1.85±0.13 (3)	9.6±2.0 (4)
Glucagon, 1 h	0.21 (2)	15.2±2.9 (5)
Glucagon, 3 h	0.37±0.04 (4)	3.8±0.8 (4)

Fed rats were treated as described in the legend to Fig. 1 and, at the indicated times, were anesthetized with pentobarbital. Blood was collected from the abdominal aorta for the analysis of plasma free fatty acids and livers were taken for determination of their glycogen content. Values represent means ±SEM for the number of animals shown in parentheses.
* AIS, anti-insulin serum.

period. The characteristic glycogenolytic effect of glucagon, however, was clearly operative as evidenced by its enhancement of net glucose production from a control value of 259±51 to 727±44 μmol per 100 g body wt.

The finding that anti-insulin serum and glucagon, when given in vivo, both induced the same increase in hepatic ketogenic capacity (Fig. 2) suggested that their strikingly different effects on plasma ketone levels (Fig. 1B) were due to differences in the effects of the two agents on the rate of delivery of free fatty acids to the liver (24) (Table I). This prediction was fulfilled when it was shown that acute elevation of plasma free fatty acid concentration by infusion of Intralipid plus heparin into glucagon-treated rats resulted in a brisk rise in plasma ketone levels (Fig. 3). Only minor elevations in this parameter were noted in animals receiving control serum, despite the fact that at the 3-h time point, plasma free fatty acids had reached levels of 2–3 mM in both groups.

Effects of anti-insulin serum and glucagon treatment in vivo on hepatic ketogenesis from (−)-octanoylcarnitine. As discussed previously (5), direct assay of carnitine acyltransferase failed to reveal significant difference in enzyme activity in liver mitochondria prepared from normal and ketotic rats. A detailed study in which the same conclusion was reached has been reported by DiMarco and Hoppel (27). Nevertheless, a considerable body of evidence has accumulated implicating this first step in fatty acid oxidation as a major site for control of ketogenesis in the intact liver (1, 2), thus raising the possibility that within its natural environment the carnitine acyltransferase system of enzymes might be under a form of control that is lost upon disruption of

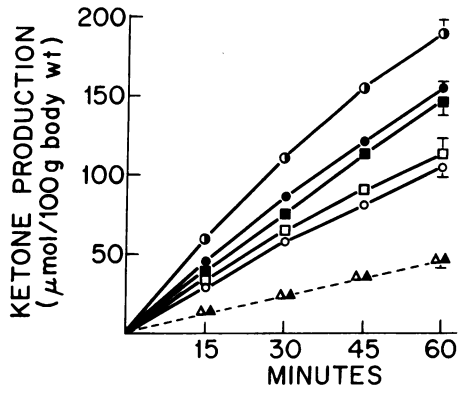

FIGURE 2 Comparison of the effects of anti-insulin serum (AIS), glucagon, and fasting on ketogenesis from oleate in the perfused rat liver. Fed animals were treated as described in the legend to Fig. 1 or were fasted for 24 h. The livers were then perfused with recirculating medium containing oleic acid at an initial concentration of 0.7 mM and rates of ketone production were determined. Treatment of rats was as follows: (▲), none; (△), control serum, 3 h; (○), AIS, 1 h; (●), AIS, 3 h; (□), glucagon, 1 h; (■), glucagon, 3 h; (◑), fasted, 24 h. Values represent means for four animals in each group. For purposes of clarity standard errors are shown only for the 60-min time point but in every case were smaller than this at earlier times.

