Effect of Glucagon on the Metabolism of Lipids and on Urea Formation by the Perfused Rat Liver

J. C. Penhos, M.D., C. H. Wu, M.D., J. Daunas, M.D., M. Reitman, B.A., and R. Levine, M.D., New York

SUMMARY

Livers obtained from fed or fasted rats were perfused with blood from fasted rats with and without the addition of glucagon and/or a hyperlipemic serum (HLS).

In addition to its well-known hyperglycemic effect, glucagon increased the blood levels of the ketone bodies and of urea, and decreased the levels of cholesterol, total lipids and NEFA.

The magnitude of the effects noted were dependent upon the previous state of the liver and the addition of the hyperlipemic serum.

In the fed liver, glucagon produced a greater degree of hyperglycemia than in the fasted liver and this effect was enhanced by the addition of a hyperlipemic serum. In fasted livers the addition of a hyperlipemic serum did not increase the hyperglycemia produced by glucagon alone.

Total lipids, cholesterol and NEFA were decreased when glucagon was added to either the fasted or fed liver, in the presence or absence of a hyperlipemic serum.

Glucagon caused a greater increase in the level of ketone bodies in the fasted than in the fed liver and this effect was increased by the addition of a serum rich in lipids.

The addition of glucagon caused an increase in the levels of urea and this effect was reduced when a hyperlipemic serum was added. DIABETES *15*:740-48, October, 1966.

A survey of the available literature on the effects of glucagon on various aspects of lipid metabolism discloses unexplained discrepancies in the results. On the one hand it has been reported that glucagon is lipolytic and in the main inhibits the synthesis of fat and cholesterol.[5,21,22,36,43,52] On the other hand there also exist data to show that glucagon lowers the concentration of fatty acids in the plasma and increases fat synthesis.[1,14,43] In the case of ketone body metabolism, the hormone is said to reduce production by liver slices,[52] lower ketone body concentration of the blood,[32] produce no change[2] or even increase liver synthesis of ketones.[5,17,22]

The investigation was undertaken in the hope that by separating extrahepatic from intrahepatic effects one would be able to achieve greater consistency. We chose to use the perfusion with blood of the isolated rat liver as the technic for studying the effects of glucagon on lipid metabolism of the liver. Lipid metabolism in such preparations has been studied by many workers[4,18,24-27,29,34,38,42,47,48] but the action of glucagon has only been observed once. The hormone did not modify the rate of uptake of fatty acids by the perfused liver.[4]

We shall report on the effects of glucagon on some aspects of lipid metabolism of the isolated livers taken from fasted and well-fed animals with and without the addition of a lipid-rich serum.

METHODS AND MATERIALS

The perfusion apparatus: This is a simplified modification of one previously described.[45] The present one (figure 2) has only two glass parts (B and C). The first piece (B) is a chamber that keeps the blood pressure constant at 15 cm. H_2O; the excess blood is allowed to overflow through a bypass (E) into the aeration chamber (C) where it flows downward over the glass wall. The second glass portion (C) has three parts: the upper part is the perfusion chamber with a separate glass door (G) that facilitates the introduction of the liver. The door is hinged to the main portion of the chamber with surgical tape. The top of this chamber is a round piece of cork or plastic (H) with a hole in the center that allows for the passage of plastic tubing 12 cm. of length (P) that connects (B) with a No. 16 needle (I) 1 cm. in length. The middle part of (C) is an aeration chamber that receives a mixture of O_2 (95 per cent) plus CO_2 (5 per cent) through (K)

From the Department of Medicine, New York Medical College, New York, New York. Dr. J. C. Penhos' present address is: Experimental Medicine Unit, Research Foundation, Washington Hospital Center, Washington, D.C. 20010.

and allows the gas mixture to pass out through (L) into a trap for CO_2. The lower part of (C) is the reservoir of blood from which it is possible to take samples via the arm (M) occluded with a cork. The perfusion chamber is separated from the aeration chamber by a convex diaphragm of glass (D) containing a few holes to produce streams of blood which drop freely from the inferior vena cava.

The peristaltic action pump (N) (American Instrument Co., Silver Spring, Maryland, Catalogue No. 4-8941) recirculates the blood from the reservoir to the filter (A) which can be any type of filter utilized in hospitals for blood transfusion.

Liver perfusion: Livers for perfusion were taken from fed or fasted male albino rats (Wistar strain) weighing about 350 to 400 gm. They were fed regular Purina chow up to the time of the experiment. The fasted rats were deprived of food for eighteen hours.

Sodium amytal (Lilly) 4 mg. per 100 gm. body weight was injected intraperitoneally as the anesthetic. A median incision was made to expose the duodenum. The bile duct was catheterized (figure 1) with a polyethylene tube (inside diameter 0.25 mm.) through a small incision in the widest part of the bile duct near its entrance into the duodenum.

