Electrolyte and Water Composition of Muscle and Liver in Hereditary Obese-Hyperglycemic Syndrome of Mice.* (21981)

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(Introduced by A. Baird Hastings.)
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The hereditary obese-hyperglycemic syndrome of mice(1) has, among other characteristics, an insulin resistant hyperglycemia and glycosuria(2). In a small proportion of obese animals, the hyperglycemia is not present but can be immediately elicited by injection of a single dose of growth hormone to which obese animals are extremely sensitive (3). Non-obese animals show the usual resistance of rodents to the diabetogenic effect of growth hormone. The syndrome is transmitted as a Mendelian recessive trait which affects both sexes in a similar manner. A number of relationships have been recognized between potassium deficiency and carbohydrate metabolism. The abnormalities of glucose production and utilization observed in insulin deficient rat liver slices incubated at a potassium concentration optimal for glycogenesis were similar to those observed in normal slices incubated in a low potassium medium(4,5,6).‡ Although no abnormality of potassium concentration was demonstrated in the liver cells of intact insulin-deficient rats (8), hyperglycemia and depletion of liver glycogen have been reported in rats maintained on a potassium deficient diet(9). Fatty acid metabolism of rat liver slices was increased by a high potassium concentration in the incubation medium(10).

These observations suggested that tissue analyses for water, fat potassium, sodium and chloride of mice with the hereditary obese-hyperglycemic syndrome and the estimation of intracellular concentrations of potassium and sodium might be of interest.

Materials and methods. Six male and 3 female mice with the hereditary obese-hyperglycemic syndrome were used. Three male and 3 female litter-mates served as controls. After a 24-hr fast (water *ad lib.*) the animals were anesthetized by intraperitoneal injection of one per cent aqueous sodium amytal. A dose of 0.2 mg per gram gave satisfactory anesthesia in 15 minutes. One obese animal required 0.3 mg per g. Blood sugar levels (11, 12) were determined on 0.02 ml of blood obtained from the tail. Blood for measurement of hemoglobin concentration was obtained in a similar manner. Serum for chloride(13) and sodium(14) determinations was separated from blood which was aspirated from the right side of the heart and placed under oil. Samples of muscle from the thigh and calf were dissected as free from adipose tissue as possible. Liver samples were obtained using care to avoid the gall bladder. Both tissue samples were minced, dried, defatted and extracted with 0.75 N nitric acid(15). Duplicate samples were used to measure the blood content of the tissues(15). The chloride content of the extracts was determined by a modification of the colorimetric micro-method of Lowry and co-workers(13). The sodium and potassium contents were determined by flame photometry(14).

Results. In Tables I and II the data from obese and control animals have been compared, all values in the latter table being calculated on a blood-free-fat-free basis. The obese animals were significantly hyperglycemic when sampled in the fed state several days before being sacrificed. The serum chloride concentration was lower in the obese animals, but this would only partially account for the difference in liver chloride concentration observed. The dry fat-free weight of

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‡ The *in vivo* intracellular potassium concentration can be maintained when liver slices are incubated only by increasing the potassium concentration of the medium to a level far higher than that of extracellular fluid(7).

TABLE I. Mean Body Weight, Blood and Serum Values.

	No. used	Age (days)	Body wt (g)	Blood: Fasting sugar (mg %)	Blood: Normal sugar (mg %)	Serum: Na (mEq/l)	Serum: Cl (mEq/l)
Obese	9	133	62 ± 7	151 ± 33	218 ± 38	145.6 ± 1.9	101.0 ± 1.8
Control	6	137	28 ± 2	94 ± 17	106 ± 13	146.3 ± .8	106.0 ± .9
p value			<.01	>.1	<.05	>.1	<.05

Digits following the ± sign are stand. error of the mean.

muscle was higher per unit of wet fat-free tissue in the obese animals. The potassium content of muscle was significantly lower per unit of dry fat-free tissue in the obese mice.

