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Effect on Cardiac Glycogen of Intravenously Administered Sodium Acetoacetate, Sodium Beta-Hydroxybutyrate and Sodium Butyrate.

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Following pancreatectomy[1,2] or in alloxan diabetes,[3] the glycogen content of the liver and skeletal muscles is rapidly depleted, but the glycogen content of the heart is increased. In some recent experiments,[4] we have shown that in rats the amount of glycogen stored by the myocardium parallels changes in the blood ketone level induced by dietary means. In general, the glycogen stores of the liver and skeletal muscles tend to vary inversely with those of the heart. These observations suggested that ketosis may be a causative factor in increasing the deposition of cardiac glycogen, though decreased insulin availability may be important. With the idea of minimizing changes in insulin activity, experiments of shorter duration were carried out.

Method. Two groups of experiments were done using adult male white rats of the Sprague-Dawley strain. The animals of the first group (Table I) were fasted for 24 hours; those of the second group (Table II) were unfasted. Each group was divided into several series, one serving as a control series, the others being subjected to intravenous infusion of 0.9% sodium chloride, 3% sodium chloride, 10% sodium butyrate, 10% dl-betahydroxybutyrate or 10% sodium acetoacetate solution. The solutions were adjusted to a pH of 7.2; the volume infused varied from 5 to 7½ ml; the duration of the infusion was 4 hours; and the time of sacrifice was one hour after termination of the infusion. The animals were anaesthetized with sodium pentobarbital and the trachea was cannulated routinely except in the uninfused control series of the first group, which was anaesthetized and sacrificed at the time the infusions were begun in the other series of the group.

Since the temperature regulating mechanism in the rat was greatly affected by the anesthetic, heat was applied in an attempt to keep the temperature of each individual animal near the normal level. At a room temperature below 30 to 31°C, supplementary heat from lamps was generally necessary.

At the time of the sacrifice, blood samples were obtained for glucose and ketone body determinations and samples of heart, liver and skeletal muscle were removed for glycogen determination. The various analyses were carried out by methods described earlier.[3]

Results and Discussion. Table I shows that in the fasted series receiving infusions of sodium *dl*-β-hydroxybutyrate and sodium butyrate, the cardiac glycogen stores were 654 ± 27 and 653 ± 34 mg % respectively, significantly above the values of 502 ± 26 and 510 ± 16 mg % found in the fasted series receiving respectively no treatment or an infusion of 0.9% sodium chloride solution.

The glycogen stores in the skeletal muscle were essentially the same in the experimental and the control series. The liver glycogen levels in the series receiving saline (532 ± 63 mg %) were significantly higher than in the untreated series (183 ± 32 mg %) or the series receiving sodium *dl*-β-hydroxybutyrate (112 ± 48 mg %). The very great standard error for values obtained for liver glycogen after sodium butyrate infusion makes it impossible to attach any significance to the data on liver glycogen in this series.

The only remarkable finding in blood ketone values was the low level of ketones in the series receiving the saline solution. In

1 Cruickshank, E. W. H., *J. Physiol.*, 1913, **47**, 1.

2 Fisher, N. F., and Lackey, R. W., *Am. J. Physiol.*, 1925, **72**, 43.

3 Lackey, Robert W., Bunde, Carl A., Gill, A. J., and Harris, Leroy C., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 191.

4 Lackey, Robert W., Bunde, Carl A., and Harris, Leroy C., *Am. J. Physiol.*, 1946, **145**, 470.

TABLE I.
Tissue Glycogen, Blood Ketones, and Blood Sugar in 24-Hour Fasted Rats Given Sodium *dl*-β-Hydroxybutyrate and Sodium Butyrate by Intravenous Infusion.

Solution infused	No. of animals	Glycogen* mg per 100 g tissue			Blood ketones mg per 100 ml‡	Blood sugar mg per 100 ml
		Liver	Heart	Skeletal muscle		
None	17	183 ± 32†	502 ± 26†	396 ± 28†	17.6	115
Isotonic saline	12	532 ± 63	510 ± 16	385 ± 22	4.6	85
Sodium β-hydroxybutyrate	9	112 ± 48	654 ± 27	421 ± 15	54	111
Sodium butyrate	13	464 ± 264	653 ± 34	362 ± 54	15.8	139

* As glucose.
† Standard error.
‡ As hydroxybutyric acid.

TABLE II.
Tissue Glycogen, Blood Ketones, and Blood Sugar in Non-Fasted Rats Given Sodium *dl*-β-Hydroxybutyrate and Sodium Acetoacetate by Intravenous Infusion.

