Hypolipemic action of glucagon in experimental endogenous lipemia in the rat

R. Philip Eaton

Department of Medicine, University of New Mexico School of Medicine, Albuquerque, New Mexico 87106

Abstract The effect of glucagon on serum lipids and very low density lipoproteins (VLDL) has been examined in the eulipemic and the hyperlipemic rat. An inhibition of amino acid incorporation into hepatic lipoprotein apoprotein was observed, with an associated decrease in circulating VLDL apoprotein, decreased serum triglyceride concentration, and a loss of the pre-β band as judged by serum lipoprotein electrophoresis. The data suggest that an important action of this hormone is to decrease the synthesis of the protein moiety of the VLDL; this may contribute to the hypolipemic action of glucagon by introducing a limitation in hepatic lipoprotein production.

Supplementary key words lipoprotein · triglyceride · apoprotein · very low density lipoprotein · protein synthesis

GLUCAGON is known to have a hypolipemic effect in man, dog, fowl, and rat (1–5). Though the mechanism of this response is not established, studies by DeOya and coworkers (5, 6), Heimberg, Weinstein, and Kohout (7), and Penhos et al. (8) have demonstrated that triglyceride production by the perfused liver is reduced by glucagon, suggesting that hepatic lipoprotein metabolism may be the site of action of this effect of glucagon in vivo. Endogenous hyperlipemia is considered to represent an abnormality in both lipid and protein physiology, and in some forms of the disease a net increased apoprotein production contributes to the pathophysiology of the lipoproteinemia (9–13). Glucagon is known to have a catabolic effect on protein metabolism and to cause a net reduction in hepatic protein production. It is possible that these effects on protein synthesis may partially mediate the reduction in circulating lipoproteins (14, 15).

An opportunity to investigate this possibility is presented by the model of endogenous hyperlipemia induced by chronic treatment of the rat with cobaltous chloride (11). This results in an increased synthesis of hepatic lipoprotein protein, increased serum levels of the apoprotein, and a pre-β lipoproteinemia indistinguishable from the "carbohydrate-induced" lipemia as previously reported (10). The present study demonstrates that glucagon is a potent hypolipemic agent in this hyperlipemic rat model, and that reduction in the synthesis and circulating concentration of lipoprotein apoprotein may contribute to this response.

METHODS

Animals

Male Sprague-Dawley rats weighing 180–200 g at the time of the study were used in all experiments. Cobalt-treated rats were prepared by daily subcutaneous injection of cobalt chloride solution (4 mmoles/ml) at a dose of 2 mmoles/100 g of body weight as previously reported (11). These injections were given in two 5-day courses separated by a 9-day period of no injections. Examination of the animals was performed 5 days after the last injection. The animals and their pair-fed controls were fed a regular Purina rat chow diet ad lib. Pair feeding was accomplished by weighing the food consumed by the cobalt-treated rats daily and providing this amount to control animals for the subsequent day. The food was placed in the containers at 4 p.m., making it available for nocturnal consumption.

An abnormal, prolonged state of nonphysiological sustained elevations in serum glucagon was desired to examine the effects of this hormone on lipoprotein

Abbreviations: FFA, free fatty acids; VLDL, very low density lipoprotein; LDL, low density lipoprotein; AIS, anti-insulin serum.

This work was presented in part at the national meeting of the American Diabetes Association on 13 June 1970.

physiology. To attain this state, control and cobalt-treated rats were given 1 mg of glucagon by subcutaneous injection at 12-hr intervals for 4 days prior to study, the final injection being 4 hr prior to killing. The glucagon preparations were obtained from Eli Lilly Co. in crystalline form and were utilized in a 1:1 aqueous emulsion in corn oil. This massive administration of a slowly released form of glucagon was intended to overcome the known vigorous activity of tissue proteinase in inactivating glucagon. Though these animals were pair-fed, their daily weight gain of 4.0 ± 0.5 g/day was decreased in comparison with control animals which averaged 5.7 ± 0.5 g/day (means ± SD of 20 rats in each group).

