trophoretic and chromatographic properties indistinguishable from Hb III. Finally, Hb "III" in the methotrexate-treated adult duck and Hb III in the embryo and duckling may be structurally identical. On the basis of current genetic concepts(12) the last possibility would imply that the folic acid antagonist has led to the recall of Hb III by derepressing the gene responsible for the synthesis of this protein.

Summary. Methotrexate-induced megaloblastic anemia led to altered proportions of Hbs I and II in the adult Peking duck and to the appearance of the 2 new hemoglobins which we have tentatively labeled Hbs "III" and "X." One of these, Hb "III," has electrophoretic and chromatographic properties indistinguishable from Hb III seen only in the embryo and duckling.

1. Cabannes, R., Serain, G., Compt. Rend. Soc. Biol., Paris, 1955, v149, 1193.

2. Saha, A., Biochem. et Biophys. Acta, 1959, v32, 258.

3. Borgese, T. A., Bertles, J. F., Science, 1965, v148, 509.

4. Warner, J. R., Rich. A., Hall, C. E., ibid., 1962, v138, 1399.

5. Huisman, T. H., Dozy, A. M., J. Chromatog., 1962, v7, 180.

6. Raymond, S., Weintraub, L., Science, 1959, v130, 711.

7. Kunkel, H. G., Wallenius, G., ibid., 1955, v122, 288.

8. Ebaugh, F. G., Jr., Fox, M. R. S., Blood, 1957, v12, 355.

9. Klein, J. R., Am. J. Physiol., 1959, v196, 656.

10. Beaven, G. H., Ellis, M. J., White, J. C., Brit. J. Haemat., 1960, v6, 201.

11. Heller, P., Yakulis, V. J., Epstein, R. B., Friedland, S., Blood, 1963, v21, 479.

12. Jacob, F., Monod, J., J. Mol. Biol., 1961, v3, 318.

Received May 21, 1965. P.S.E.B.M., 1965, v119.

Carbohydrate and Protein Metabolism in Rats During Chronic Administration of Glucagon and Epinephrine.* (30419)

SARA J. MACDONALD, KATHARINE O. DE BOER AND B. DE BOER
Department of Physiology and Pharmacology, School of Medicine, University of North Dakota, Grand Forks

Both epinephrine and glucagon are effective against insulin hypoglycemia and when given to the normal animal usually produce a temporary rise in blood sugar. However, it is thought that their hyperglycemic and glycogenolytic actions involve different carbohydrate reserves. In the normal untreated animal, glucagon, which is secreted into the portal vein, acts almost exclusively on hepatic glycogen, whereas epinephrine, which is diluted and widely distributed by the blood, acts principally on muscle glycogen stores(1). Since glucagon reduces plasma amino acid concentration(2) and increases nitrogen excretion(3), it is almost certain that gluconeogenesis from protein also occurs, either as a direct effect or as a result of liver glycogen depletion(4). This may account in part, for the adverse effect of glucagon administration on growth(5).

Although there have been many studies of metabolic effects of epinephrine and glucagon, we have not seen data comparing the effects of these hormones on both carbohydrate and protein metabolism in the same animal. The purpose of the present experiments was to study changes in liver and muscle glycogen, blood glucose, and plasma amino acids in fed and fasted rats during chronic administration of epinephrine and/or glucagon. Changes in body weight were also recorded.

Materials and methods. Adult male Sprague-

* This investigation was supported in part by a grant from Eli Lilly and Co. The Department was also the recipient of a supply grant from NIH in conjunction with a NIH fellowship for Miss MacDonald, primary author.

