STUDIES OF THE EFFECT OF EXERCISE IN DIABETES.

II. LACTIC ACID FORMATION IN PHLOREHIZIN DIABETES.

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In a previous publication (1) we have reported the results of observations upon the response of human diabetics to exercise. It was found that as the result of exertion, severe or moderately severe diabetics produce lactic acid which accumulates in the circulating blood. For an equivalent amount of work the accumulation was somewhat greater than that observed in normal individuals. The experiments demonstrated that diabetics of a considerable grade of severity can accomplish work by the normal mechanism of muscular contraction; i.e., by the formation of lactic acid. From the theoretic standpoint, however, the experiments could not be conclusive, since in no case was the diabetes complete. It seemed possible that all the diabetics studied had retained enough of the normal carbohydrate metabolism to accomplish the muscular exercise in the usual manner. It was our desire to determine whether completely diabetic individuals could form lactic acid, and, if not, by what chemical mechanism the process of muscular contraction was accomplished. Complete diabetes, especially since the introduction of insulin therapy, is a great rarity, and in those few complete diabetics who have been carefully observed a partial ability to metabolize carbohydrate may return at any time. Furthermore, on account of the extremely critical condition of such patients exercise sufficient to show lactic acid would not be justifiable. To study the response to exercise during the com-
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pletely diabetic state, it, therefore, became necessary to use either phlorhizinized or depancreatized animals.

For this study phlorhizin diabetes was selected, not only because of the greater ease of preparation, but because animals treated in this way are in general stronger and more capable of exercise. Regardless of the mechanism which may be involved in phlorhizin diabetes, it is established that the loss of the function of carbohydrate oxidation is as complete as in any known condition. The non-protein respiratory quotients of completely phlorhizinized animals indicate that fat alone is oxidized and the D:N ratios are the same as in complete human diabetes. They are higher and usually more constant than in animals which have been depancreatized.

In addition to phlorhizin some animals were also given repeated injections of adrenalin on the day preceding the exercise experiment.

Sansum and Woodyatt (2), in extension of the previous work of A.I. Ringer in 1910 give evidence that following adrenalin the glycogen reserves of the body are exhausted even when the period of phlorhizin and fasting corresponds to the shortest that we employed. They found that ether and nitrous oxide caused an increased excretion of glucose in phlorhizin dogs, but not in animals which received adrenalin in addition to phlorhizin. This was interpreted to mean that adrenalin caused a complete exhaustion of the glycogen deposits so that narcotics could no longer induce an increased glycosuria. As another point in favor of this view, they confirmed the findings of A. I. Ringer that in phlorhizinized dogs adrenalin first causes a greater excretion of glucose through the urine with the result that D:N ratios are high. Later doses of adrenalin do not raise the D:N ratios, presumably because there is no more glycogen to be expelled. For our purposes we only insist upon the point that the glycogen store is materially reduced by treatment with adrenalin.

In the following pages an attempt has been made to answer two main questions: (1) May an animal which has lost the power of oxidizing carbohydrate retain the ability to form lactic acid? (2) May lactic acid be formed in a completely phlorhizinized animal whose glycogen stores are minimal? Incidentally, observations have been made on the glucose content of the blood and on the acetone content of blood and urine before and after exercise.

Preparation of Animals.—Since there were no observations in the literature upon the lactic acid response of normal animals to strychnine convulsions, it was necessary to obtain such data.
These animals represent our control group. Animals of another series were fasted and given subcutaneously 1.0 gm. of Merck’s phlorhizin in 10 cc. of sterile Mazola oil, every 24 hours until the D:N ratios indicated that the diabetes was complete. The phlorhizin had been recrystallized in the laboratory before administration. Two dogs were prepared by a method similar to that described by Sansum and Woodyatt (2). These animals, after 3 days of fasting, were given, subcutaneously, 1.0 gm. of phlorhizin in oil and 15 minims of adrenalin (Parke, Davis and Co.). The adrenalin dosage was repeated every 3 hours for 24, after which the experiment with strychnine was performed. Four dogs were given still more thorough preparation. After 3 days of fasting they received the usual dose of phlorhizin in oil until D:N ratios indicated complete diabetes. Adrenalin, 15 minims, was then administered every 3 hours for 27 hours. From 3 to 6 hours later the usual experiment with strychnine was begun.

Even after prolonged treatment with phlorhizin complete D:N ratios did not appear in five dogs. Of these, two were discarded; the others (Dogs 5, 6, and 9) were used in exercise experiments.