Nature of treatment or solution infused	No. of animals	Glycogen* mg per 100 g tissue			Blood ketones mg per 100 ml‡	Blood sugar mg per 100 ml
		Liver	Heart	Skeletal muscle		
Untreated	10	1660 ± 200†	451 ± 34†	434 ± 15†	3.2	129
Dummy operation	16	2368 ± 276	510 ± 17	504 ± 24	3.4	116
Isotonic saline	11	3381 ± 309	615 ± 27	604 ± 16	7.6	134
Hypertonic saline	11	2537 ± 330	627 ± 27	551 ± 20	8.5	127
Sodium β-hydroxybutyrate	12	807 ± 251	681 ± 42	566 ± 37	22	136
Sodium acetoacetate	11	1051 ± 254	724 ± 45	614 ± 52	17.3	159

* As glucose.
† Standard error.
‡ As hydroxybutyric acid.

this the blood sugar level was also lower. Increases in the blood sugar level followed administration of sodium butyrate but not administration of β-hydroxybutyrate,

The rather large increase in cardiac glycogen in the series receiving sodium butyrate and sodium *dl*-β-hydroxybutyrate, without a concomitant increase in glycogen deposition in either the liver or the skeletal muscle, supports our previously stated concept of a relationship between ketonemia and increased glycogen in the myocardium. The increase in blood sugar level after the administration of sodium butyrate has been observed before[4,5] and may be related to the increased cardiac glycogen; but observations that the elevation of the blood sugar level was not great and that the muscle glycogen stores were not increased suggest that the underlying factors which selectively increased the cardiac glycogen in this case were the same as those in the ketosis of prolonged fasting, which is not accompanied by an increase in blood sugar level.[4] In the fasted series in which *dl*-β-hydroxybutyrate was given, the increased cardiac glycogen was not associated with an increase in blood sugar.

In the fasted series infused with saline solution there was no change in the skeletal muscle, or cardiac glycogen. The increase observed in the liver glycogen may have been attributable to the decrease in muscular activity incident to anesthesia or to the fact that in fasting rats the liver glycogen stores are depleted in the early stages of the fast, but tend to be replenished as the fast is prolonged.[6] The pronounced lowering of blood ketones and blood sugar in this series is difficult to explain, but may be related to the effect of sodium chloride on the insulin producing mechanism or the anti-insulin or anti-ketogenic mechanism, an action which

[5] Markees, Silvio, *Klin. Wchnschr.*, 1941, **20**, 1260.

[6] Bunde, Carl A., and Lackey, Robert W., unpublished data.

has been discussed by McQuarrie[7] and Lewis, McKee, and Longwell.[8]

Table II records data from unfasted animals. The cardiac glycogen levels of 681 ± 42 mg % and 724 ± 45 mg % found in the experimental series receiving sodium β-hydroxybutyrate and sodium acetoacetate, respectively, were the highest observed. These series had also the lowest liver glycogen stores (807 ± 251 and 1051 ± 254 mg % respectively) and the highest blood ketone levels; the muscle glycogen levels (566 ± 37 and 614 ± 52 mg %) differed little from those found in the control series receiving the hypertonic and the isotonic saline solution (551 ± 20 mg % and 604 ± 16 mg % respectively). Thus the same parallelism is observed between the occurrence of ketonemia and increased cardiac glycogen stores in this as in the fasted series of animals.

Quite unexpectedly, the non-fasted animals receiving isotonic or hypertonic sodium chloride solution showed higher values for cardiac glycogen, skeletal muscle glycogen, blood ketones and blood sugar than did animals subjected only to dummy operations. These effects were contrary to those observed after similar saline infusion in fasted animals. The meaning of this difference is not apparent to us. The increase in blood sugar observed in the series receiving sodium acetoacetate raises the same question of inter-relationship with cardiac glycogen storage as that discussed in connection with the fasted series receiving sodium butyrate.

No attempt was made in these studies to control the factor of alkalosis which must have followed our administration of the sodium salts of acids capable of being metabolized. Deuel and coworkers[9] have concluded that alkalosis is inimical to hepatic glycogenesis and this may explain the lowering of liver glycogen in certain of our series. Long and Evans[10] observed a lowered cardiac glycogen in rats with pronounced alkalosis, induced by giving them sodium bicarbonate. In our animals cardiac glycogen increased in spite of this adverse condition.

The data presented seem to give some additional support to the idea that there exists a parallelism between blood ketone levels and the deposition of glycogen in the myocardium. Since an artificially produced ketonemia may increase the glycogen stores in the heart within a few hours, either with or without a preliminary fast of short duration, the relationship between ketonemia and cardiac glycogen storage may well be a direct one rather than one indirectly attributable to the decreased insulin availability resulting from conditions giving rise to endogenous ketonemia.

Summary. In experiments carried out on adult male white rats under sodium pentobarbital anaesthesia, intravenous administration of ketone bodies resulted in an increase in the glycogen stores of the heart. Concomitant increase in the glycogen stores of the liver and skeletal muscle did not occur.

7 McQuarrie, Irvine, *Essays in Biology*, p. 413, University of California Press, Berkeley and Los Angeles, 1943.

8 Lewis, Robert C., Jr., McKee, Frances S., and Longwell, Bernard B., *J. Nutrition*, 1944, **27**, 11.

9 Deuel, Harry J., Jr., Butts, Joseph S., Blunden, Harry, Cutler, Charles H., and Knott, Leslie, *J. Biol. Chem.*, 1937, **117**, 119.

10 Long, C. N. H., and Evans, G. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 186.