Chemical analyses

Chemical determinations were performed on individual serum samples obtained after decapitation of the unanesthetized animals (fed ad lib.) in the morning and separation after clot formation at 5°C. Serum glucose and FFA were determined as previously described (16). Serum triglycerides were measured according to the method of Carlson (17), cholesterol by the procedure of Leffler (18), and serum insulin and glucagon by a double antibody radioimmunoassay technique (19). Serum lipoprotein electrophoresis was performed as previously described by Eaton and Kipnis (16). Quantitative serum lipoprotein fractionation was performed by flotation ultracentrifugation of the serum at density 1.006. 1.5 ml of serum, from individual rats, was placed in a microultracentrifuge tube (Beckman model 303369), and 1 ml of saline was layered on top. After ultracentrifugation in a Spinco no. 40.3 head for 35 min, the top 0.5 ml, containing chylomicrons, was removed. The infranatant solution was again placed in a microultracentrifuge tube, with a saline overlay, and spun at 40,000 rpm for 24 hr. The top 0.5-ml fraction containing the VLDL was isolated by the tube-slicing technique and analyzed for protein, cholesterol, and triglyceride. Although it is customary to recentrifuge lipoprotein fractions after the initial flotation, in our micromethod we chose instead to centrifuge in a 1:1 dilution with saline to achieve a uniform VLDL preparation. Evaluation of this VLDL preparation by paper lipoprotein electrophoresis revealed a single oil red O-staining band with the appropriate migration described for both human and rat VLDL (16).

Since VLDL is the major transport form of endogenous serum triglyceride, this isolated fraction might be expected to demonstrate a definable triglyceride complement associated with the apoprotein moiety. In examining this possibility, we observed a constant triglyceride-to-protein ratio of 11.6 ± 2 μg/μg of protein in the VLDL fraction of six normal rats and of six lipemic cobalt-treated rats (11). Though the apoprotein content of the lipemic VLDL was four times that of normal rat serum (240 μg/ml vs. 60 μg/ml), the triglyceride component was consistently proportionate to the protein concentration. The constancy of this ratio in the isolated VLDL over a range of concentrations of apoprotein suggests reasonable reproducibility of the methodology without variable contamination with nontriglyceride-carrying serum proteins. These observations support the constancy of composition and confidence of purity of the VLDL fractions that were used in these studies. Clearly, further steps in purification might well be utilized in the isolation of VLDL under certain circumstances. However, to determine the effect of glucagon on VLDL concentration in a large group of about 50 rats, we felt this modified technique to be justifiable.

In vitro protein synthesis was measured, as previously described (10), in liver slices prepared with a Stadie-Riggs microtome and incubated for 45 min at 37°C in Krebs bicarbonate buffer, pH 7.4, containing 3% bovine albumin, 100 mg/100 ml of glucose, and [^{14}C]leucine, 0.1 μCi (New England Nuclear Corp., Boston, Mass.). The rats were killed by decapitation, and the liver was perfused in situ with 10 ml of ice-cold saline before removal. The slices were washed in 30 ml of ice-cold buffer for 2–4 min; 1 g of slices (wet wt) was then transferred to the appropriate incubation flasks. The flasks were gassed for 10 min with 95% O_2–5% CO_2 and capped for the duration of the incubation. After incubation, the medium and liver slices were homogenized in Bellco glass homogenizers and extracted with 2 ml of ice-cold saline three times. The lipoproteins present in the incubation medium and saline tissue extract were isolated by modifications of the methods previously used by Bungenberg de Jong and Marsh (20) and Radding and Steinberg (21) in their studies of lipoprotein synthesis by in vitro liver preparations. The saline extracts were then adjusted to d = 1.006 and centrifuged for 60 min in a no. 40 rotor in the model L Spinco ultracentrifuge at 50,000 *g;* the floating fat and chylomicron fraction were removed by tube slicing (22). The infranatant solution was then taken to d = 1.063 with KBr according to the method of Havel, Eder, and Bragdon (23) and recentrifuged for 18 hr at 100,000 *g*. The top 1.5-cm fraction was removed by tube slicing and the lipoproteins present (VLDL and LDL) were precipitated by the addition of 10 ml of 20% trichloroacetic acid at 5°C. After three washes with 5 ml of 10% trichloroacetic acid and three washes with 5 ml of 5% trichloroacetic acid, the precipitates were extracted twice with 10 ml of 1% trichloroacetic acid in 95% ethanol to remove any remaining albumin. The preparation was then resuspended in 5 ml of 5% trichloroacetic acid, and the resulting precipitate was then extracted