When the bile was flowing, the median abdominal incision was extended transversely to the right and to the left. The liver was freed by cutting the ligaments that connect it to the stomach and diaphragm. The portal vein was exposed and the pyloric vein was identified and ligated. This ligature serves as a reference for the cephalad extension of the portal cannula.

Holding (with a clamp) the still untied portal vein, a small incision, below the origin of the splenic vein, was made with sharp scissors and one end of a polyethylene tubing connected by a No. 16 needle to the perfusion chamber was introduced up to the level of the pyloric vein, and tied with two ligatures, one below the pyloric vein and the other below the splenic vein.

As soon as the first ligature was made, the heparinized blood in the tube was allowed to go into the organ. The interruption of the liver circulation lasts only for three to five seconds. All the connections of the

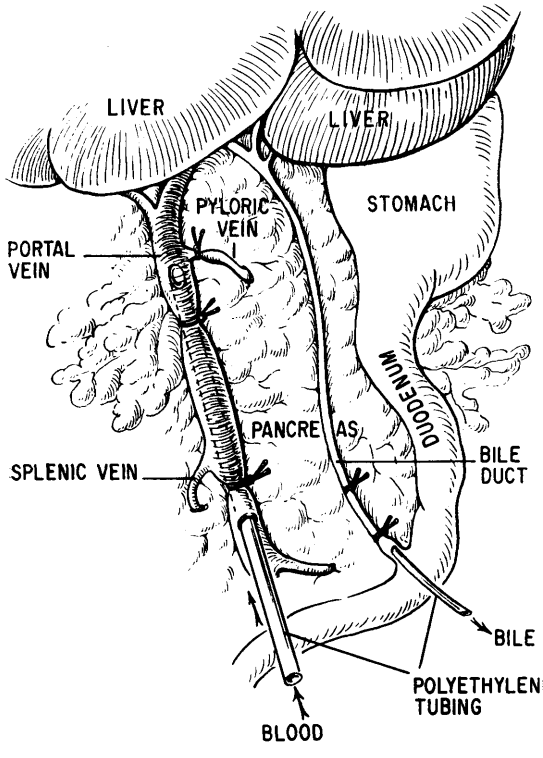

FIG. 1. Position of the cannulae in the portal vein and bile duct.

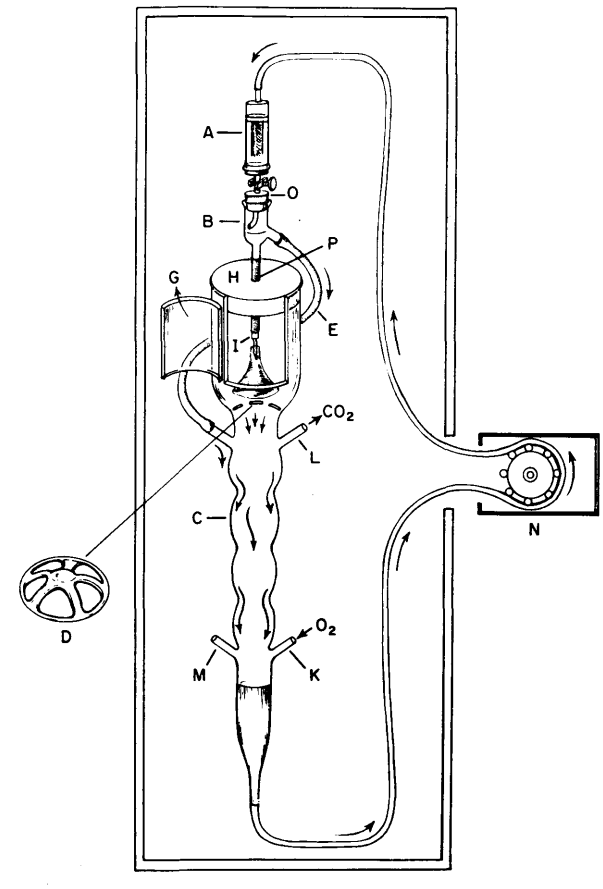

FIG. 2. Liver perfusion apparatus. For details see under "Methods."

liver were then severed without cannulating the inferior vena cava. The polyethylene tubing was shortened and re-attached to a needle (No. 16) that was connected to the perfusion chamber (figure 2). The liver remains hanging, touching the glass diaphragm slightly. The transfer of the liver from the animal takes another three to five seconds.

Sequence of manipulations: forty ml. of a heparin solution (10 mg. per cent in sterile saline 0.9 per cent) was introduced via the perfusion chamber to the reservoir and the pump was switched on. The object of this is to wet the entire apparatus. After ten minutes all the saline solution was removed through needle (I), and the pump switched off.

