hereditary obese hyperglycemic syndrome and in littermate controls. Only 12% of the excess weight of obese animals was found to be represented by water. Blood volume was not increased in obese animals. The total body water and percentage of weight represented by water in young obese animals was similar to those in non-obese animals of the same weight.

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Tissue Glycogen Storage as Affected by Acetoacetate. (20207)

HERBERT C. TIDWELL, MARY E. NAGLER, AND CAROLYN DUNKELBERG.

From the Department of Biochemistry, Southwestern Medical School of the University of Texas, Dallas.

After extensive studies on the effect of ketone bodies on carbohydrate metabolism, Nath and collaborators(1,2) believe that the gradual accumulation in the system of the intermediary fat metabolites, the ketone bodies, is responsible for the onset of hypoglycemia and for the gradual development of a decreased sugar tolerance in the experimental animal. These substances were reported(3) to cause an inactivation of endogenous insulin both *in vitro* as well as *in vivo*. More recently a rapid and continuing depletion of muscle and liver glycogen was found after daily injections of β-hydroxy-butyric acid had been given over a prolonged period(4). Also acetoacetate *in vitro* markedly accelerated glycogenolysis in normal rat liver slices(5). A disturbed phosphorylation was suggested as a reason for the decreased glycogen storage in these tissues. The last two studies were cited as evidence against the suggestion of Tidwell and coworkers(6,7) that the hypoglycemia after large injections of the ketone bodies might be the result of an increased glycogenesis promoted by the preferential utilization of the ketone bodies(8).

The availability of the tissues after the prolonged daily injections of acetoacetate(7) prompted us to determine the glycogen content of these muscle and liver tissues. When the glycogen values were found to be quite normal, it seemed desirable to investigate further the proposed effect of the ketone bodies on insulin action and on glycogenolysis in liver tissue, both *in vitro*. No evidence was obtained to support the contention that acetoacetate causes an inactivation of insulin or promotes glycogenolysis in the muscle or liver tissue.

Experimental. The female albino rats used in the first part of this study were those from a previous investigation(7). They had received daily intraperitoneal injections of 100 mg of acetoacetate, or an equivalent amount of propionate, per kilo of body weight the first week and these amounts were increased by 20 mg per kilo per week for 21 weeks. Similar rats which had received only the commercial stock diet for the same period were used as additional controls. At the end of this period, the rats were sacrificed by decapitation, and pieces of their livers and leg muscles were promptly removed for glycogen determinations(9). The sugar was measured after hydrolysis of the glycogen by the method of Nelson(10).

Surviving hemidiaphragms from normal unfasted rats were used to determine the effect of acetoacetate upon insulin action. The procedure of Villee and Hastings(11) was fol-

lowed with a few minor changes. The hemidiaphragms were portioned out for incubation in 4 buffer mixtures which had been prepared as follows: a) buffer mixture(12), b) buffer mixture containing 50 mg % of acetoacetate, c) the (b) mixture with 0.05 unit per ml of insulin, and d) buffer with same amount of insulin but no acetoacetate. All the buffer mixtures contained 200 mg % of glucose and were adjusted to a pH of 7.0. After incubation for 2 hours, the glycogen content of the tissues and the sugar loss from the incubating media were determined. Surviving rat *liver slices were incubated* in Krebs-Ringer-phosphate buffer solutions(13), containing either 0, 50 or 125 mg % of acetoacetate, to determine the effect of this ketone body on the glycogen of the tissue. Part of this test was repeated with the buffer used by Stadie and Zapp(12) because it contained glucose. In both cases, the liver slices were prepared according to the method of Deutsch(14). Approximately 175 mg of the tissue were incubated for 1 hour under the same conditions as were the hemidiaphragms. Again, the glycogen of the tissues and the sugar content of the media at the start and the end of this period were determined. A part of an earlier study(7) was repeated with the *following modifications*. Equivalent amounts of isotonic saline or a similar volume of isotonic glucose replaced the acetoacetate and propionate. They were injected daily in gradually increasing amounts over a period of 7 weeks. Fasting blood sugar levels were determined each week and glucose tolerances at the beginning and end of the study. Apparent *differences were tested* for by the t method of Fisher(15), and only those with a P value less than 0.01 (usually a t value of 3 or more) were considered significant.