TABLE 1. Effect of glucagon injection in eulipemic control rats and in cobalt-lipemic rats[a]

Parameter	Control Rats		Cobalt-lipemic Rats	
	Basal	After Glucagon[b]	Basal	After Glucagon[b]
Serum triglyceride, mg/100 ml	67 ± 6	31 ± 3	173 ± 5	35 ± 6
Serum VLDL apoprotein, μg/ml	60 ± 14	<20	240 ± 60	<20
Serum cholesterol, mg/100 ml	61 ± 6	39 ± 4	78 ± 5	62 ± 4
Serum FFA, mmoles/l	383 ± 43	952 ± 51	745 ± 28	634 ± 18
Serum insulin, μU/ml	25 ± 2	41 ± 8	21 ± 3	40 ± 12

[a] All values are means ± SD of 20–30 rats.
[b] Glucagon administered at pharmacological excess, 2 mg/day × 4.

sequentially with 5 ml of chloroform–ether–ethanol, 5 ml of acetone, and 5 ml of ether. The aliquots of final white precipitate were weighed, dissolved in Hyamine, added to toluene containing 0.5% 2,5-diphenyloxazole, and counted in a Tri-Carb liquid scintillation counter. The results are expressed in terms of total incorporation (counts/minute) per gram wet weight of liver.

RESULTS

Prior to glucagon treatment, cobalt-treated rats demonstrated a marked lipemia as previously reported and characterized (11). Their basal serum triglyceride concentration was elevated to 173 ± 5 mg/100 ml (mean ± SD of 20 rats [11]; control = 67 ± 6 mg/100 ml). The lipemia was associated with a marked pre-β band on lipoprotein electrophoresis (Fig. 1) and with an elevation in serum VLDL apoprotein to 240 ± 60 μg/ml (control = 60 ± 14 μg/ml).

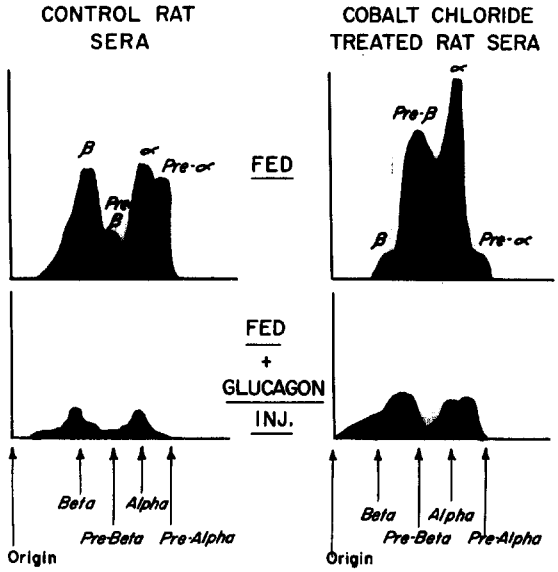

FIG. 1. Effect of glucagon injection (2 mg/day × 4) on the electrophoretic patterns of serum lipoproteins from control and cobalt-lipemic rats.

As shown in Table 1 and Fig. 1, the chronic administration of glucagon resulted in a significant reduction in the serum triglyceride, cholesterol, VLDL apoprotein, and pre-β-migrating lipoprotein band in the cobalt-lipemic rats and a lowering of these parameters even in control eulipemic rats. These latter animals demonstrated a reduction to 46% of normal triglyceride concentration ($P = 0.001$), while hyperlipemic animals demonstrated a reduction to 20% of levels of untreated rats ($P = 0.001$). Total serum cholesterol was also reduced by glucagon injection, but the changes were not as great as those seen for triglycerides. In both control and cobalt-lipemic animals, the change in lipoprotein pattern in response to chronic glucagon injection was identical, as shown in Fig. 1. In both cases all lipoprotein species, including the pre-β peak, were greatly reduced, with only faint peaks remaining in the β and α positions and occasionally a small peak in the pre-α region.

