groups of malate and succinate as would be expected if acetoacetate was being utilized only by the established path (acetoacetate → 2 "acetate" → 4 CO_2 via the tricarboxylic acid cycle). This is taken as corroborative evidence for our previous conclusion that ketone bodies *per se* are not responsible for the net increase in cardiac glycogen noted in ketonemia.

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Effect on Cardiac Glycogen of Intravenously Administered Sodium Acetoacetate-3-C^{14}. (22346)

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In dogs made diabetic by pancreatectomy the glycogen content of liver and skeletal muscle decreases while that of heart increases (1). A similar pattern has been observed in rats injected with large amounts of acetoacetate or B-hydroxybutyrate(2). It becomes a matter of some importance to establish whether the ketone bodies *per se* are being metabolized in a manner directly responsible for this net increase in cardiac glycogen or are merely acting in some secondary role. In the former instance a new and yet unrecognized mechanism would have to be operating, since the conventional metabolic path (acetoacetate → 2 "acetate" → $4CO_2$ via the tricarboxylic acid cycle) is not compatible with a net increase in any component. If ketone bodies on the other hand are acting only in some indirect manner, that role too must be defined. The data published by Lackey *et al.* (2) simply notes the quantitative increase of cardiac glycogen without throwing any light on possible mechanisms. The present paper is the first in a series designed to define this particular metabolic area. Here we have made the direct test of whether ketone bodies *per se* go to heart glycogen. We have injected into rats large amounts of acetoacetate-3-C^{14} and subsequently isolated the cardiac glycogen to determine the quantity and distribution of C^{14} in the glucose molecule. By this means strong inferences concerning the metabolic path can be obtained.

Materials and methods. Sodium acetoacetate-3-C^{14} was synthesized according to established methods(3) and put into a solution at pH 7.4 such that per ml there was 0.88 mM (11%) of acetoacetate containing an amount of C^{14} giving 600,000 cts/min. The quantity of isotopic material available limited the study to 3 rats. The nature of the results is such that valid conclusions are clearly possible despite the small number of animals involved. A 200 g rat, warmed by a heat lamp, was anesthetized with nembutal, the jugular vein exposed, and the fine cannula of an intravenous drip apparatus introduced. In each of our 3 experiments the gravity flow ceased after about 2½ hours at which time 3 ml (2.6 mM and 1,800,000 cts/min.) of the ace-

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toacetate solution had been introduced. In one rat we used a hypodermic syringe to inject an additional 2 ml into the tail vein over a period of half an hour. In all instances a total of 4 hours elapsed before the animal was sacrificed. Following this, the heart was removed, washed with saline and dropped into hot 30% KOH together with 35 mg of purified glycogen made from rat liver. The carrier material was necessary in view of the fact that a single rat heart contains only about 2 mg of glycogen which is an inadequate amount for isolation, purification and degradation. The dilution does not interfere with evaluation of radioactivity since counting was done on the total sample which was highly purified (4), degraded to CO_2 by persulfate oxidation (5) and assayed as $BaC^{14}O_3$.

Results. According to Lackey *et al.*(2) the infusion of acetoacetate can cause as much as a 60% increase in heart carbohydrate. In the average rat this represents formation of approximately 1.5 mg of glycogen over and above that normally present. We would not expect our experiments to effect this much synthesis since we introduced only about 60% as much ketone as the original workers. However, assuming that we obtained only 10% as much synthesis and assuming that no more than one C^{14} from the acetoacetate was incorporated into each glucose of the glycogen molecule, we would have expected radioactivity in the order of 500 counts per minute. In none of our experiments did the glycogen molecule contain any traces of radioactivity. We are forced to conclude therefore, that the increase in heart glycogen accompanying ketonemia does not represent a direct conversion of ketone bodies to carbohydrate. The mechanism of this effect is still an open question.

Summary. The intravenous injection of large amounts of acetoacetate-3-C^{14} has been effected under conditions reported to cause a net increase in heart glycogen. Isolation and degradation of the cardiac glycogen failed to divulge any trace of radioactivity. It thus appears that the net increase in heart carbohydrate accompanying ketonemia represents some secondary and yet undetermined influence of ketone bodies.

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Feasibility and Safety of Frequent Plasmapheresis of the Same Human Donors.* (22347)

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Plasmapheresis is the obtaining of whole blood from a donor with the retention of the plasma; the red blood cells being returned to the donor. This has been accomplished in the past in both man and other animals by well known technics(1-5). These are laborious, time consuming, and have many other obvious drawbacks inherent in the methods which would prevent large scale use. The present study is concerned with plasmapheresis in man utilizing the ADL-Cohn Blood

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