The contamination with LH is only slightly higher than that of Van Dyke. As set forth in Step III the LH and TSH content can be lowered further. Repeated ammonium sulfate precipitation of Step III material by the method of Van Dyke *et al.*(1) does not improve the biological activity or reduce the contaminants. As is shown in Table I the contamination with other hormones is negligible.

Summary. 1. A simple method has been developed for the recovery of FSH from residues resulting from the acid acetone extraction of swine pituitary glands. 2. The biological activity of the preparations obtained compare favorably with those of other investigators.

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Total Body Water and Blood Volume in Hereditary Obese-Hyperglycemic Syndrome of Mice.* (20206)

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In the course of an investigation on factors influencing fatty acids and cholesterol turnover in mice with the hereditary obese hyperglycemic syndrome(1), it became necessary to determine total body water and blood volume in these animals as well as in non-obese littermates. Results of these determinations present sufficient interest to justify separate publication.

Material and method. Three types of animals were used: obese and non-obese mice (age 4 to 6 months) and "young" adult obese mice (age 2 to 3 months) chosen so that their body weights would overlap with those of the large non-obese mice. Obese animals are clearly recognizable by their characteristic shape after they reach a weight of 20 to 25 g. The range for the non-obese animals was 24.5 to 33.0 g; that for the obese 31.0 to 105.4 g.

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In determination of total body water, deuterium dilutions were measured with a Nier Consolidated Mass Spectrometer. Weighed amounts of heavy water from .377 to .696 g were injected intraperitoneally. Tail vein samples of blood were collected in oxalated glass capillaries, sealed and kept frozen until analysis. In a series conducted to determine optimal experimental conditions, blood samples were taken at 5-minute intervals for 30 minutes, then at 30-minute intervals for 2 hours. Heavy water levels in blood were found to increase steadily during the first half hour and to remain constant during the following one and one half hours, thus indicating that equilibrium had been reached. Table I gives a typical set of values. On the basis of these results, in the main experimental series, blood samples were taken at 30-minute intervals during the 2 hours following heavy water

TABLE I. Atoms % Excess Deuterium in Blood of Mice as a Function of Time after Heavy Water Injection.

Time (min.)	5	10	15	20	30	60	90	120
Atoms % excess	.03	.10	.16	.17	.18	.18	.19	.18

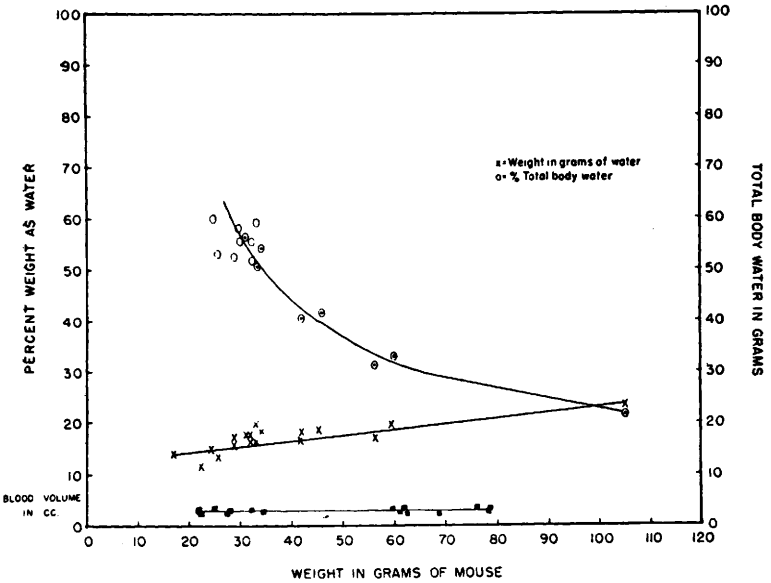

FIG. 1. Total body water, blood volume and % weight as water as a function of body weight.

injection and results averaged. Blood volume was determined by dilution of Evans blue(3).

Results and discussion. Fig. 1 gives the variation of total body water and percentage weight represented by water as a function of body weight. It is readily seen that:

1) The total body water increases only very slowly with increasing body weight. On the average, water represented only 12% of excess body weight in the older obese mice.

2) Consequently, the proportion of the weight corresponding to water decreases steadily with increasing body weight. For example, in two female littermates, one weighing 24.5 g (mouse No. 1) and the other 105.4 g (mouse No. 17), the percentage of the body weight represented by water was respectively 60% and 22%. Of the 80.9 g excess body weight carried by the obese sister only 8.9 represented water.

3) The young obese animals have a total body water and percentage body weight represented by water similar to that of the non-obese animals of the same weight. Values relating to these animals fall in the same curves as those relating to older non-obese and obese animals. Young obese animals are thus already distinguishable by their shape and by metabolic anomalies(1) from older obese at a weight which is still in the normal range and corresponds to a normal lean mass. At the same age, however, a few determinations on young non-obese animals (not included in this series) show, as expected, smaller weight, and higher weight represented by water (up to 75%), than do either obese mice of the same age or older non-obese mice.

Fat contents corresponding to the determined water content can be calculated by an empirical formula in a number of mammalian species(2,4). The extremes of percentage weight represented by fat, corresponding to mice No. 1 and No. 17 respectively (female littermates) are 18 and 70%. The median for the non-obese animals is 25%; that for the obese 45%.

Blood volume data are presented in Fig. 1. No correlation seems to exist between weight and blood volume in these animals. This result suggests that the small progressive increase in total body water, representing about 12% of the excess weight, represents the hydration of the excess adipose tissue. The lack of an increase in blood volume in obese animals may be related to the metabolic inertia of excess adipose tissue and to the absence of any increment in oxygen consumption in obese animals(5,6).

Summary. Total body water and blood volume were determined in mice with the

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)

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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-