To explore the significance of this qualitative decrease in the pre-β lipoprotein which is responsible for the bulk of the endogenous lipemia in these rats, serum was fractionated to examine the VLDL fraction quantitatively (Table 1). This fraction, which is known to correspond to the pre-β-migrating lipoproteins (16), was reduced in all glucagon-treated rats to levels below the limits of sensitivity of our methodology. The apoprotein content of the VLDL was too low to be accurately evaluated by these quantitative microultracentrifugation techniques. The range in values was 5–25 μg/ml in specimens from eight animals. This extremely low protein recovery in the VLDL of the glucagon-treated rat supports the effectiveness of the isolation methodology in excluding extraneous serum proteins from this ultracentrifugal fraction. Although this fraction from normal and cobalt-lipemic rat sera contained significant amounts of both triglyceride and cholesterol as previously reported (11), these lipids were present in concentrations of less than 15 mg/100 ml in sera from glucagon-injected animals. As judged by lipoprotein electrophoresis, chylomicrons were persistently present in the sera of glucagon-treated animals, also contributing to the final triglyceride serum concentration in the range of 10–20

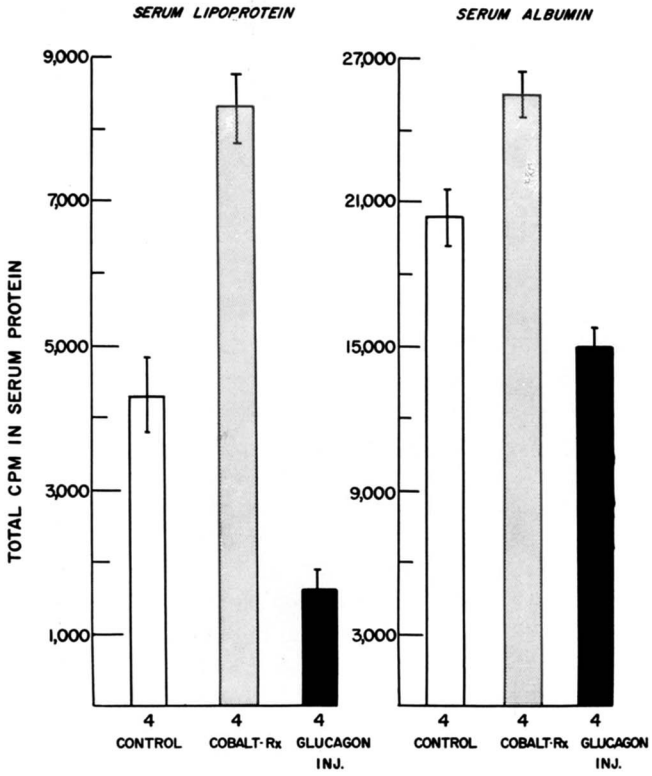

FIG. 2. Effect of glucagon injection in cobalt-lipemic rats on [^{14}C]leucine incorporation into serum VLDL–LDL and albumin in vivo, expressed as total incorporation into protein contained in 10 ml of serum. All values are means ± SD of four fed animals (see Methods).

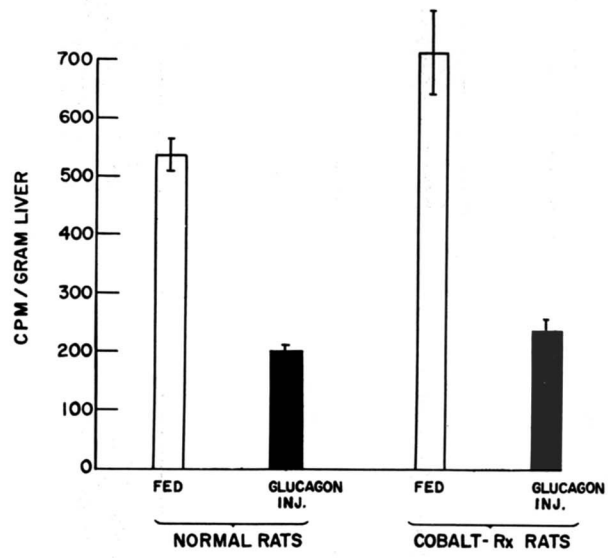

FIG. 3. Effect of glucagon injection on [^{14}C]leucine incorporation into tissue VLDL–LDL in normal and in cobalt-lipemic rat liver in vitro. All values are means ± SD of four animals.

mg/100 ml. Thus, the absence of a pre-β-migrating lipoprotein band on electrophoresis corresponds with the very low recovery of VLDL apoprotein and lipid by ultracentrifugal techniques.