Results and discussion. Similar amounts of glycogen were found in the livers of the rats on the stock diet and those receiving the special diets(7) along with prolonged injections of propionate or acetoacetate (Table I). This was also true of the leg muscle of these 3 groups of animals. In a previous report(7), they were shown to have had similar food intakes and growth curves with only slight alterations in their utilization of carbohydrate during the 21 weeks of the study. These results are not in agreement with those of Nath and Chakrabarti(4), who reported that smaller amounts of the ketone body injected over a shorter period caused a rapid and sustained depletion of liver and muscle glycogen.

TABLE I. Muscle and Liver Glycogen after Prolonged Propionate and Acetoacetate Injections, in 3 groups of about 10 rats each.

Body wt. g	Treatment	Glycogen, mg/g Liver	Muscle
244	S*	43.7 ± 6.0†	5.45 ± .57
249	Sp + propionate	48.3 ± 3.0	5.50 ± .21
255	Sp + acetoacetate	42.9 ± 4.4	5.42 ± .17

* S = stock diet; Sp = special diet.
† Stand. errors of the mean are included.

The ability of small amounts of insulin to promote glycogenesis in surviving rat diaphragm tissue(16) was used to test the effect of acetoacetate on insulin action (Table II).

The amount of glucose lost from the mixture and the glycogen of the tissue after incubation were unchanged by the presence of 50 mg % of acetoacetate. The addition of 0.05 unit per ml of insulin to these mixtures increased glycogen storage and glucose disappearance rate in both of them. Again, the glucose and glycogen changes in the 2 media were the same and there was no demonstrable effect of the ketone body upon the insulin action. The slightly, but not significantly, higher average value for muscle glycogen in the presence of acetoacetate suggests that more insulin would have sufficiently increased the rate of glucose oxidation to allow the depressing effect of the ketone body to be observed (17). In any case, there was no indication of any hindrance of the glycogenic action of the insulin of the tissue or of that added to the medium.

A similar but rapid hydrolysis of the liver glycogen (65 to 70% in 1 hour) occurred in the buffer media containing either 200 mg % or no glucose (Table III). Also, the rate of glycogenolysis was not affected by the presence of 50 or 125 mg % of acetoacetate in the absence of glucose, but it was markedly decreased by the latter amount of acetoacetate when the sugar was present. These findings were substantiated by the relative increases in the glucose of the incubation media. We are

TABLE II. Effect of Acetoacetate upon Insulin Action as Measured by Glycogen Formation. 4 groups of rats, 10-13 rats in each.

Tissue wt, mg	Buffer containing	Glucose loss from media, mg/g/hr	Tissue glycogen content, mg/g	"t" value†
151.9	Glucose	2.04 ± .15*	2.67 ± .11	
156.7	Glucose HAcAc	1.87 ± .18	2.60 ± .16	.74
156.8	Glucose insulin HAcAc	3.17 ± .17	4.29 ± .22	6.93
153.2	Glucose insulin	3.10 ± .16	3.89 ± .18	5.78

* Stand. errors of the mean are included.
† Usually a "t" value of 3 or greater indicates that difference from control value is statistically significant.

TABLE III. Liver Glycogen as Affected by Acetoacetate.

No. tests	Buffer mixture	Acetoacetate content, mg %	Increase in glucose of media, mg/g	Tissue glycogen content, mg/g	Glycogen hydrolyzed, %	"t" value
5	K*	0	23.5 ±1.0†	13.4 ± .1	65.7 ± .3	
6	K	50	25.4 ± .8	11.8 ± .5	69.8 ± 1.2	1.65
7	K	125	24.7 ± .7	13.0 ± .7	66.7 ± 1.5	.65
10	S	0	19.8 ± .8	18.8 ± .9	64.4 ± 1.7	
10	S	125	12.5 ± .4	26.0 ±1.3	50.8 ± 2.4	4.59

* K = Krebs-Ringer phosphate; S = Stadie-Zapp.
† Stand. errors of the mean are included.

not able to account for our failure to confirm the findings of Nath and Chakrabarti, who reported the amount of glycogenolysis, under conditions similar to those of the first part of Table III, to be accelerated 44 and 70% with 2 and 5 mg of acetoacetate respectively.