The next step consists of the careful introduction of 50 ml. of whole blood, collected as described below. The pump is again switched on and adjusted sufficiently to initiate the circulation. The mixture of O_2 and CO_2 is then sent through (K). The clamp (O) under the filter (A) is adjusted to have a level of blood of 2 cm. in the filter to avoid bubble or foam formation. The blood runs through the needle (I) and through the bypass (E). At the moment when the liver is connected to the needle the tube (P) is clamped. As soon as the liver hangs in place, the clamp is taken out and the blood is allowed to circulate through the liver.

Preparation of the perfusate: For the purpose of having a high level of NEFA, donor rats (after anesthesia) were injected, via the inferior cava, with 0.5 mg. of heparin per rat. After five minutes, blood was taken from the aorta with a No. 18 needle. All the blood was collected in a beaker chilled with ice. The blood donor rats were males and of the same approximate weight as the experimental animals. The total volume of blood utilized in the perfusion was 50 ml.

Preparation of the lipid-rich serum: Male rats weighing 350 to 400 gm., fasted for eighteen hours, were anesthetized as previously described. A median abdominal incision was made and all of the viscera were displaced to the right side. A neutral synthetic fat emulsion (Lipomul without glucose) was injected intravenously, very slowly, via the inferior vena cava (1 ml./100 gm. body weight).

After fifteen minutes, blood was collected from the aorta and allowed to clot. The serum obtained was utilized to add to the perfusate (1 ml./10 ml. of blood) in those experiments where a high level of lipids was required.

A period of thirty minutes of equilibration was selected for the liver perfusion to provide for adjustment in view of the vasoconstrictor factor of the liver[8]; of the levels of sugar, total lipids, cholesterol, and NEFA; and the change that may take place in the clearing factor.[38]

Crystalline glucagon (Lilly, 258-234-167-1) was dissolved in normal saline with the *p*H adjusted to 8.0 in a concentration of 0.1 mg. per ml. A total dose of 0.2 mg. was used; 0.1 mg. at the start of the perfusion and 0.1 mg. at the thirty-minute "equilibration" time. The dosage used far exceeds normal concentrations of glucagon in blood. The effects obtained may thus not reflect the exact physiological actions of this hormone.

The following chemical methods were used: blood sugar,[41] total lipids,[10] cholesterol,[35] nonesterified fatty acids,[13] ketone bodies,[6] and urea.[40] Samples were obtained from the perfusate before the liver was placed in the perfusion chamber (—30 min.), after thirty minutes of equilibration (0 minutes), and then at 5, 30, 60, and 90 min. after the period of equilibration.

RESULTS

From the data in table 1 it can be seen that after thirty minutes of equilibration, the level of the blood sugar increased more markedly when the perfusion was done using a "fed" liver than a "fasted" organ. After the first thirty minutes of equilibration, the blood sugar level increases slightly in the control experiments using livers from fasted animals. The addition of glucagon or of hyperlipemic serum (HLS) increased the final level but adding both together produced no additional effect.

The level of the blood sugar increased markedly when the perfusion was done with livers obtained from fed rats. This effect is strongly reinforced by the addition of glucagon and to a lesser degree by HLS. The combination of both produced a strong additional effect.

The level of total lipids in the perfusate (table 2) decreased during the period of equilibration (—30 to 0 min.). After that period when "fed" livers were perfused there was an increase in the level of the total lipids in the perfusate. A similar but less pronounced effect was seen when the livers came from fasted rats. This rise was inhibited by the addition of glucagon to the perfusion (fed livers).

When HLS was added the total lipids reached a very high level, which decreased very rapidly and with more intensity in the fasted livers. The addition of glucagon produced a decrease in the level of total lipids only in the perfusion of the fed livers.

The level of cholesterol increased following the equili-

TABLE 1

Modifications of the levels of blood sugar in the perfusates of rat liver perfusions.
Each figure represents the average of four perfusions with its corresponding range.

Parameter		Blood sugar mg. per 100 ml.											
Liver condition		Fast						Fed					
Minutes		—30	0	5	30	60	90	—30	0	5	30	60	90
Control	Aver.	93	143	148	165	171	166	89	331	370	390	322	284
	Range	88-100	117-182	118-184	134-206	136-212	135-190	80-100	250-386	288-424	280-512	268-386	242-355
Plus glucagon	Aver.	91	158	165	179	214	250	88	445	477	613	679	755
	Range	88-98	140-172	141-177	155-190	186-228	206-270	76-98	409-486	419-542	525-714	556-857	583-940
Plus hyper-lipemic serum	Aver.	94	150	159	169	204	220	94	292	328	393	416	430
	Range	88-104	118-210	125-216	131-222	151-250	184-260	72-110	205-344	205-475	226-623	253-655	253-690
Plus glucagon and hyper-lipemic serum	Aver.	95	150	163	177	231	236	98	467	473	714	813	1,044
	Range	88-105	131-172	145-180	164-186	190-230	220-256	90-109	415-574	415-574	500-1,050	650-1,214	845-1,280

TABLE 2

Modification of the levels of total lipids in the perfusates of rat liver perfusions.
Each figure represents the average of four perfusions with its corresponding range.