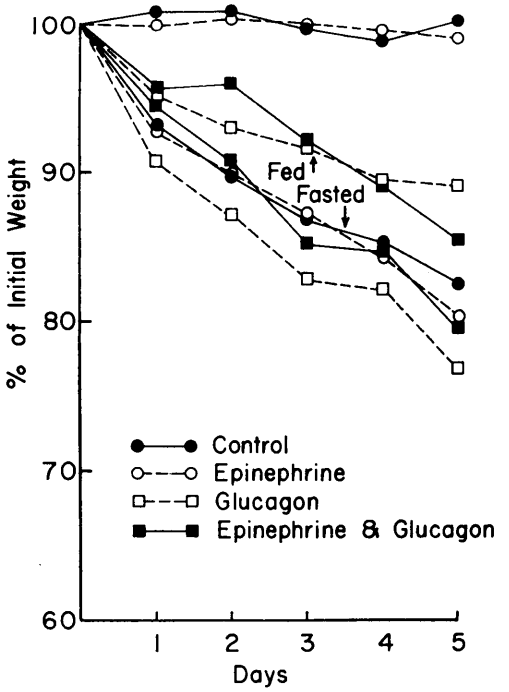

FIG. 1. Changes in body weight of fed and fasted rats during chronic administration twice daily of peanut oil 0.1 ml (control) or peanut oil containing epinephrine 0.1 mg, glucagon 0.93 mg or both epinephrine and glucagon.

Dawley rats were fed (food and water *ad libitum*) or fasted (water only) 1-5 days under otherwise identical conditions. Each animal received twice daily a subcutaneous injection of 0.1 ml peanut oil (control) or 0.1 ml peanut oil containing epinephrine 0.1 mg, glucagon 0.93 mg or both epinephrine and glucagon. Animals were sacrificed at 24-hour intervals, 12 hours after the last injection.

Rats to be killed were administered pentobarbital 60 mg/kg. Approximately 20 minutes later, the abdominal cavity was entered and liver for glycogen determination removed within 30 seconds and placed in KOH. Muscle tissue was obtained immediately thereafter and treated in the same manner. The method of Good, Kramer and Somogyi (6) was used for both liver and muscle glycogen. Heparinized blood was used for glucose and amino acid determinations. The Somogyi-Nelson(7) colorimetric technique was used for blood sugar. The method of Frame, Russell and Wilhelmi(8) was used for blood amino acid determinations.

Results. Although data were collected at 24-hour intervals for 5 days, the major changes, except for body weight, occurred within the first 24 hours. The data from glycogen, glucose, and amino acid determinations for days 1-5 have been pooled.

Changes in body weight based upon initial readings for the same animals are shown in Fig. 1 for the 5-day experimental period. Glucagon with or without epinephrine significantly decreased body weight in fed animals. Epinephrine alone did not alter body weight. Fasted animals of all groups sustained a body weight loss of approximately 20% in 5 days. The glycogen group lost more weight than other fasted animals, but the difference was not statistically significant.

The results of glycogen, glucose, and amino acid determinations are summarized in Table I. The glycogen content of the liver in fasted controls was about one-third that of fed controls. Liver glycogen was reduced by hormone administration in both fed and fasted animals, but the reduction was not statistically significant in the fasted group.

Muscle glycogen levels were lowered by fasting but were not significantly changed by hormone administration in either fed or fasted animals. Plasma amino acid levels were the same in fed and fasted controls. Hormone administration lowered these levels in both fed and fasted animals, glucagon alone or with epinephrine being more effective than epinephrine alone.

Blood glucose levels were lower in fasted than in fed animals. Neither hormone produced significant differences in blood glucose. The absence of hyperglycemia may be explained by the fact that the samples were taken 12 hours after the last hormone injection. Peak hyperglycemia usually occurs within 2 hours after glucagon or epinephrine injection(5,9,10).

Discussion. The use of pentobarbital anesthesia to expedite the taking of samples did not appear to alter carbohydrate parameters. The control levels of liver glycogen in both fed and fasted animals were intermediate to those reported by other workers(9,10,11,12). Blood glucose values were in the normal range.