cellular integrity. To test this postulate we have employed (−)-octanoylcarnitine as the substrate for ketone body production in perfused livers from normal and ketotic rats (5). The rationale for its use is depicted schematically in Fig. 4. Oleic acid, a representative of physiological fatty acids, requires esterification to carnitine and reconversion to the CoA derivative before entering the oxidation sequence (Fig. 4A). It is oxidized at low rates in livers from fed rats but at high rates in livers from ketotic animals (2, 5, 16). On the other hand, free octanoic acid traverses the mitochondrial membrane without requiring a carnitine transport mechanism (Fig. 4B) and is oxidized to acetyl-CoA at the same rate in livers from fed, fasted, and diabetic animals (5, 15).[4] However, when the medium-chain fatty acid is esterified to carnitine (Fig. 4C), carnitine acyltransferase II is required for its oxidation. Rates of oxidation of (−)-octanoylcarnitine can, therefore, be utilized to assess the activity of carnitine acyltransferase II in the perfused liver. As previously shown, the enhancement in hepatic long-chain fatty acid oxidation characteristic of fasted rats is accompanied by a proportional increase in the oxidation of (−)-octanoylcarnitine (5). This suggests that the carnitine acyltransferase reaction constitutes the rate-limiting step in fatty acid oxidation and that it is the primary site for activation of this process in livers from ketotic animals. To test whether a similar relationship would hold after short-term treatment with anti-insulin serum or glucagon, livers were perfused for 15 min in the absence of oleate, after which (−)-[1-^{14}C]octanoylcarnitine was added to the medium and the quantity and specific activity of the total ketones formed were measured. As seen from the data of Table II, after 3 h of anti-insulin or glucagon treatment, the ability of the liver to convert the labeled (−)-carnitine ester into acetoacetate and β-hydroxybutyrate had increased almost fivefold. Moreover, the rate attained equaled 70% of that seen in livers from 24-h fasted rats; this degree of stimulation was similar to that observed when oleic acid served as the ketogenic substrate (Fig. 2). The parallelism between the enhancement of oxidation of long-chain fatty acids and of (−)-octanoylcarnitine caused by the three treatment schedules is further illustrated by comparison of the quantities of labeled and unlabeled C_2 units converted into acetoacetate and β-hydroxybutyrate. Clearly, there was a direct proportionality between the rates of oxidation of the (−)-carnitine ester and of endogenously derived fatty acids, as reflected by the constancy of the ketone body specific activity (Table II).

Relationship between glycogen content of the liver and its ketogenic capacity. In previous time-sequence studies of the effects of the starvation and refeeding on a va-

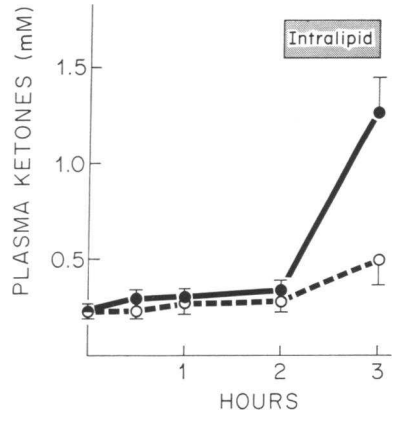

FIGURE 3 Effects of acute elevation of plasma free fatty acid concentration on plasma ketone levels in rats receiving a glucagon infusion. Fed animals received guinea pig serum with (●) or without (○) glucagon for a period of 3 h as described in the legend to Fig. 1. At the 2-h time point another 50 U of heparin was given and an infusion of Intralipid was started at a rate of 20 μl per min. A further 50 U of heparin was given after 2.5 h. Values represent means ±SEM for four animals in each group.

[4] Whereas rates of oxidation of octanoate are similar in all three conditions, rates of ketone body synthesis from this substrate differ in livers from normal and ketotic animals. As discussed elsewhere, this is due to altered disposal of acetyl-CoA through the tricarboxylic acid cycle and the pathways of lipogenesis and ketogenesis (5, 15).