Parameter		Total lipids mg. per cent											
Liver condition		Fast						Fed					
Minutes		—30	0	5	30	60	90	—30	0	5	30	60	90
Control	Aver.	203	129	141	144	144	151	202	160	157	152	180	204
	Range	168-244	125-135	126-166	130-170	130-154	136-175	164-274	135-198	126-174	124-174	147-228	185-230
Plus glucagon	Aver.	192	147	135	127	136	144	155	123	115	117	119	119
	Range	162-228	126-185	110-171	101-162	109-167	119-172	153-163	108-145	108-119	105-129	105-129	108-141
Plus hyper-lipemic serum	Aver.	184	140	408	242	196	148	222	189	466	242	224	220
	Range	164-200	124-166	382-424	185-310	154-234	127-161	199-256	141-224	334-540	207-272	195-276	183-260
Plus glucagon and hyper-lipemic serum	Aver.	197	143	406	261	179	145	183	138	415	254	192	151
	Range	162-257	108-210	324-524	252-268	158-210	110-190	153-215	115-191	384-460	169-354	126-260	126-177

bration period (table 3). The increase was enhanced by the addition of HLS, and inhibited by the addition of glucagon.

The levels of NEFA decreased in all the experiments during equilibration. After that, the levels increased more in fed than in fasted livers (table 4). The addition of HLS produced at five minutes a strong increase in the level of NEFA, which decreased during the ninety

TABLE 3

Modifications of the levels of cholesterol in the perfusates of rat liver perfusions.
Each figure represents the average of four perfusions with its corresponding range.

Parameter		Cholesterol mg. per cent											
Liver condition		Fast						Fed					
Minutes		—30	0	5	30	60	90	—30	0	5	30	60	90
Control	Aver.	62	54	54	60	64	67	57	48	51	56	60	65
	Range	53-86	49-58	52-64	54-66	61-72	61-79	53-63	44-53	48-57	50-64	55-64	62-68
Plus glucagon	Aver.	58	45	45	50	48	40	56	52	60	55	53	48
	Range	46-72	38-50	38-52	42-56	40-57	32-53	53-59	49-57	52-67	52-61	48-59	46-53
Plus hyper-lipemic serum	Aver.	64	58	92	87	82	78	65	52	90	91	88	86
	Range	59-73	57-65	83-99	76-94	75-99	73-81	59-73	50-55	77-105	77-101	76-96	73-94
Plus glucagon and hyper-lipemic serum	Aver.	60	49	76	73	62	57	62	51	85	75	85	60
	Range	52-69	42-57	65-82	66-82	53-69	46-64	52-76	36-69	67-93	56-82	49-72	51-77

TABLE 4

Modifications of the levels of NEFA in the perfusates of rat liver perfusions.
Each figure represents the average of four perfusions with its corresponding range.

Parameter		NEFA uEq./ml.											
Liver condition		Fast						Fed					
Minutes		—30	0	5	30	60	90	—30	0	5	30	60	90
Control	Aver.	1.17	.30	.32	.31	.36	.48	1.06	.28	.35	.40	.52	.85
	Range	.92-1.48	.27-.38	.27-.44	.18-.40	.22-.55	.24-.64	.82-1.52	.22-.36	.27-.48	.27-.54	.29-.78	.49-1.26
Plus glucagon	Aver.	1.43	.31	.27	.24	.17	.16	1.36	.37	.31	.27	.21	.21
	Range	1.22-1.77	.24-.42	.22-.33	.18-.33	.09-.28	.09-.23	1.01-1.61	.24-.45	.24-.42	.19-.35	.15-.27	.13-.27
Plus hyper-lipemic serum	Aver.	1.28	.52	3.12	2.12	1.33	.99	1.12	.41	3.43	1.99	1.54	1.35
	Range	1.07-1.60	.32-.77	2.88-3.92	1.46-2.60	1.08-1.94	.38-.93	1.09-1.58	.33-.54	3.39-3.57	1.78-2.37	1.04-1.89	.89-1.61
Plus glucagon and hyper-lipemic serum	Aver.	1.34	.28	3.04	1.51	.62	.47	1.33	.48	3.30	2.06	.90	.61
	Range	.98-1.70	.26-.32	2.48-3.50	1.11-2.11	.46-.79	.32-.69	.94-1.60	.23-.84	2.66-3.98	1.95-2.22	.64-1.15	.45-.90

minutes of the perfusion. Addition of glucagon to the perfusate intensified the decrease of the NEFA levels in the fasted and fed livers, with or without the addition of HLS.

