the electrophoretic pattern of the serum in nephrotic patients soon after diuresis induced by ACTH, or ACTH plus urea. The pattern which is characteristic of nephrotic serum (low albumin and large, unresolved α_2 and β globulin peaks) reverts toward the normal after diuresis. The serum albumin rises sharply and the α_2 and β globulin region shows clear resolution. 2. Possible explanations of these abrupt changes in the electrophoretic patterns and concentrations of serum proteins are discussed.

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Acetoacetate-Induced Changes in Blood Lactic and Blood Ascorbic Acids; Prevention by Insulin and Amellin. (19075)

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Diabetes is frequently associated with acidosis, a symptom characterized by a lowering of the blood alkali reserve. It has recently been shown by the authors(1) that the injection of β-hydroxybutyrate into normal rats caused a depletion in liver and muscle glycogen and an increase in the tissue lactic acid. Green and Brosteaux(2) reported that pyruvic acid almost completely inhibits the enzymatic oxidation of lactic acid *in vitro,* and Holmes and Holmes(3) have observed a direct relationship between the blood sugar value and the brain lactic acid content in both hypo and hyperglycemic conditions. The increase of lactic acid in diabetes has been observed by Maranon and his associates(4) and Genes(5). Marenzi(6) has observed that the injection of anterior pituitary extract into dogs causes an increase in blood sugar and blood lactate values. Vit. C has been found by Sigal and King(7) and Banerjee and Ghosh(8,9) to play a role in carbohydrate metabolism. Sigal and King(7) have shown that the successive stages of Vit. C depletion are associated with a corresponding rise in the fasting blood sugar level and a distinctly lowered glucose tolerance; the administration of Vit. C restores the carbohydrate metabolism to normal. The injection of acetoacetate in a dose of 18 mg per 100 g body weight has recently been reported by Nath and his associates(10) to cause a marked depression in the blood Vit. C level of rabbits. An attempt was therefore made to study the cumulative effects of acetoacetate on the lactic acid and the Vit. C contents of the blood of rabbits.

Experimental. In the first set of experiments (Group A), 6 healthy male rabbits

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TABLE I. Effect of Acetoacetate on Blood Lactic Acid and Ascorbic Acid.

Group A							Group B			
			Lactic acid, mg/100 cc blood		Ascorbic acid, mg/100 cc plasma				Lactic acid, mg/100 cc blood	
No. of rabbits	Day of acetoacetate inj.*	Days of treatment with amellin	Avg value	Stand. dev.	Avg value	Stand. dev.	No. of rabbits	Day of acetoacetate inj.*	Avg value	Stand. dev.
6	0		6.2	.86	2.5	.36	20	0	7.9	1.01
6	10		8.9	2.16	2.2	.20	20	10	11.3	1.54
6	20		12.3	2.74	1.7	.10	19	30	18.5	1.48
6	40		15	2.80	1	.06	18	50	24.7	1.94
6	60		16	2.28	.83	.14	16	70	30.1	1.57
4	80	20 (10 mg daily)	7.2	.88	—	—	15	90	36.7	1.76

* Daily dose of acetoacetate injected: Group A. 1st to 10th day—25 mg/kg; 11th to 20th day—50; 21st to 30th—75; 31st to 40th—100; 41st to 50th—125; 51st to 60th—150; 61st to 80th—175.
Group B. 1st to 10th—50 kg; 11th to 30th—100;31st to 50th—150; 51st to 70th—225; 71st to 90th—250; 91st to 100th—275; 111th to 130th—300.

(each weighing about 2 kg) with a fasting blood sugar level between 108-112 mg/100 cc were injected daily with acetoacetate (intraperitoneally) in the doses given in Table I.

In a second group of 20 animals (Group B) higher doses of acetoacetate were injected for a considerably longer period. The animals were anaesthetized with Nembutal, the blood was collected periodically and lactic acid was determined by the method of Barker and Summerson(11). Preliminary determinations on the plasma Vit. C were carried out in Group A by the method of Bessey(12).