TABLE I. Liver and Muscle Glycogen, Blood Glucose, and Plasma Amino Acid Levels in Fed and Fasted Rats Following Chronic Administration of Glucagon and/or Epinephrine.

	Liver glycogen, g/100 liver			Muscle glycogen, g/100 muscle		Plasma amino acids, mg/100 ml blood			Blood glucose, mg/100 ml blood		
	Mean*	S.D.	P	Mean	S.D.	Mean	S.D.	P	Mean	S.D.	P
Fed series:											
Control	3.96	± 1.01		.46	± .08	8.31	± 1.53		129.0	± 13.9	
Glucagon	1.83	± 1.14	<.01	.48	± .08	6.86	± 1.52	>.05	122.0	± 20.1	<.4
Epinephrine	2.56	± 1.47	<.05	.46	± .08	7.78	± 1.02	>.4	134.0	± 20.3	>.5
Glucagon and epinephrine	2.57	± 2.12	<.10	.42	± .16	6.13	± .97	<.01	120.0	± 14.9	>.2
Fasted series:											
Control	1.11	± 1.07		.37	± .09	8.28	± .84		113.0	± 27.8	
Glucagon	.74	± .17	<.5	.36	± .14	6.83	± 1.22	<.01	108.0	± 18.6	>.5
Epinephrine	.20	± .18	>.1	.31	± .06	7.34	± .69	>.02	100.0	± 7.1	>.2
Glucagon and epinephrine	.51	± .13	<.3	.30	± .05	6.61	± 1.07	<.01	100.0	± 15.4	>.2

* Values based on 7-10 animals. S.D. = Standard deviation from mean. P = Probability compared with control for that group.

Fed animals were provided with food and water *ad libitum*; fasted rats, water only. Control animals received 0.1 ml peanut oil; experimental rats, 0.93 mg glucagon and/or 0.1 mg epinephrine suspended in 0.1 ml peanut oil administered subcutaneously twice daily for 1-5 days.

In our experiments, body weight loss by fed animals as a result of glucagon administration was in agreement with findings of other workers, who have reported that glucagon-treated animals lost more weight than pair fed controls(5,10). That suppression of appetite and decreased food intake is not the full explanation of weight loss was supported by the observation that liver and muscle glycogen and blood glucose levels were not reduced to the levels of fasting controls. For example, the mean liver glycogen level of glucagon-epinephrine animals, which had lost an average of 10% of body weight, was the same as for the epinephrine group, which lost no weight and more than twice that of fasted controls. If weight loss in glucagon animals were a starvation effect, these parameters should have approached fasting levels (Table I).

Epinephrine and glucagon produce acute changes in carbohydrate parameters which differ from the results with chronic administration. In this connection Costa *et al*(11) reported that 2 hours after an injection of glucagon, both fed and fasted rats showed glycogen levels below controls in liver and above controls in muscle, whereas 2 hours after epinephrine administration glycogen levels were higher than controls in liver and lower than controls in muscle. With both hormones peak hyperglycemia occurs within 2 hours(5,9,10).

With chronic administration, on the other hand, the effect of these hormones on carbohydrate parameters appears to be modified by other factors. Stimulation of insulin secretion is no doubt one of these factors(1, 11). In fed animals liver glycogen levels have been reported to be higher than controls with chronic administration of glucagon and lower with epinephrine, the reverse of the acute effect, with no alteration or an occasional increase in muscle glycogen stores(9,10,11,12).

Our fed animals did not show increased levels of liver glycogen with chronic administration of glucagon. Instead all hormone injected groups had lower-than-control levels of liver glycogen in both fed and fasted animals. Costa *et al*(11) reported that the decrease in liver glycogen following glucagon administration persisted for 12 hours in fasted animals, with a subsequent increase to higher-than-control levels at 24 hours. Our results suggest that continued glucagon administration prevents this rebound effect. Although muscle glycogen and blood glucose levels were lower in fasted animals hormone administration did not alter these parameters significantly in our animals.