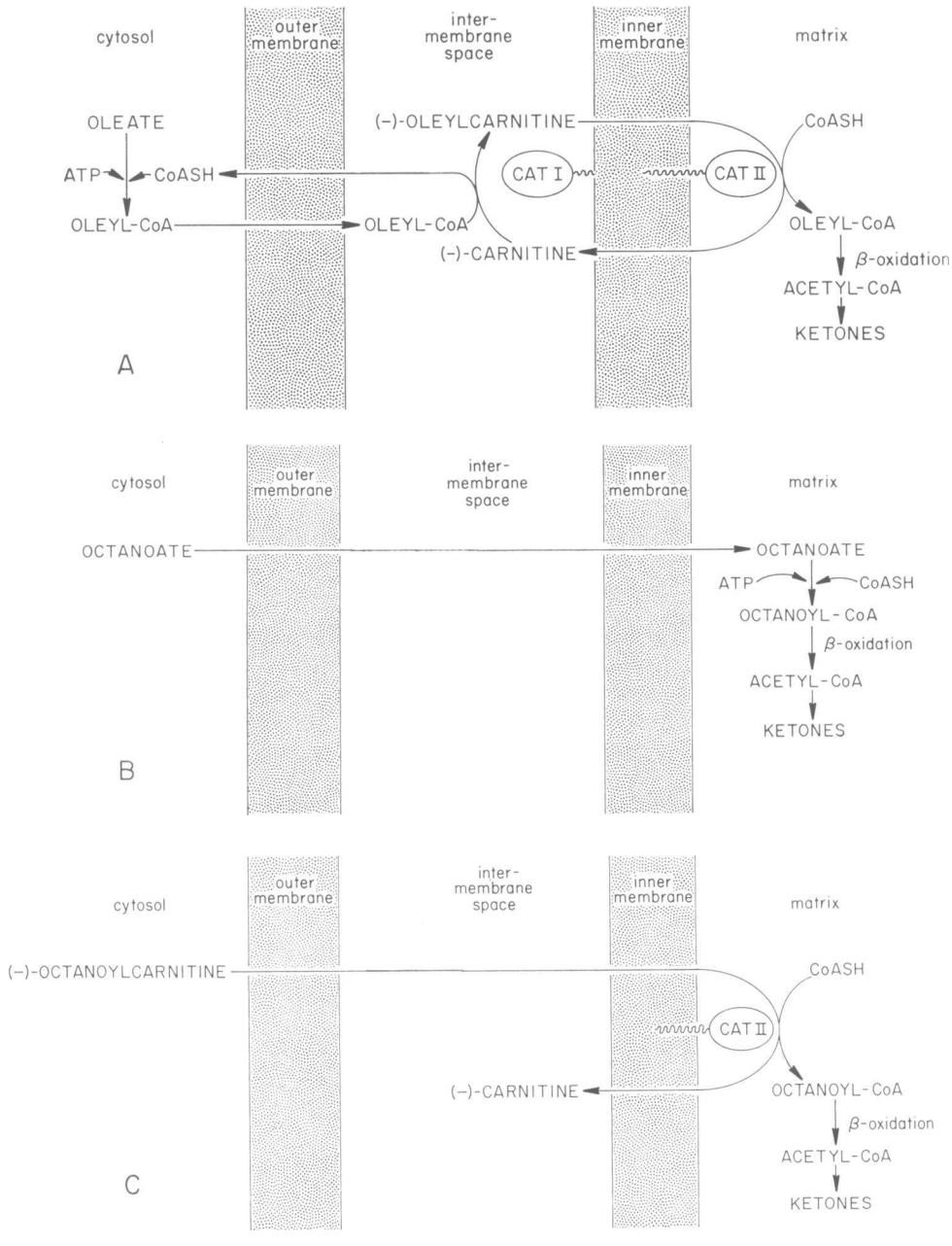

FIGURE 4 Schematic representation of the steps involved in the mitochondrial oxidation of oleic acid (A), octanoic acid (B), and (−)-octanoylcarnitine (C).

riety of metabolic parameters in the rat (2), it was found that the ability of the perfused liver to convert oleic acid into ketone bodies changes in the opposite direction to fluctuations in the tissue glycogen content. It became apparent from those studies that after withdrawal of food from the animals, relatively little change took place in ketogenic capacity until liver glycogen content had fallen below a level of approximately 30 mg per g wet wt of tissue, after which further reduction in glycogen was accompanied by a sharp increase in long-chain fatty acid oxidation. As seen from the data of Table I, essentially the same relationship was observed between the two parameters in animals treated with anti-insulin serum or glucagon.

Effects of low-dose glucagon in fed rats. As noted above, large doses of glucagon were employed in the experiments described so far to determine the rapidity with which the liver could be switched from a nonketo-

