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Tissue Electrolytes in Alloxan-Diabetic Rats with Ketoacidosis.* (19443)

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Metabolic balance studies on diabetic human subjects and experimental animals have afforded abundant evidence that the development of ketonemic acidosis is attended with large urinary losses of water, electrolytes and nitrogen. The nature of the losses suggests that increased cellular catabolism in acidosis leads to a considerable depletion of intracellular materials without breakdown of whole tissues. It may be surmised that functional derangements which occur during severe and prolonged acidosis depend less on changes in constituents of extracellular fluids, much studied and well known, than on changes of intracellular constituents, which have been relatively little studied. The investigations reported here were undertaken to amplify existing knowledge by direct analysis of tissues.

Following is a preliminary report of changes in liver and skeletal muscle of alloxan-diabetic rats in severe ketonemic acidosis. More extensive studies on other animals and other tissues will be reported later.

Methods. Adult white rats from Rockland Farm, weighing around 300 g, fed Purina Chow diet, were kept in glass metabolism cages for collecting urine(1). Three groups, of 36 to 40 rats in each, were studied: a control group, not treated with alloxan, and two alloxan-treated groups. In the latter, diabetes was induced by intravenous injections of alloxan, 50 mg/kg, after a 24- to 30-hour fast. One group was allowed food 24 hours after the injection of alloxan, then starved to induce ketoacidosis. The second alloxan-treated group was fed and treated with NPH insulin, 3 to 5 units per day, until the animals recovered their original weight, usually a period of 7 to 10 days; then ketoacidosis was induced by withholding food and insulin. In both groups severe ketoacidosis developed as a rule within 3 to 4 days, as described in a previous paper from this laboratory(2). All were anesthetized by intraperitoneal injection of nembutal for the taking of blood (drawn from the inferior vena cava) and tissue samples. The whole liver was removed, the gall bladder dissected away, and excess blood removed by blotting with filter paper. The thigh muscles were stripped, dissected free from gross fat and connective tissue. The tissues were transferred as quickly as possible to weighing bottles. Tissue water was determined by weighing and drying the samples at 105°C to constant weight. The dried tissues, liver and thigh muscles, were ground to obtain a homogenous powder. Fat content was determined by extracting the powder with petroleum and ethyl ether in a Soxhlet reflux apparatus. Weighed samples of the fat-free powder were used for determinations of Cl by the method of Van Slyke(3), and of nitrogen by the microkjeldahl method; samples were dry ashed for determinations of sodium and potassium with the Weichselbaum-Varney flame photometer. Samples of blood

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TABLE I. Data on Blood Serum, Muscle, and Liver of Normal Rats and Diabetic Rats with Ketoacidosis. Mean of 12 determinations with standard deviations in each group.

Groups	Sugar, mg/100 cc	Ketones,* mg/100 cc	pH	CO_2, meq/l
		Serum		
Normal	121†		7.45	28.4
	±15		±.04	±2.2
Acidotic 1	686	174	7.08	9.5
	±126	±73	±.10	±2.9
Acidotic 2	746	211	6.99	8.8
	±199	±98	±.06	±2.0

Groups	Fat % of total solids	Muscle: Per 100 g fat free solids — H_2O, g	N, g	Cl, meq	Na, meq	K, meq
Normal	7.0	308.4	14.77	5.10	8.22	45.0
	±1.1	± 4.4	±.56	± .57	± .65	±1.84
Acidotic 1	10.0	272.8	15.16	5.25	8.26	38.23
	±2.4	±15.0	±.25	±1.06	±1.43	±2.44
Acidotic 2	5.5	271.4	15.34	5.23	8.87	39.69
	±1.5	± 5.9	±.68	±1.43	±1.01	±2.51

Groups	Fat % of total solids	Liver: Per 100 g fat free solids — H_2O, g	N, g	Cl, meq	Na, meq	K, meq
Normal	7.34	265.3	11.94	10.34	8.78	32.9
	±1.8	±27.9	±.74	±2.91	±1.24	±3.25
Acidotic 1	38.9	330.4	13.62	11.71	11.41	32.0
	±16	±33.4	±.37	±3.82	±1.73	±4.55
Acidotic 2	20.7	310.8	13.50	11.18	11.26	34.4
	±10.9	±21.7	±.36	±1.57	±1.47	±2.62

Acidotic Group 1: Rats inj. with Alloxan, fed one day, then allowed to develop ketoacidosis immediately, receiving no insulin or food.

Acidotic Group 2: Rats inj. with Alloxan, fed and treated with insulin 7 days; acidosis induced by withholding insulin and food.

* Ketones expressed as acetone.

† "Normal" blood sugar values determined 1 hr after feeding.

were delivered from a syringe under oil for determinations of serum pH(4) and CO_2 content(5). Sugar in whole blood or serum was determined by the method of Nelson(6). Ketones in diluted plasma were estimated by a micromethod(7) employing the acetone test tablets or powder commonly used for qualitative tests for acetone in urine, with appropriate color standards.

Results. Data from analyses of blood, skeletal muscle and livers of the control group and two acidotic groups of rats are listed in Table I. High values for serum sugar and ketones, and low values for serum pH and CO_2 content were approximately the same in the two acidotic groups. Muscle of both acidotic groups showed a lowered content of water and of potassium, while the chloride and sodium values were not significantly different from those of the controls; the slightly higher value for nitrogen content is probably significant (p between 0.05 and 0.02). In liver the fat content was greatly increased, higher in group 1 than in group 2; the content of water, sodium and nitrogen was significantly increased, but the potassium and chloride values were not changed, compared with the controls.

Discussion. The high fat content of muscle in the acidotic group 1 may be ascribed to immediate cellular toxic effects of the alloxan apart from its diabetogenic action. In relation to fat-free solids the potassium concentration decreased 15 and 12% respectively in the two groups. It is of interest to note here that a number of investigators(8-10) studying the effects of prolonged low-potassium diets, designed to produce potassium deficiency, have

found increases in the sodium content of muscle when the potassium content decreased. The failure in the present experiments of sodium to increase with lowered potassium may be attributed to the dehydration and acidosis and to the short duration of these experiments.

The high fat content of the livers of the acidotic rats is presumably associated with the development of severe ketosis and with toxic effects of alloxan, which is known to produce some hepatic necrosis. Although the degree of ketonemia and acidosis seemed alike in the two groups, the fat content of the livers was less in the second group when ketosis was induced after 7 days of feeding and insulin control, suggesting some recovery from early toxic effects of the alloxan. Increased contents of water and sodium may be considered to be interdependent. The lack of change of potassium in the livers of the acidotic animals conforms with observations by other investigators mentioned above that potassium deficiency (induced by diet) was not attended with any change in liver potassium.

The finding in the acidotic rats of a higher than normal nitrogen content in liver and muscle, relative to the total solids, is in accord with the concept that acidosis leads to a greater loss of labile non-protein constituents than of protein from body tissues.

Summary. Data are reported from analyses of tissues of normal rats and of alloxan-diabetic rats with severe ketoacidosis induced by withholding food and insulin 3 to 4 days. In muscle of the acidotic rats, compared with the normal controls, the concentration of water and of potassium decreased, and the concentration of nitrogen increased slightly, with values for sodium and chloride showing no significant change. In liver there was a gain of water, sodium and nitrogen, with no change in concentration of chloride or potassium. Livers of the acidotic rats showed a greatly increased fat content.

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ERRATUM

Article 19259, Vol 79, p. 21, title should read "Ineffectiveness of Parenteral Pvridoxine . . ."