In association with the reduction in VLDL and triglyceride concentrations, a rise in serum FFA to 952 ± 51 mmoles/l in control animals and a continued elevation to 634 ± 18 mmoles/l in cobalt-lipemic animals were observed.

The administration of glucagon to normal rats raised the basal levels of serum immunoreactive glucagon to 800–1500 pg/ml (six animals) and resulted in an elevation in serum immunoreactive insulin to 41 ± 10 μU/ml in all animals.

Effect of hepatic protein production

Hepatic lipoprotein protein production was examined in vivo and in vitro with [^{14}C]leucine, as shown in Figs. 2 and 3. As previously discussed in detail (11), the hepatic pool of leucine is unchanged by cobalt treatment, fasting, glucose feeding, alloxan diabetes, or glucagon administration (24). Serum was collected 4 hr after the intravenous injection of [^{14}C]leucine, and the heparin-precipitable VLDL–LDL and albumin were isolated as previously described (10). Production of lipoprotein protein is described in terms of the total amount of radioactive leucine incorporated into the particular protein in 10 ml of serum 4 hr after administration of the isotope. Accumulation of radioactivity in a serum protein thus represents the net effect of hepatic synthesis, secretion into the blood, and removal from the blood. We have previously reported that this incorporation is increased twofold in cobalt-treated rats compared with controls. As shown in Fig. 2, glucagon injection in cobalt-treated animals reduced the elevated level of incorporation into serum VLDL–LDL from 8313 ± 1200 cpm/10 ml serum to a minimal level of 1610 ± 600 cpm/10 ml serum. Similarly, incorporation into serum albumin in vivo was reduced by glucagon from a level of 25505 ± 2000 cpm/10 ml serum to 14382 ± 2000 cpm/10 ml serum in cobalt-treated rats. This reduction in incorporation of [^{14}C]leucine into serum proteins after glucagon treatment may represent decreased hepatic protein synthesis, decreased hepatic protein secretion, increased peripheral utilization of these serum proteins, or a combination of all three events.

To assist in further distinction between these explanations for this action of glucagon, studies were performed in vitro with liver slices to examine [^{14}C]leucine incorporation into VLDL–LDL protein. In this system, no peripheral metabolism and removal of the newly synthesized radioactive lipoproteins occurs, and incorporation into VLDL–LDL proteins contained both within the tissue and secreted into the medium is evaluated as a single process. Thus, alterations in hepatic

secretion of protein is excluded as a factor influencing the final incorporation of [^{14}C]leucine into protein, and only protein synthesis determines the amount of radioactivity recovered. As shown in Fig. 3, glucagon injection prior to killing the animals reduced incorporation into VLDL–LDL in liver from both normal and cobalt-treated rats to approximately equal levels of 1400 cpm/g liver (preglucagon = 3752 ± 100 cpm/g liver in controls and 4963 ± 500 cpm/g liver in cobalt-treated rats, expressed as means ± SD of four animals in each group). This suppression of hepatic synthesis of VLDL–LDL protein is consistent with the fall in circulating VLDL protein observed in glucagon-treated rats.

DISCUSSION

A synchronous relationship between hepatic apoprotein synthesis and the secretion of lipoproteins has been demonstrated in several animal studies (22, 25–29). The inhibition of the synthesis of hepatic lipoprotein protein by actinomycin D, carbon tetrachloride, ethionine, or puromycin results in a failure of the hepatic "lipoprotein secretory mechanism," leading to a decrease in the concentration of lipids in the plasma and frequently to the accumulation of lipid in the liver as the triglyceride portion of the lipoprotein continues to be synthesized. Similarly, in several rat models of endogenous hyperlipemia, increased hepatic protein synthesis has been reported, consistent with an important role of apoprotein synthesis on hepatic lipoprotein production, viz., nephrotic hyperlipemia, hereditary obese hyperlipemia, carbohydrate-induced hyperlipemia, ethanol-induced hyperlipemia, and cobalt-induced hyperlipemia (9–13). We now report that the hormone glucagon causes a depression in hepatic apoprotein synthesis and a decrease in circulating VLDL apoprotein consistent with a mediation of the hypolipemic action of glucagon at the level of protein synthesis.