genic to a ketogenic mode. It was felt desirable, however, to test the effects of the hormone when administered in much smaller quantities such that its blood level might be expected to remain within the physiological range. To this end another group of fed rats was treated in a manner identical with that described in the legend to Fig. 1 except that the amount of glucagon administered was reduced by a factor of 200. As shown in Table III, such treatment resulted in a mean plasma glucagon concentration of 231 pg per ml at the 3-h time point, a value approximately twice that found in untreated rats in our previous studies (17). The observed plasma insulin concentration, 34 μU per ml, represents a 23% decrease from that found earlier in untreated animals (17). Although no significant changes in plasma glucose or ketone body levels were seen during the course of the infusion (data not shown), it should be noted that after 3 h the ketogenic capacity of the liver had clearly been enhanced, this time by a factor of almost 3 (Table III). Once again, the oxidation of long-chain fatty acids and of (−)-octanoylcarnitine had been stimulated to the same extent, consistent with the observations of Table II. In addition, determination of liver glycogen levels on three animals treated in this manner yielded a mean value of 19.6±1.6 mg per g wet wt. Thus, the degree of enhancement of fatty acid oxidation and reduction of the glycogen content of the liver were of the same magnitude in animals receiving the small dose of glucagon for 3 h as in those treated with the larger dose of the hormone or with insulin antibodies for a 1-h period (Fig. 1; Tables I and III).

DISCUSSION

Four points emerging from these studies warrant emphasis. First, it is evident that the enhancement in hepatic long-chain fatty acid oxidation and ketogenesis that occurs in the rat after about 6 h of starvation (2) can be induced acutely by infusion of fed animals with insulin antibodies or glucagon. In fact, only 1 h of treatment with either agent was sufficient to cause an abrupt switch in liver metabolism from a nonketogenic to a ketogenic profile (Fig. 2).

Second, since anti-insulin serum produced a ketotic state in vivo (Fig. 1) it was not surprising to find that livers taken from such animals exhibited a ketogenic set (Fig. 2, Table II). An unexpected observation, however, was that a ketogenic liver could be obtained from a nonketotic animal, i.e., from those animals treated with glucagon (Figs. 1 and 2; Tables II and III). The absence of ketonemia in the latter group almost certainly resulted from a stimulation of insulin secretion

TABLE II

Comparison of the Effects of Anti-Insulin Serum and Glucagon Treatment of Fed Rats with Those of Fasting on Ketogenesis from (−)-Octanoylcarnitine in the Perfused Liver

Treatment	Labeled C_2 units ↓ ketones (A)	Total C_2 units ↓ ketones (B)	Unlabeled C_2 units ↓ ketones (B-A)	Sp act of ketones (A/B)
	μmol/100 g body wt			
None	10.5±1.4	52.0±7.1	41.5±5.7	0.20±0.01
AIS, 3 h	49.2±2.6	252±9.0	203±6.0	0.20±0.00
Glucagon, 3 h	47.0±1.6	238±10.0	191±11.0	0.20±0.01
Fasted, 24 h	68.7±2.5	297±5.5	228±4.6	0.23±0.01

Fed rats were treated as described in the legend to Fig. 1 and, at the indicated times, were anesthetized with pentobarbital. Livers were perfused with recirculating fluid containing no added fatty acid for a period of 15 min at which time (−)-[1-^{14}C]octanoylcarnitine (sp act 0.4 μCi per μmol) was added to bring its initial concentration to 1.25 mM. The term "labeled C_2 units → ketones" represents the micromoles of labeled substrate converted into ketones, multiplied by 4. The term "total C_2 units → ketones" equals the total quantity of ketones formed, multiplied by 2. Values represent means±SEM for four or five animals in each group and refer to events taking place between 15 and 60 min of perfusion.

TABLE III

Effects of Low-Dose Glucagon on Hepatic Ketogenic Capacity in Fed Rats

Treatment	Plasma insulin	Plasma glucagon	Perfusions with oleate: Total ketone production	Perfusions with (−)-[1-^{14}C]octanoylcarnitine: Labeled C_2 units ↓ ketones (A)	Total C_2 units ↓ ketones (B)	Unlabeled C_2 units ↓ ketones (B-A)	Sp act of ketones (A/B)
	μU/ml	*pg/ml*		*μmol/100 g body wt*			
None*	44±5	124±14	45.0±2.0	10.5±1.4	52.0±7.1	41.5±5.7	0.20±0.01
Glucagon	34±2	231±19	129.0±19	26.8±1.9	139.0±11.1	112.2±9.3	0.19±0.005

Fed rats received an infusion of guinea pig serum containing 0.5 μg per ml of glucagon at a rate of 200 μl per min for 5 min followed by 10 μl per min for 3 h as described in the legend to Fig. 1. Livers were subsequently perfused either with oleic acid for 1 h or with (−)-[1-^{14}C]octanoylcarnitine for 45 min as described in the legends to Fig. 2 and Table II and rates of ketone body synthesis were measured. Arterial blood was collected from an identically treated group and the plasma was analyzed for insulin and glucagon. Values represent means±SEM for four or five animals used in each determination.