The levels of the ketone bodies increased more in fasted than in fed livers during the perfusion. This increase is magnified by the addition of glucagon or HLS. The addition of both factors produced an additive ef-

TABLE 5

Modifications of the levels of ketone bodies in the perfusates of rat liver perfusions. Each figure represents the average of four perfusions with its corresponding range.

Parameter		Ketone bodies mg. per cent											
Liver condition		Fast						Fed					
Minutes		—30	0	5	30	60	90	—30	0	5	30	60	90
Control	Aver.	6	23	25	31	35	44	4	15	16	20	25	28
	Range	5-7	18-28	22-29	28-34	31-39	37-55	2-6	13-17	15-17	16-25	22-32	23-32
Plus glucagon	Aver.	5	31	33	58	67	76	6	24	26	37	42	47
	Range	3-8	27-36	28-36	52-62	60-73	69-81	3-10	20-27	24-28	27-63	27-68	37-70
Plus hyperlipemic serum	Aver.	6	28	35	64	94	106	6	15	18	39	49	58
	Range	5-8	22-40	31-47	42-93	67-131	88-139	4-7	10-24	11-25	33-53	41-68	43-82
Plus glucagon and hyperlipemic serum	Aver.	6	32	43	59	125	137	7	23	25	47	68	83
	Range	3-11	13-52	36-60	67-107	99-168	104-169	3-10	15-28	21-30	26-69	42-97	60-10[illegible]

TABLE 6

Modifications of the levels of urea in the perfusates of rat liver perfusions. Each figure represents the average of four perfusions with its corresponding range.

Parameter		Urea mg. per cent											
Liver condition		Fast						Fed					
Minutes		—30	0	5	30	60	90	—30	0	5	30	60	90
Control	Aver.	29	32	37	48	54	69	28	32	34	40	49	53
	Range	26-31	30-36	32-43	37-56	48-64	63-78	24-31	30-34	33-37	37-44	42-52	50-58
Plus glucagon	Aver.	31	33	46	59	71	83	27	32	37	43	50	62
	Range	24-35	30-36	45-49	52-72	60-88	75-92	23-30	28-36	35-40	39-45	46-54	60-66
Plus hyperlipemic serum	Aver.	29	36	37	48	53	61	27	32	35	42	47	54
	Range	26-31	31-38	35-40	40-56	49-60	56-70	23-30	29-37	33-39	36-45	42-50	50-60
Plus glucagon and hyperlipemic serum	Aver.	30	39	44	53	60	67	28	31	35	36	49	54
	Range	23-35	30-42	34-50	51-57	56-65	60-70	26-30	29-34	30-36	33-41	48-53	52-60

fect (table 5).

During the perfusion the level of urea (table 6) increased more significantly in livers obtained from fasted rats. The addition of HLS did not change these values, but the addition of glucagon magnified the increments in the levels of urea.

This last effect was inhibited by the simultaneous addition of glucagon and HLS.

DISCUSSION

The higher carbohydrate output of the fed liver was observed also by Haft and Miller,[20] Mortimer,[39] and Heimberg et al.[27]

The increase in the level of the glycemia when HLS was added could be explained by a sparing effect of the lipids on the utilization of carbohydrates. Heimberg et al.[27] did not find changes in the glucose level of the perfusate after adding chylomicra or a synthetic neutral fat emulsion. In our hands the direct addition of a synthetic neutral fat emulsion also gave negative results.

The intense uptake of fat during the period of equilibration was observed by numerous investigators in liver perfusions.[25-27,29,34,38,47] After this period, the increase in the level of total lipids that took place was probably due to release of triglycerides,[27,47] cholesterol and phospholipids.

The increase in the uptake of lipids, produced by the addition of HLS, was further stimulated by glucagon in fed livers, showing that the hormone had an effect in a liver (fed) set in an anabolic direction but not in the fasted organ in which breakdown reactions may predominate.

Glucagon inhibits the rise of cholesterol when HLS was added. This effect was observed in vivo[3,11,12,44] and in vitro by Berthet[5] who considered that glucagon inhibited the synthesis of cholesterol in liver slices. It is also possible that glucagon accelerates the catabolism of cholesterol; or, since it is ketogenic, one of the precursors of cholesterol such as B-hydroxy-B-methylglutaryl-CoA is diverted to form ketone bodies rather than to initiate the formation of cholesterol.