Liver glycogen was shown previously(13) to be markedly depleted by injection of acetoacetate, but restored to a considerable extent by the simultaneous injection of acetoacetate plus either insulin or amellin.* Since blood lactate may be utilized to form liver glycogen, a preliminary study was carried out (Group A, Table I) to determine the effect of amellin on the blood lactate level. A more detailed study was carried out in Group B. After these animals had received acetoacetate injections for 90 days, the rabbits were divided into 3 groups; the first was kept as the control, the second given 10 mg of amellin per kg/day and the third 0.5 unit of insulin per kg/day. The acetoacetate injections were continued and the lactic acid content of the blood was determined after an additional 20 and 40 days. The results are shown in Table II.

Discussion. The results suggest that acetoacetic acid may inhibit the oxidation of lactic acid. However, the large increase in blood lactic acid could have been the result of non-utilization or of partial utilization of lactic acid by the liver for the synthesis of glycogen. This deficiency may be due to inactivation of endogeneous insulin, induced by the injection of ketone bodies(14). Severe depletion of liver glycogen has recently been observed by Bondy and Sheldon(15) in human diabetics by liver biopsy. Foglia(16) has shown that in subtotally depancreatectomized rats, the liver could not form glycogen during severe diabetes. That insulin lack may be associated with increased blood lactic acid is indicated by the work of Petiteau(17), who observed that the blood lactate increased much faster after hepatectomy in pancreatectomized dogs than in dogs with an intact

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* This is a complex protein-like plant substance which contains a purine base, a thio sugar, and a phosphate group(13).

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TABLE II. Effect of Insulin and Amellin in Utilizing Blood Lactic Acid.

Group No.	No. of animals	Days of acetoacetate inj.*	Days of treatment with Amellin (10 mg/kg)	Days of treatment with Insulin (.5 U/kg)	Lactic acid, mg/100 cc blood Avg values	Lactic acid, mg/100 cc blood Stand. dev.
I	5	90			36.2	2.04
"	5	110			54.2	3.31
"	3	130			62.4	1.64
II	5	90	0		37.1	1.63
"	5	110	20		22.5	1.50
"	5	130	40		7.6	.46
III	5	90		0	36.9	1.43
"	5	110		20	11.8	.91
"	5	130		40	29.6	.87

* Dose as in Table I (Group B).

pancreas.

Banerjee and Ghosh(8) observed that guinea pigs placed on a scorbutic diet for 21 days showed a decreased glucose tolerance as compared with controls receiving a diet supplemented with ascorbic acid. Banerjee (9) observed that in scorbutic animals the insulin content of the pancreas was reduced and the β-cells showed definite signs of degeneration. Mosenthal and Loughlin(18) found plasma Vit. C levels of less than 0.8 mg % in 43% of more than 100 diabetic children studied. We have observed the diminution of ascorbic acid in the blood of our experimental animals, even though they received sufficient Vit. C along with their food.

After 90 days of administration of sodium acetoacetate, the blood lactate was markedly increased. Injection of amellin for 40 days, even along with the acetoacetate, reduced the lactic acid to normal. Insulin produced a transient beneficial effect in the utilization of lactate during the first 20 days, but was unable to prevent the accumulation of blood lactate when the administration of acetoacetate plus insulin was prolonged (40 days).

Summary. 1. The daily injection of 50 mg/kg of sodium acetoacetate into normal rabbits in increasing doses markedly increased the blood lactate level to an average of 36.7 mg/100 cc (control = 7.9 mg/100 cc) after 90 days. 2. In a second group of animals receiving about half the dosage, the plasma ascorbic acid decreased from 2.50 to 0.83 mg/100 cc after 60 days. 3. Simultaneous injection of amellin prevented the accumulation of blood lactate. In animals which had received sodium acetoacetate for 90 days, amellin restored the blood lactate level to normal within 40 days, in spite of the continued injection of increasing amounts of the acetoacetate. 4. In an experiment otherwise identical, insulin was not as effective as amellin; when it was injected, the blood lactate diminished initially but later increased. 5. The amellin treated animals were healthier than the insulin treated ones.

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