Our observation of an action of glucagon in decreasing amino acid incorporation into hepatic lipoprotein apoprotein is consistent with earlier observations on this catabolic action of glucagon. This event is clearly not specific for lipoprotein, but represents a generalized inhibition of hepatic protein synthesis and increased protein breakdown as shown in our data for albumin synthesis. As recently reviewed by Miller (14), glucagon has a marked effect on increasing hepatic urea production, increasing net hepatic proteolysis, and decreasing amino acid incorporation into nonspecific hepatic protein (7, 8, 14, 15). Furthermore, a synchronous inhibition of triglyceride release from the perfused liver as urea production is increased has been reported with glucagon in vitro (7, 8). Therefore, the hypolipemic action of glucagon in vivo may be partially attributed to its effects on decreasing hepatic lipoprotein apoprotein synthesis, thus limiting hepatic lipoprotein production.

The extent to which these effects are mediated by the action of glucagon in inhibiting protein formation in the liver is not established, since glucagon is also reported to depress triglyceride synthesis in the liver (5–8), an action which could be rate limiting in the assembly of the final lipoprotein complex. An independent effect on decreasing hepatic triglyceride synthesis cannot be excluded by our data; if present, this effect would complement the depression of apoprotein synthesis. Such a response would be consistent with the observations of the physiological states of fasting and of high-carbohydrate feeding (10, 11, 30), in which synchronous changes in synthesis of both the triglyceride and protein moieties of hepatic lipoprotein have been observed, and in which corresponding changes in glucagon activity have been suggested.

In the normal perfused liver, triglyceride production is reported to be directly proportional to the perfusate concentration and uptake of FFA by the liver (7, 31). In the intact cobalt-lipemic rat, the usual elevated FFA level is only minimally reduced by glucagon (14%), while the serum triglyceride concentration is reduced by 75% (Table 1). Furthermore, in normal rats, the serum FFA level is actually increased twofold in response to glucagon (Table 1), making it difficult to ascribe the resulting reduction in serum triglyceride concentration to unavailability of FFA precursors. In addition to this potential effect on modifying the availability of triglyceride precursors, glucagon has been shown to stimulate FFA metabolism to ketone bodies while simultaneous triglyceride production is decreased (7). Thus, modulation of FFA availability may be exerted by glucagon at the level of alternate pathways of utilization relative to triglyceride production. This suggestion is consistent with the studies of Woodside and Heimberg (31) in livers from diabetic rats. Using anti-insulin serum (AIS) to produce acute insulin deficiency (and presumably relative glucagon excess), they demonstrate that, at any perfusate FFA concentration, triglyceride production is reduced in AIS liver compared with normal liver. Furthermore, insulin administration in vivo to AIS rats resulted in normalization of hepatic triglyceride production in spite of identical availability of FFA in the perfusion system (31). Thus, the supply of FFA substrate is a prerequisite for triglyceride production, but glucagon and insulin also appear to regulate further lipoprotein production.

Since the secretion of lipoprotein requires synchronized synthesis and assembly of both the lipid and protein moieties, it is possible that an action of glucagon in blocking FFA conversion to triglyceride may result in

a secondary inhibition of protein synthesis. Similarly, a direct effect of glucagon on inhibition of protein synthesis may also result in a secondary reduction of FFA conversion to triglyceride. Our data do not resolve the potential relative contributions of these two events, but they do demonstrate that inhibition of apoprotein synthesis is one manifestation of the ultimate hepatic response to glucagon challenge.