* Values taken from Tables I and II, except those for plasma insulin and glucagon which were taken from reference 16.

due to the initial activation of glycogenolysis and consequent rise in blood glucose concentrations caused by glucagon administration (24, 28, 29). The elevation of plasma insulin would inhibit lipolysis and thereby diminish free fatty acid turnover such that the liver is deprived of substrate for ketone body synthesis (Table I). Consistent with this formulation is the fact that acute elevation of plasma free fatty acids by infusion of Intralipid plus heparin resulted in a sharp rise in plasma ketone levels in glucagon-treated animals (Fig. 3). This effect of Intralipid was considerably less pronounced in animals treated with control serum, in keeping with the finding that under these circumstances the ketogenic potential of the liver was low (Fig. 2). These observations serve to emphasize the fact that two obligatory requirements for the development of the ketotic state are an accelerated mobilization of free fatty acids from fat depots to the liver and an alteration in the metabolic set of this tissue such that a greater fraction of the fatty acids taken up enter the pathway of β-oxidation rather than that leading to the formation of triglycerides and phospholipids, the major disposal route in the nonketotic state (2, 16, 30, 31). The data also indicate that this alteration in hepatic fatty acid metabolism does not necessarily require an insulin-deficient state in vivo for its induction. This follows from the observation that insulin concentrations after 3 h of infusion with small quantities of glucagon were only minimally depressed. On the basis of studies from other laboratories (28, 29), it is likely that insulin concentrations were actually elevated at earlier time points. The fact that the modest increase in the blood glucagon to insulin ratio produced under these conditions elicited the switch of liver metabolism from a nonketogenic to a ketogenic profile (Table III) is interesting in light of the proposed bihormonal role of glucagon and insulin in glucose homeostasis (9–13, 32). It thus seems probable that hepatic fatty acid metabolism and ketogenesis are under the same bihormonal control.

Third, the observation that the stimulation of hepatic long-chain fatty acid oxidation, whether induced acutely by anti-insulin serum or glucagon, or over the longer term by starvation, resulted in a proportional acceleration in the oxidation of (−)-octanoylcarnitine, suggests that a common denominator to all three situations was an activation of carnitine acyltransferase II, which catalyzes the conversion of acylcarnitine into acyl-CoA (3, 4).[5] Whether this was accompanied by a simultaneous activation of carnitine acyltransferase I, which is responsible for the prior conversion of acyl-CoA into acylcarnitine (3, 4) is not yet clear.

Finally, although the present experiments give no indication as to the mechanism whereby this enzyme system might be regulated, it would appear that some component of hepatic carbohydrate metabolism plays an important role. This suspicion is based on the fact that irrespective of the method used to alter its rate, fatty acid oxidation invariably correlated in a reciprocal manner with changes in the glycogen content of the liver. A similar relationship is seen in the newborn rat; during the suckling period hepatic ketone production is elevated while the tissue glycogen level is low, whereas the converse situation obtains shortly after weaning (35, 36). These observations regarding the interrelationship between glycogen content and ketogenesis are reminiscent of much older studies in the literature (37, 38) and raise the intriguing possibility that glycogen might play a direct role in the regulation of carnitine acyltransferase activity. On the other hand, the inverse correlation between the two parameters did not manifest itself during the course of perfusion of livers from fed animals with glucagon added directly to the medium. Over a 1-h period, the hormone caused a fall in glycogen content of approximately 25 mg per g wet wt of tissue (data not shown) without a concomitant stimulation of ketogenesis. Similar observations were reported by Exton, Corbin, and Park (39) and by Raskin, McGarry, and Foster (40). Consideration must, therefore, be given to the possibility that in vivo anti-insulin serum and glucagon elicit the formation of a factor(s) that is involved in the activation of hepatic fatty acid oxidation but that is missing from the medium used in liver perfusion studies. Should this be the case, fluctuations in liver glycogen content as they occur in vivo might simply reflect changes in the level of another metabolite, perhaps related to glycogen, that serves to inhibit fatty acid flux through the carnitine acyltransferase step.