The drep in NEFA levels after the addition of glucagon has been observed in human beings,[1,14,15,46] showing that glucagon not only has a role in the removal of triglycerides (from the blood stream) but also of NEFA. Similar effects were observed by injecting glucose in man,[15] but this was probably due to secretion of insulin triggered by hyperglycemia. It would seem also according to the recent work of Sammols et al.[51] that glucagon itself promotes the secretion of insulin. However, in our experiments the effects on NEFA levels cannot be due to insulin since the liver was completely isolated. In patients with hepatitis[14] glucagon produced a drop in NEFA without hyperglycemia. In unpublished experiments by the senior author, addition of glucose to the perfusate in rat liver perfusions did not produce the same effect as did glucagon.

If we consider that glucagon increases the output of FFA from the epididymal fat in vitro[21,52] we must consider that the liver under the effect of this hormone has an uptake of fatty acids faster than their release from adipose tissue. Other experiments in vitro, using liver slices,[22,23] demonstrated the inhibitory effect of glucagon on the synthesis of fatty acids from acetate, glucose or fructose.

The intense decrease in the level of NEFA following glucagon (when HLS was added) clearly demonstrates that the hormone produces a stimulation of the uptake of free fatty acids by the liver.

The published studies of the effect of glucagon on the production of ketone bodies are contradictory. In studies on liver slices in vitro, some investigators report that glucagon increased the formation of ketone bodies[5,22] and others report that ketone bodies were decreased.[51] Experiments in animals showed that glucagon was ketogenic,[16] nonketogenic,[2] or even that it reduces ketonemia.[32] In human beings some workers confirmed that glucagon was ketogenic[7,28,50] and others reported no change in ketone bodies.[8] In our liver perfusion, glucagon was conclusively ketogenic and this effect was intensified by addition of HLS. We consider that the contradictory results obtained in vivo were due to the compensatory mechanism of insulin secretion produced by hyperglycemia. The amount of ketone bodies produced under the effect of glucagon could partly explain the fall in the values of the lipids during the perfusion. It may be that the effect of glucagon on glycogenolysis produced depletion of glycogen which forced the liver to utilize lipids. In other words, the effect of glucagon on lipid metabolism in the liver may be a secondary or indirect effect, because the fed liver with greater reserves of glycogen maintains a lower production of ketone bodies.

The protein catabolic effect of glucagon has been studied in human beings,[30,50] in animals,[31,32,49] and also in rat liver perfusions.[37] The addition of a hyperlipemic serum had a sparing effect on protein catabolism (urea production) produced by glucagon. It is evident that the energetic requirements of the liver, when glucose utilization is prevented, are met by fat before proteins are catabolized. Glucagon not only produces glycogenolysis but also seems to inhibit or reduce the utilization of the circulating glucose in the liver.[37]

ACKNOWLEDGMENT

This investigation was supported in part by Research Grant AM-09196-01 from the National Institute of Health, and in part by a grant from Cyanamid Interna-

tional, Pearl River, New York.

The authors wish to thank Dr. T. J. Vecchio of The Upjohn Company for the preparation of the Lipomul without glucose, and Drs. Mary A. Root and W. R. Kirtley of the Lilly Research Laboratories for the gift of the glucagon. The technical assistance of Randolph White is appreciated.

REFERENCES

[1] Albrink, M. J., Fitzgerald, J. R., and Man, E. B.: Effect of glucagon on alimentary lipemia. Proc. Soc. Exp. Biol. Med. *95*:778, 1957.

[2] Amatruda, T. T., Jr., Chase, J. W., and Engel, F. L.: The role of endocrine glands in ketones. II. Ketonemia following insulin hypoglycemia. J. Clin. Invest. *41*:758, 1961.

[3] Amatuzio, D. S., Grande, F., and Wada, S.: Effect of glucagon on serum lipids in essential hyperlipemia and in hypercholesterolemia. Metabolism *11*:1240, 1962.

[4] Aydin, A., and Sokal, J. E.: Uptake of plasma-free fatty acids by the isolated rat liver: Effect of glucagon. Amer. J. Physiol. *205*:667, 1963.

[5] Berthet, J.: Some aspects of the glucagon problem. Amer. J. Med. *26*:703, 1959.

[6] Bessman, S. P., and Anderson, M.: Estimation of citric acid and ketone bodies by the salicylaldehyde-acetone reaction. Fed. Proc. *16*:154, 1957.

[7] Bogoch, A., Dufy, M. H., and McIntosh, H. W.: The hyperglycemic effect of 24-hours intravenous administration of glucagon in diabetes mellitus. Canad. Med. Ass. J. *78*:583, 1958.