In Table III a correction has been applied to the data for the increment of water injected with the sodium amytal used for anesthesia. Derived values have been calculated for the amount of extracellular water in muscle, assuming that all chloride was extracellular(15). This permitted calculation of the concentration of potassium and sodium in intracellular water. No significant differences in these derived data between the obese and control mice were observed. The column headed "$(H_2O)_E$ less adipose tissue" will be discussed later.

Derived values have been calculated for the amount of extracellular water in liver, assuming that all the sodium was extracellular(16). This permitted calculation of the concentration of potassium and chloride in intracellular water. Here, as in muscle, no significant differences between the two groups were observed.

Discussion. Correction of the data for the increment of water injected with sodium amytal was necessary before comparing obese with control mice (Table III) because this increment was proportionally greater in the obese animals. With one exception the water injected was proportional to body weight, but the obese group contained less water per unit of body weight than their controls. To apply such a correction the following relationship obtained on mice with the same abnormality by Mayer and Hagman was used(17):

Grams of Total Body Water = 12 + (Grams of Body Weight x 0.11). Data were also reported by these authors showing that 15 minutes after intraperitoneal injection of deuterium oxide, the volume of dilution for the isotope labeled water had reached 88% of its equilibrium volume. These findings permitted calculation of the approximate water content of cells and extracellular fluid prior to the injection of aqueous sodium amytal solution. Specifically, the original serum water and tissue water values were multiplied by the following factor:

$$\frac{\text{Total Body Water}}{\text{Total Body Water} + (\text{Injected Water} \times 0.88)}$$

Application of this factor to the data increased the difference between the two groups with re-

TABLE II. Mean Values for Muscle and Liver; Original Data. The values are reported per kilo of wet fat-free, blood-free tissue or of dry fat-free, blood-free tissue.

	Per kg wet fat-free tissue: Dry fat-free wt (kg)	Wt of fat (kg)	Cl (mEq)	Na (mEq)	K (mEq)	Per kg dry fat-free tissue: K (mEq)
Muscle:						
Obese	.244 ± .002	.202 ± .041	14.4 ± .7	23.9 ± 1.1	108.4 ± 1.5	445 ± 6
Control	.235 ± .002	.075 ± .011	15.7 ± .7	23.7 ± .9	111.3 ± .9	474 ± 6
p value	<.01	<.05	>.1	>.1	>.1	<.01
Liver:						
Obese	.267 ± .002	.145 ± .021	26.8 ± .9	35.0 ± 1.3	84.7 ± 1.4	318 ± 6
Control	.259 ± .006	.103 ± .019	31.6 ± 2.0	39.1 ± 2.6	85.5 ± 2.5	329 ± 6
p value	>.1	>.1	<.05	>.1	>.1	>.1

Digits following the ± sign are stand. error of the mean.

TABLE III. Mean Values for Muscle and Liver Corrected for Water Injected with Anesthetic. Values are reported per kilo of wet fat-free, blood-free tissue or of intracellular water.

	Per kg wet fat-free tissue					Per kg intracellular water		
	Dry fat-free wt (kg)	Wt of fat (kg)	$(H_2O)_E$ (kg)	$(H_2O)_E$ less adipose tissue (kg)	$(H_2O)_C$ (kg)	K_C (mEq)	Na_C (mEq)	Cl_C (mEq)
Muscle:								
Obese	.256 ± .002	.212 ± .043	.133 ± .008	.107 ± .007	.612 ± .008	183 ± 3	7.1 ± 1.1	
Control	.241 ± .002	.077 ± .011	.138 ± .006	.129 ± .006	.622 ± .006	182 ± 3	5.0 ± 1.1	
p value	<.01	>.01	>.1	<.05	>.1	>.1	>.1	
Liver:								
Obese	.279 ± .001	.152 ± .022	.228 ± .007		.493 ± .008	176 ± 3		5.3 ± 1.3
Control	.265 ± .007	.105 ± .019	.256 ± .018		.479 ± .012	179 ± 4		6.9 ± 1.3
p value	<.1	>.1	>.1		>.1	>.1		>.1