We have not examined the possibility that accelerated clearance of serum lipoproteins may contribute to the hypolipemic action of glucagon. Caren and Corbo (32) have reported that a transfer of lipids to blood platelets does occur with glucagon injection and may account for a portion of the loss of lipoprotein lipid. A potentiation of the action of lipoprotein lipase could result in increased disposal of lipoproteins, as has been suggested for the hypolipemic action of clofibrate (33). However, DeOya, Prigge, and Grande (6) have reported that hepatectomy prevents the hypolipemic response to glucagon in fowl, suggesting that a direct effect on peripheral lipoprotein clearance may not mediate this action of glucagon.

Finally, glucagon could influence serum lipoprotein levels by decreasing appetite, thereby reducing caloric intake.[1] Our animals gained weight more slowly than control animals, in spite of identical caloric intake. Thus, a generalized catabolic effect of glucagon was apparent which was not corrected by parallel food ingestion. The possibility of multiple loci of action must thus be maintained until further data are available. Nevertheless, the hypolipemic action of glucagon reported here is not unique to the rat. It has similarly been reported in alimentary and carbohydrate-induced lipemia in man[1] (34), in familial lipemia (4, 35), and in the sustained lipemia complicating von Gierke's disease, Niemann-Pick disease, and in relapsing pancreatitis (36).

This investigation was supported by the U.S. Public Health Service grant 5 R01 HE12085 and by Career Development Award 1 K04 HE 35843. The technical assistance of I. Pommer is gratefully acknowledged.

Manuscript received 26 June 1972; accepted 16 January 1973.

REFERENCES

1. Caren, R., and L. Corbo. 1960. Glucagon and cholesterol metabolism. *Metabolism.* **9:** 938–945.
2. Salter, J. M. 1960. Metabolic effects of glucagon in the Wistar Rat. *Amer. J. Clin. Nutr.* **8:** 535–539.
3. Paloyan, E., and P. V. Harper, Jr. 1961. Glucagon as a regulating factor of plasma lipids. *Metabolism.* **10:** 315–323.
4. Amatuzio, D. S., F. Grande, and S. Wada. 1962. Effect of glucagon on the serum lipids in essential hyperlipemia and in hypercholesterolemia. *Metabolism.* **11:** 1240–1249.
5. DeOya, M., W. F. Prigge, D. E. Swenson, and F. Grande. 1971. Role of glucagon on fatty liver production in birds. *Amer. J. Physiol.* **221:** 25–30.
6. DeOya, M., W. F. Prigge, and F. Grande. 1971. Suppression by hepatectomy of glucagon-induced hypertriglyceridemia in geese. *Proc. Soc. Exp. Biol. Med.* **136:** 107–110.
7. Heimberg, M., I. Weinstein, and M. Kohout. 1969. The effects of glucagon, dibutyryl cyclic adenosine 3′,5′-monophosphate, and concentration of free fatty acid on hepatic lipid metabolism. *J. Biol. Chem.* **244:** 5131–5139.
8. Penhos, J. C., C. H. Wu, J. Daunas, M. Reitman, and R. Levine. 1966. The effect of glucagon on the metabolism of lipids and on urea formation by the perfused rat liver. *Diabetes.* **15:** 740–748.
9. Baraona, E., and C. S. Lieber. 1970. Effect of chronic ethanol feeding on serum lipoprotein metabolism in the rat. *J. Clin. Invest.* **49:** 769–778.
10. Eaton, R. P., and D. M. Kipnis. 1969. Effect of glucose feeding on lipoprotein synthesis in the rat. *Amer. J. Physiol.* **217:** 1153–1159.
11. Eaton, R. P. 1972. Cobalt chloride-induced hyperlipemia in the rat: effects on intermediary metabolism. *Amer. J. Physiol.* **222:** 1550–1557.
12. Marsh, J. B., and D. L. Drabkin. 1960. Experimental reconstruction of metabolic pattern of lipid nephrosis: key role of hepatic protein synthesis in hyperlipemia. *Metabolism.* **9:** 946–955.
13. Zucker, L. M. 1965. Hereditary obesity in the rat associated with hyperlipemia. *Ann. N.Y. Acad. Sci.* **131:** 447–458.
14. Miller, L. L. 1965. Direct actions of insulin, glucagon, and epinephrine on the isolated perfused rat liver. *Federation Proc.* **24:** 737–744.
15. Pryor, J., and J. Berthet. 1960. The action of adenosine 3′,5′-monophosphate on the incorporation of leucine into liver protein. *Biochim. Biophys. Acta.* **43:** 556–557.
16. Eaton, R. P., and D. M. Kipnis. 1969. Effects of high-carbohydrate diets on lipid and carbohydrate metabolism in the rat. *Amer. J. Physiol.* **217:** 1160–1168.
17. Carlson, L. A. 1963. Determination of serum triglycerides. *J. Atheroscler. Res.* **3:** 334–336.
18. Leffler, H. H. 1959. Estimation of cholesterol in serum. *Amer. J. Clin. Path.* **31:** 310–313.
19. Eaton, R. P., M. Conway, and M. Buckman. 1972. Role of α-adrenergic blockade on alanine-induced hyperglucagonemia. *Metabolism.* **21:** 371–373.
20. Bungenberg de Jong, J. J., and J. B. Marsh. 1968. Biosynthesis of plasma lipoproteins by rat liver ribosomes. *J. Biol. Chem.* **243:** 192–199.
21. Radding, C. M., and D. Steinberg. 1960. Studies on the synthesis and secretion of serum lipoproteins by rat liver slices. *J. Clin. Invest.* **39:** 1560–1569.
22. Seakins, A., and D. S. Robinson. 1963. The effect of the administration of carbon tetrachloride on the formation of plasma lipoproteins in the rat. *Biochem. J.* **86:** 401–407.
23. Havel, R. J., H. A. Eder, and H. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
24. Mallette, L. E., J. H. Exton, and C. R. Park. 1969. Effects of glucagon on amino acid transport and utilization in the perfused rat liver. *J. Biol. Chem.* **244:** 5724–5728.