[8] Bondy, P. K., and Cardillo, L. R.: The effect of glucagon on carbohydrate metabolism in normal human beings. J. Clin. Invest. *34*:494, 1956.

[9] Brauer, R. W., Leong, G. F., and Pessotti, R. L.: Vasomotor activity in the isolated perfused rat liver. Amer. J. Physiol. *174*:304, 1953.

[10] Bragdon, J.: The determination of blood lipids. J. Biol. Chem. *190*:513, 1951.

[11] Caren, R., and Corbo, L.: Pancreatic alpha cell function in relation to cholesterol metabolism. J. Clin. Endocr. *16*:507, 1956.

[12] Caren, R., and Corbo, L.: Glucagon and cholesterol metabolism. Metabolism *9*:938, 1960.

[13] Dole, V. P., and Meinertz, H.: Microdetermination of long chain fatty acids in plasma and tissues. J. Biol. Chem. *234*:2595, 1960.

[14] Dreiling, D. A., Bierman, E. L., DeBons, A. F., Elsbach, P., and Schwartz, I. L.: Effect of ACTH, hydrocortisone and glucagon on plasma non-esterified fatty acid concentration, NEFA in normal subjects and in patients with liver disease. Metabolism *11*:572, 1962.

[15] Eymer, K. P., Schwartz, K., and Weinges, K. F.: The behavior of esterified and non-esterified fatty acids in the serum under the influence of glucagon and insulin in man. Klin. Wschr. *39*:631, 1961.

[16] Foa, P. P., and Galansino, G.: Glucagon Chemistry and Function in Health and Disease. Springfield, Ill., Charles C Thomas, 1962.

[17] Foa, P. P., and Weinstein, H. R.: Metabolic effect of an insulin-free pancreatic extract. Amer. J. Physiol. *163*:711, 1950.

[18] Gidez, L. I., Roheim, P. S., and Eder, H. A.: Effect of plasma free fatty acid concentrations on triglyceride synthesis by perfused liver. Fed. Proc. *21*:289, 1962.

[19] Greene, E. C.: Anatomy of the Rat. New York, Hafner Publ. Co., 1959, pp. 227.

[20] Haft, D. E., and Miller, L. L.: Alloxan diabetes and demonstrated direct action of insulin on metabolism of isolated perfused rat liver. Amer. J. Physiol. *192*:33, 1958.

[21] Hagen, J. H.: Effect of glucagon on the metabolism of adipose tissue. J. Biol. Chem. *236*:1023, 1961.

[22] Haugaard, E. S., and Haugaard, N.: The hyperglycemic-glycogenolytic factor on fat metabolism of liver. J. Biol. Chem. *206*:641, 1954.

[23] Haugaard, E. S., and Stadie, W. C.: The effect of hyperglycemic-glycogenolytic factor and epinephrine on fatty acid synthesis. J. Biol. Chem. *200*:753, 1953.

[24] Heimberg, M., and Fizette, N. B.: The action of norepinephrine on the transport of fatty acids and triglycerides by the isolated perfused rat liver. Biochem. Pharmacol. *12*:392, 1963.

[25] Heimberg, M., Meng, H. C., and Bradley, D.: Effect of adrenocortical and hypophyseal hormones on uptake of neutral fat by isolated perfused liver. Amer. J. Physiol. *195*:678, 1958.

[26] Heimberg, M., Meng, H. C., and Park, C. R.: Effect of sex, fasting, and alloxan diabetes on the uptake of neutral fat by isolated perfused rat liver. Amer. J. Physiol. *195*:673, 1958.

[27] Heimberg, M., Weinstein, I., Klaussner, H., and Watkins, M. L.: Release and uptake of triglycerides by isolated perfused rat liver. Amer. J. Physiol. *202*:353, 1962.

[28] Helmer, O. M., Kirtley, W. R., and Ridolfo, A. S.: Clinical and metabolic changes induced by glucagon in patients with rheumatoid arthritis. J. Lab. Clin. Med. *50*:824, 1957.

[29] Hillyard, L. A., Cornelius, C. E., and Chaikoff, I. L.: Removal by the isolated rat liver of palmitate-1-C-14 bound albumin and of palmitate-1-C-14 and cholesterol-4-C-14 in chylomicrons from perfusion fluid. J. Biol. Chem. *234*:2240, 1959.

[30] Izzo, J. L.: Metabolic effects of continued administration of sulfonylurea derivatives in selected diabetic subjects: Interrelations with glucagon. Ann. N.Y. Acad. Sci. *74*:582, 1959.

[31] Izzo, J. L., and Glasser, S. R.: Comparative effects of glucagon, hydrocortisone and epinephrine on the protein metabolism of the fasting rat. Endocrinology *61*:189, 1961.