Digits following the ± sign are stand. error of the mean.
Symbols: $(H_2O)_E$ extracellular water; $(H_2O)_C$ intracellular water; K_C intracellular potassium; Na_C intracellular sodium; Cl_C intracellular chloride.

spect to the dry fat-free weight of both tissue (muscle "p value" <0.01, liver "p value" <0.1). The serum chloride difference recorded in Table I was halved when the correction was applied.

It was apparent on dissection and confirmed by data in Tables II and III that considerably more adipose tissue was mingled with striated muscle fibres in the obese than in the control muscle samples. Data have been collected by Lowry and Hastings showing that adipose tissue from rat omentum contained 83% fat and 10% extracellular water(18). Assuming similar findings for adipose tissue of the obese and control mice, values were derived for $(H_2O)_E$ of muscle fibres after deducting the fraction associated with adipose tissue in the muscle samples (Table III). This approximation of the "true" $(H_2O)_E$ for muscle fibres gave a significantly lower value in the obese mice. The potassium concentration per unit of dry fat-free muscle was 6% lower in the obese group (Table II). However, $(H_2O)_C$ per unit of dry fat-free muscle was 7% lower in the fat animals after correction for injected water. "$(H_2O)_E$ less Adipose Tissue" was 22% lower when expressed per unit of dry fat-free muscle. This dehydration occurred even though water was available throughout the fasting period.

Studies in man have shown that the hyperglycemia and glycosuria following hypertonic glucose infusion is associated with sodium diuresis and a lesser degree of potassium diuresis(19). One may presume that the same type of electrolyte losses occurred in the obese mice and that while in the fasting state, dehydration was necessary to preserve the osmolarity of body fluids.

Summary. 1. Samples of striated muscle and liver from fasted mice with the hereditary obese-hyperglycemic syndrome were analyzed for water, fat, potassium, sodium, and chloride. 2. Some degree of intracellular dehydration and a greater degree of extracellular dehydration was observed without change in concentration of intracellular potassium.

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Local Effects on the Feather Papilla of Thyroxine and of Progesterone.* (21982)

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Shedding and renewal of plumage occurs in birds in well-defined association with sexual and seasonal events. Normally, the standing lifeless feather is gradually expelled from the mouth of the follicle as a new feather arises following an activation which is initiated in the resting papilla, an organ that uninterruptedly occupies the internal well of the follicle and which is the actual site of the externally manifest cyclical events. Seasonal inertia of the papilla is a familiar phenomenon in laboratories utilizing experimentally the regenerating plumage. Forcible removal of the standing feather causes a tearing of the apical tissue of the papilla with haemorrhage into the lumen of the follicle(1). The wound stimulus is sufficient to assure immediate activation of the papilla in the capon whereas in the cock and hen, regeneration usually fails outside of the environs of molt. We may speak at such times of a "quiescent papilla." The papilla has long been shown to respond by growth to oral or parenteral administration of thyroid substance(2; and many others). The dosage requisite for this reaction was less as the season of normal molt approached(3), showing the gradual course of natural changes which culminate in that event. Recently it has been established that progesterone, when given as a single intramuscular injection of a preparation having slow absorption from local depots (Reposito Progesterone), also will cause a general activation of the feather papillae in adult fowl(4). There was no hint in these experiments that progesterone action was here in any sense *via* a stimulation of the bird's thyroid; this was checked in the feather structure where excess thyroid has long been known to produce specific, readily recognized consequences(5; and many others). The similar end effects seem accordingly independently due to either progesterone or to thyroxine although some common intermediate participation is not ruled out.

This possibility suggested following the consequences of separate local applications of either of the two substances to the papilla;

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