[5] As pointed out previously (5), it is possible that separate enzymes are involved in the transfer of long- and medium-chain (−)-carnitine esters across the mitochondrial membrane (33, 34). Should this be the case it would appear that both activities are subject to coordinate control.

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REFERENCES

1. McGarry, J. D., and D. W. Foster. 1972. Regulation of ketogenesis and clinical aspects of the ketotic state. *Metab. (Clin. Exp.).* **21**: 471–489.
2. McGarry, J. D., J. M. Meier, and D. W. Foster. 1973. Effects of starvation and refeeding on carbohydrate and lipid metabolism *in vivo* and in the perfused rat liver. *J. Biol. Chem.* **248**: 270–278.
3. Kopec, B., and I. B. Fritz. 1973. Comparison of properties of carnitine palmitoyltransferase I with those of carnitine palmitoyltransferase II, and preparation of an-

tibodies to carnitine palmitoyltransferases. *J. Biol. Chem.* **248**: 4069–4074.
4. Brosnan, J. T., B. Kopec, and I. B. Fritz. 1973. The localization of carnitine palmitoyltransferase on the inner membrane of bovine liver mitochondria. *J. Biol. Chem.* **248**: 4075–4082.
5. McGarry, J. D., and D. W. Foster. 1974. The metabolism of (−)-octanoylcarnitine in perfused livers from fed and fasted rats. Evidence for a possible regulatory role of carnitine acyltransferase in the control of ketogenesis. *J. Biol. Chem.* **249**: 7984–7990.
6. Weinhouse, S., R. H. Millington, and B. Friedman. 1949. The effect of carbohydrate on the oxidation of fatty acids by liver slices. *J. Biol. Chem.* **181**: 489–498.
7. Lossow, W. J., and I. L. Chaikoff. 1954. Carbohydrate sparing of fatty acid oxidation. I. The relation of fatty acid chain length to the degree of sparing. II. The mechanism by which carbohydrate spares the oxidation of palmitic acid. *Arch. Biochem. Biophys.* **57**: 23–40.
8. Lossow, W. J., G. W. Brown, Jr., and I. L. Chaikoff. 1956. The action of insulin in sparing fatty acid oxidation. A study with palmitic acid-1-C^{14} and octanoate-1-C^{14}. *J. Biol. Chem.* **220**: 839–489.
9. Mackrell, D. J., and J. E. Sokal. 1969. Antagonism between effects of insulin and glucagon on the isolated liver. *Diabetes.* **18**: 724–732.
10. Unger, R. H. 1974. Alpha- and beta-cell interrelationships in health and disease. *Metab. (Clin. Exp.).* **23**: 581–593.
11. Parrilla, R., M. N. Goodman, and C. J. Toews. 1974. Effect of glucagon: insulin ratios on hepatic metabolism. *Diabetes.* **23**: 725–731.
12. Cherrington, A. D., R. Kawamori, S. Pek, and M. Vranic. 1974. Arginine infusion in dogs. Model for the roles of insulin and glucagon in regulating glucose turnover and free fatty acid levels. *Diabetes.* **23**: 805–815.
13. Sakurai, H., R. Dobbs, and R. H. Unger. 1974. Somatostatin-induced changes in insulin and glucagon secretion in normal and diabetic dogs. *J. Clin. Invest.* **54**: 1395–1402.
14. McGarry, J. D., and D. W. Foster. 1973. Acute reversal of experimental diabetic ketoacidosis in the rat with (+)-decanoylcarnitine. *J. Clin. Invest.* **52**: 877–884.
15. McGarry, J. D., and D. W. Foster. 1971. The regulation of ketogenesis from octanoic acid. The role of the tricarboxylic acid cycle and fatty acid synthesis. *J. Biol. Chem.* **246**: 1149–1159.
16. McGarry, J. D., and D. W. Foster. 1971. The regulation of ketogenesis from oleic acid and the influence of antiketogenic agents. *J. Biol. Chem.* **246**: 6247–6253.
17. Meier, J. M., J. D. McGarry, G. R. Faloona, R. H. Unger, and D. W. Foster. 1972. Studies of the development of diabetic ketosis in the rat. *J. Lipid Res.* **13**: 228–233.