[1] Bierman, E. L. Personal communication.

25. Wilcox, H. G., M. Fried, and M. Heimberg. 1965. Lysine incorporation into serum-lipoprotein protein by the isolated perfused rat liver. *Biochim. Biophys. Acta.* **106:** 598–602.
26. Fried, M., H. G. Wilcox, G. R. Faloona, S. P. Eoff, M. S. Hoffman, and D. Zimmerman. 1968. The biosynthesis of plasma lipoproteins in higher animals. *Comp. Biochem. Physiol.* **25:** 651–661.
27. Jones, A. L., N. B. Ruderman, and M. G. Herrera. 1967. Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver. *J. Lipid Res.* **8:** 429–446.
28. Buckley, J. T., T. J. Delahunty, and D. Rubinstein. 1968. The relationship of protein synthesis to the secretion of the lipid moiety of low density lipoprotein by the liver. *Can. J. Biochem.* **46:** 341–349.
29. Faloona, G. R., B. N. Stewart, and M. Fried. 1968. The effects of actinomycin D on the biosynthesis of plasma lipoproteins. *Biochemistry.* **7:** 720–725.
30. Fallon, H. J., and E. L. Kemp. 1968. Effects of diet on hepatic triglyceride synthesis. *J. Clin. Invest.* **47:** 712–719.
31. Woodside, W. F., and M. Heimberg. 1972. Hepatic metabolism of free fatty acids in experimental diabetes. *Israel J. Med. Sci.* **8:** 309–316.
32. Caren, R., and L. Corbo. 1970. Transfer of plasma lipid to platelets by action of glucagon. *Metabolism.* **19:** 598–607.
33. Bierman, E. L., J. D. Brunzell, J. D. Bagdade, R. L. Lerner, W. R. Hazzard, and D. Porte, Jr. 1970. On the mechanism of action of Atromid-S on triglyceride transport in man. *Trans. Ass. Amer. Physicians.* **83:** 211–224.
34. Albrink, M. J., J. R. Fitzgerald, and E. B. Man. 1957. Effect of glucagon on alimentary lipemia. *Proc. Soc. Exp. Biol. Med.* **95:** 778–780.
35. Amatuzio, D. S., and F. Grande. 1963. Essential hyperlipemia: the effect of glucagon. *Minn. Med.* **46:** 1088–1091.
36. Paloyan, E., N. Dumbrys, T. F. Gallagher, Jr., R. E. Rodgers, and P. V. Harper. 1962. The effect of glucagon on hyperlipemic states. *Federation Proc.* **21:** 200. (Abstr.)