[32] Kalant, N.: Metabolic effects of the pancreatic hyperglycemic factor. Proc. Soc. Exp. Biol. Med. *86*:617, 1954.

[33] Kane, J. P., Longcope, C., Pavlatos, F. C., and Grodsky, G. M.: Studies on carbohydrate metabolism in idiopathic hypertriglyceridemia. Metabolism *14*:471, 1965.

[34] Kay, R., and Entenman, C.: The synthesis of "chylomicron-like" bodies and maintenance of normal blood sugar levels by the isolated, perfused rat liver. J. Biol. Chem. *236*: 1006, 1961.

[35] Leffler, H. H.: Estimation of cholesterol in serum. Amer. J. Clin. Path. *31*:310, 1959.

[36] Lipsett, M. B., Hengel, H. R., and Bergenstal, D. M.:

Effect of glucagon on plasma unesterified fatty acids and in nitrogen metabolism. J. Lab. Clin. Med. 56:342, 1960.

[37] Miller, L. L.: Some direct actions of insulin, glucagon and hydrocortisone on the isolated perfused rat liver. Rec. Progr. Hormone Res. 17:539, 1961.

[38] Morris, B., and French, J. E.: The uptake and metabolism of C-14 labelled chylomicron fat by the isolated perfused liver of the rat. Quart. J. Exp. Physiol. 43:180, 1958.

[39] Mortimore, G. E.: Effect of insulin on release of glucose and urea by isolated rat liver. Amer. J. Physiol. 204: 699, 1963.

[40] Natelson, S.: Microtechniques of Clinical Chemistry. Springfield, Ill., Charles C Thomas, 1961, p. 434.

[41] Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375, 1944.

[42] Nestel, P. J., and Steinberg, D.: Fate of palmitate and of linoleate perfused through the isolated rat liver at high concentrations. J. Lipid Res. 4:461, 1963.

[43] Orth, R. D., Odell, W. D., and Williams, R. H.: Some hormonal effects on the metabolism of acetate-1-C-14 by rat adipose tissue. Amer. J. Physiol. 198:640, 1960.

[44] Paloyan, E., and Harper, P. V., Jr.: Glucagon as a regulatory factor of plasma lipids. Metabolism 10:315, 1961.

[45] Penhos, J. C., and Krahl, M. E.: Stimulus of leucine incorporation into perfused liver protein by insulin. Amer. J. Physiol. 204:140, 1963.

[46] Penick, S. B., and Hindle, L. E.: The effect of glucagon, phenmetrazine and epinephrine on hunger, food intake and plasma non-esterified fatty acids. Amer. J. Clin. Nutr. 13:110, 1963.

[47] Rodbell, M., Scow, R. O., and Chernick, S. S.: Removal and metabolism of triglycerides by perfused liver. J. Biol. Chem. 239:385, 1964.

[48] Roheim, P. S., Haft, D. E., Gidez, L. I., White, A., and Eder, H. A.: Plasma lipoprotein metabolism in perfused rat livers. II. Transfer of free and esterified cholesterol into the plasma. J. Clin. Invest. 42:1277, 1963.

[49] Salter, J. M., Davidson, I. W. F., and Best, C. H.: The pathologic effects of large amounts of glucagon. Diabetes 6: 248, 1957.

[50] Salter, J. M., Ezrin, C., Laidlaw, J. C., and Gornall, A. G.: Metabolic effects of glucagon in human subjects. Metabolism 9:753, 1960.

[51] Sammols, E., Marri, G., and Marks, V.: Promotion of insulin secretion by glucagon. Lancet 2:415, 1965.

[52] Stewart, R. D., and Rottman, E.: Effect of pancreatic extracts on ketone body production of rat liver. Endocrinology 53:192, 1953.

[53] Vaughan, M., and Steinberg, D.: Effect of hormones on lipolysis and esterification of free fatty acids during incubation of adipose tissue in vitro. J. Lipid Res. 4:193, 1963.

Serum Magnesium Levels in the Newborn Child

(*Continued from page 739*)

Chem. 60:311, 1924; L. I. Gardner et al., *Pediatrics* 5: 228, 1950). It is not often appreciated that the average human infant is literally being subjected to a phosphorus load when offered a formula based on cows' milk. One needs only to calculate the phosphorus intake of a 70-kg. adult on a diet consisting of 100 ml. of cows' milk per kilogram body weight per day, to realize the magnitude of the load. It would be of interest to determine serum magnesium and calcium in adults under such circumstances.

The Titan yellow method is subject to certain limitations, but the same can be said for most methods for magnesium analysis. Anast is aware of these limitations, but satisfied himself that precision was sufficient for the purposes of this study.

From *Nutrition Reviews,* Vol. 23, No. 5, May 1965, pp. 133-34