Similar fasting blood sugar levels and glucose tolerances were obtained after prolonged injections of isotonic saline solutions, containing sodium equivalent to that of the previously injected acetoacetate and propionate(7) or of equal volumes of isotonic glucose. As with the propionate and acetoacetate, the fasting blood sugar levels were lowered and a slightly reduced glucose tolerance was observed at the end of the injection period. Hence, these results do not support our suggestion(7) that the injected sodium might have some influence in lowering the blood sugar levels of these rats. There still remains a difference in the availability of the sodium from sodium chloride and that from the salt of an oxidizable organic acid.

An inactivation of insulin or interference in the phosphorylation process(2) by the ketone bodies is not shown by the effect of their prolonged injection upon the fasting blood sugar levels, the glucose tolerance or the glycogen content of the liver and muscle tissues. Neither does the presence of acetoacetate appear to inhibit the glycogen formation promoted by traces of insulin nor to increase glycogenolysis in liver tissue. It even seemed to decrease this process in the latter case when glucose was available to the tissue. Furthermore, the gradual onset of a hypoglycemia and a slightly reduced glucose tolerance follows not only the prolonged injection of increasing amounts of acetoacetate, but also those of propionate, sodium chloride and glucose. Hence it appears that one must conclude that any effect of the ketone bodies on carbohydrate metabolism is not likely to be the result of their inactivation of insulin.

After completion of this paper, Chari and Wertheimer(18) confirmed our results with similar concentrations of glucose, but found an increased effect of acetoacetate with decreasing amounts of glucose. Unlike their results with the diaphragm, the ketone body was found to have no effect upon the glycogen of liver slices when glucose was not added, and to aid in maintaining the liver glycogen when the sugar was present.

Summary. 1. Glycogen contents of rat liver and leg muscle were unchanged after daily injection of acetoacetate and propionate in increasing amounts for 21 weeks. 2. Ability of very small amounts of insulin to promote glycogenesis in surviving hemidiaphragms was

not inhibited by 50 mg % of acetoacetate. 3. A rapid hydrolysis of the glycogen of liver slices was unaffected by 125 mg % of acetoacetate in the buffer in the absence of glucose, but glycogenolysis was markedly decreased if both were present. 4. Acetoacetate in these concentrations does not appear to inactivate insulin, to inhibit glycogen storage in the muscle or liver tissue, or to affect appreciably the utilization of carbohydrate as measured by the glucose tolerance.

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Epinephrine Hypertensive Effects Before and After Cocaine. (20208)

ROBERT V. BROWN AND GALE C. BOXILL.* (Introduced by J. P. Quigley.)
From the Division of Pharmacology, University of Tennessee, Memphis.

Numerous reports have indicated that cocaine increases the pressor potency of epinephrine(1-11). The questionable experimental methods employed leave this claim unsettled. The following work investigates the situation in the dog.

Procedures. Mongrel dogs were anesthetized by the intravenous injection of 30 mg of pentobarbital sodium/kg of body weight. This agent was chosen because the control blood pressure remains within physiological limits during anesthesia. Atropine sulfate, 1.0 mg/kg, was given intravenously; when necessary, additional atropine sulfate, 0.2 mg/kg/dose, was given until tetanic stimulation of the distal end of the cut left vagus failed to alter blood pressure. Blood pressure was recorded by a mercury manometer from the left common carotid artery. After securing a control record, the first dose of epinephrine was injected into the right external jugular vein. The next dose of epinephrine was injected only after the blood pressure had stabilized at approximately the preinjection level; all doses were rapidly injected, *i.e.*, within 15 seconds. Each of the 10 dogs in series 1 received repeated identical doses of epinephrine;† among the dogs, the range covered was 0.125-12.5 μg/kg. The results provide estimates of the variability of responses to such repeated doses. In series 2 each dog received the progressively increasing

* Present address: College of Pharmacy, University of Georgia, Athens.

† Eastman Kodak Co., epinephrine, containing some arterenol.