Plasma amino acids, though unchanged by fasting, were decreased by hormones, glucagon being more effective than epinephrine. Because liver glycogen levels were lower, it is not possible to ascertain whether this was a direct hormonal effect or the result of gluconeogenesis to replace liver glycogen stores. It seems unlikely that the latter is the case, in view of the fact that lowering of liver glycogen by fasting was not accompanied by a decrease in plasma amino acids.

It has been suggested by Foa and Galansino(1) that the main effect of glucagon is the transfer of glucose from the liver to the periphery, whereas the net effect of epinephrine is the reverse. Although this may be true in the untreated animal and appears to occur shortly after glucagon and epinephrine administration(11), it does not appear to be the principal effect of chronic administration. In our experiments both hormones depleted liver glycogen stores without significant increase in muscle glycogen or blood glucose. The hormones did not exhibit synergism or antagonism. Carbohydrate parameters were altered more by fasting than by hormone administration. Blood amino acid levels were, however, unchanged with fasting but lowered by glucagon and epinephrine.

Summary. Changes in body weight and in metabolic parameters were studied during chronic (twice daily) administration of glucagon 0.93 mg and/or epinephrine 0.1 mg to adult male rats. During the 5-day experimental period, fed control and epinephrine-treated animals lost no weight. Glucagon and glucagon-epinephrine animals lost 10-15% of their initial body weight. Fasted animals, control and hormone injected, lost approximately 20% of their initial body weight during the 5-day period. The weight loss by hormone-treated animals was not significantly greater than for controls. Liver glycogen was lowered by fasting and hormone administration. Muscle glycogen and blood glucose levels were lowered by fasting only. Plasma amino acid levels were not altered by fasting but were lower in hormone-treated animals. Changes in carbohydrate parameters usually associated with acute administration of glucagon and epinephrine, *i.e.*, hyperglycemia and glycogen shift from/to liver, to/from muscle, were not observed in adult rats during chronic administration of these hormones.

1. Foa, P. P., Galansino, G., Glucagon: Chemistry and Function in Health and Disease, Charles C Thomas, Springfield, Ill., 1962, p126.

2. Bocek, R. M., Peterson, R. D., Beatty, C. H., Fed. Proc., 1960, v19, 149.

3. Kalant, N., Proc. Soc. Exp. Biol. and Med., 1954, v86, 617.

4. Davidson, I. W. F., Salter, J. M., Best, C. H., Am. J. Clin. Nutrition, 1960, v8, 540.

5. Salter, J. M., Davidson, I. W. F., Best, C. H., Diabetes, 1957, v6, 248.

6. Good, C. A., Kramer, H. Somogyi, M., J. Biol. Chem., 1933, v100, 485.

7. Somogyi, M., ibid., 1945, v160, 69.

8. Frame, E. G. Russell, J. A., Wilhelmi, A. E., ibid., 1943, v149, 255.

9. Root, M. A., Endocrinology, 1956, v59, 340.

10. Salter, J. M., Am. J. Clin. Nutrition, 1960, v8, 535.

11. Costa, E., Galansino, G., Foa, P. P., Proc. Soc. Exp. Biol. and Med., 1956, v91, 308.

12. Root, M. A., ibid., 1954, v87, 108.

Received May 24, 1965. P.S.E.B.M., 1965, v119.

Luteotropic Response in Pituitary-Ovarian Isografts in Male Mice.* (30420)

HENRY C. BROWNING AND WILMA D. WHITE
Department of Anatomy, University of Texas, Dental Branch, Houston, Texas

Intraocular ovarian isografts in intact male mice develop mature follicles but show only sporadic luteinization. Administration of a single dose of bovine LH gives a quantitative luteal response(1,2). Similar grafts in ovariectomized females show cyclic formation of mature and luteinized follicles(3). The cor-

* Supported by USPHS Research Grant CA-02880.