18. Good, C. A., H. Kramer, and M. Somogyi. 1933. The determination of glycogen. *J. Biol. Chem.* **100**: 485–491.
19. Bremer, J. 1968. Long-chain acylcarnitines. *Biochem. Prep.* **12**: 69–73.
20. Wright, P. H., D. R. Makulu, and I. J. Posey. 1968. Guinea pig anti-insulin serum. Adjuvant effect of H. pertussis vaccine. *Diabetes.* **17**: 513–516.
21. Meng, H. C., and B. Edgren. 1963. Source of plasma free fatty acids in dogs receiving fat emulsion and heparin. *Am. J. Physiol.* **204**: 691–695.
22. Armin, J., R. T. Grant, and P. H. Wright. 1960. Acute insulin deficiency provoked by single injections of anti-insulin serum. *J. Physiol. (Lond.).* **153**: 131–145.
23. Balasse, E. O., D. M. Bier, and R. J. Havel. 1972. Early effects of anti-insulin serum on hepatic metabolism of plasma free fatty acids in dogs. *Diabetes.* **21**: 280–288.
24. Williamson, J. R. 1967. Effects of fatty acids, glucagon and anti-insulin serum on the control of gluconeogenesis and ketogenesis in rat liver. *Adv. Enzyme Regul.* **5**: 229–255.
25. Tarrant, M. E., R. H. S. Thompson, and P. H. Wright. 1962. Some aspects of lipid metabolism in rats treated with anti-insulin serum. *Biochem. J.* **84**: 6–10.
26. Tarrant, M. E., R. Mahler, and J. Ashmore. 1964. Studies in experimental diabetes. IV. Free fatty acid mobilization. *J. Biol. Chem.* **239**: 1714–1719.
27. DiMarco, J. P., and C. Hoppel. 1975. Hepatic mitochondrial function in ketogenic states. Diabetes, starvation and after growth hormone administration. *J. Clin. Invest.* **55**: 1237–1244.
28. Whitty, A. J., K. Shima, M. Trubow, and P. Foà. 1969. Effect of glucagon and of insulin on serum free fatty acids in normal and depancreatized dogs. *Proc. Soc. Exp. Biol. Med.* **130**: 55–61.
29. Liljenquist, J. E., J. D. Bomboy, S. B. Lewis, B. C. Sinclair-Smith, P. W. Felts, W. W. Lacy, O. B. Crofford, and G. W. Liddle. 1974. Effects of glucagon on lipolysis and ketogenesis in normal and diabetic men. *J. Clin. Invest.* **53**: 190–197.
30. Fritz, I. B. 1961. Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. *Physiol. Rev.* **41**: 52–129.
31. Mayes, P. A., and J. M. Felts. 1967. Regulation of fat metabolism in the liver. *Nature (Lond.).* **215**: 716–718.
32. Unger, R. H., and L. Orci. 1975. The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet.* **1**: 14–16.
33. Lee, L. P. K., and I. B. Fritz. 1970. Hepatic ketogenesis during development. *Can. J. Biochem.* **49**: 599–605.
34. Solberg, H. E. 1971. Carnitine octanoyltransferase. Evidence for a new enzyme in mitochondria. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **12**: 134–136.
35. Lockwood, E. A., and E. Bailey. 1971. The course of ketosis and the activity of key enzymes of ketogenesis and ketone-body utilization during development of the postnatal rat. *Biochem. J.* **124**: 249–254.
36. Aranda, A., E. Blázques, and E. Herrera. 1973. Liver components, blood glucose and ketone bodies in fed and starved suckling rats. *Horm. Metab. Res.* **5**: 350–355.
37. Blixenkrone-Møller, N. 1938. Respiratorischer Stoffwechsel und Ketonbildung der Leber. *Z. Physiol. Chem.* **252**: 117–136.
38. Mirsky, I. A. 1942. The etiology of diabetic acidosis. *J. Am. Med. Assoc.* **118**: 690–694.
39. Exton, J. H., J. G. Corbin, and C. R. Park. 1969. Control of gluconeogenesis in liver. IV. Differential effects of fatty acids and glucagon on ketogenesis and gluconeogenesis in the perfused rat liver. *J. Biol. Chem.* **244**: 4095–4102.
40. Raskin, P., J. D. McGarry, and D. W. Foster. 1974. Independence of cholesterol and fatty acid biosynthesis from cyclic adenosine monophosphate concentration in the perfused rat liver. *J. Biol. Chem.* **249**: 6029–6032.