In the individual experiments of each group the details of preparation varied considerably and may be found in the protocols.

Character of Exercise.—It was our original intention to study the effects of work performed on a treadmill. In a short trial, an animal (Dog 7), treated with phlorhizin and also with adrenalin for 1 day, would not continue at work of this sort. Previous experiments both on normal men and normal dogs had shown that a considerable amount of work was necessary before an accumulation of lactic acid could be demonstrated in the circulating blood. Since in our experiments the accumulation was the only criterion of lactic acid formation, it became necessary to use artificial means of causing vigorous muscular contraction. For this purpose, we employed strychnine. Our aim was to produce by carefully graduated dosage a condition of hypersensitivity for a long period before convulsions occurred. In this we were aided by directions given to us by Dr. Robert Hatcher of the Department of Pharmacology. The first dose of strychnine was 0.3 mg. per kilo of body weight; second dose in 15 minutes,
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0.1 mg. per kilo; third dose in 15 minutes, 0.05 mg. per kilo; this last to be repeated every 15 minutes until sensitivity was produced. The dosage necessary to produce sensitivity varied greatly in the different animals. After 2.2 mg. one normal dog became hypersensitive. In one of the phlorhizinized animals of almost twice the size 10.3 mg. were necessary before hypersensitivity developed. The degree of sensitivity of the animal was determined by tapping on the table with a percussion hammer. When a stroke of the hammer caused a vigorous contraction of the muscles of all four extremities that time was taken as the beginning of exercise. Work consisted of the response elicited by 60 to 70 taps per minute. When strychnine was given by this method it was possible to maintain a state of hypersensitivity for 1 to 2 hours before death.

Blood Samples.—A sample of blood was always taken before the administration of strychnine. Subsequently, blood was drawn at varying intervals after the exercise had been started; the time of blood taking being regulated as far as possible to correspond to the greatest amount of exercise. In certain experiments several samples were taken to demonstrate different stages in the exertion. The period following a severe tonic convulsion was considered especially desirable. For blood samples the femoral artery and vein were used. In one experiment, not published, a cannula was inserted into the femoral vein. Soon after the vein was tied muscular contractions were reduced to a minimum; apparently the collateral circulation was not sufficient. In the other experiments blood was obtained by needle puncture.

The receivers for blood contained 2.0 mg. of neutral potassium oxalate per 1 cc. of blood, and sometimes, 0.1 mg. of sodium fluoride per cc. The latter was added to check glycolysis. Analyses for sugar and lactic acid were started within 5 minutes of the time the blood was drawn.

Methods.

Lactic acid was determined by the method of Clausen (3). In four of the experiments analysis for blood sugar was made by Benedict’s method (4). Later determinations were made.
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by the method of Folin and Wu (5). The results obtained by this method were uniformly lower. Nitrogen in the urine was done by the Kjeldahl method, sugar in the urine by the method of Benedict (6). Acetone in the urine and blood was determined by the method of Van Slyke (7).

EXPERIMENTAL.

Twelve dogs were studied. They fall into three groups: (1) control, (2) dogs receiving phlorhizin alone, and (3) animals receiving combined phlorhizin and adrenalin treatment. Since we cannot express the exertion of strychnine contractions in terms of mechanical units the degree of exercise will be consistently described as poor, fair, vigorous, and convulsions. In most instances this judgment represents the opinion of the same two observers throughout. In regard to blood samples, it will be understood that unless otherwise specified in the following protocols, arterial blood was withdrawn from the femoral artery and venous blood from the corresponding vein. Also, except where the reverse is explicitly stated the vessels had previously been exposed by incision, so that there could be no doubt whether blood was coming from artery or vein.

Dog 1.—Male. Control. June 14, 1923. Weighed about 6.5 kilos. Poorly nourished. No preparation in the preliminary period. The blood samples were obtained by puncture of the femoral vessels which had not previously been exposed by incision. The sample before exercise probably came from a vein. Analysis: Lactic acid 34.3 mg. Sugar 120 mg. The first dose of strychnine was 2.2 mg. Total 2.6 mg. Hypersensitivity developed after the first dose of strychnine. Exercise was fair. Blood was drawn from the artery in two samples at 25 and 27 minutes after the beginning of exercise. The pooled blood was analyzed. Lactic acid 73.5 mg. Sugar 158 mg. Immediately after arterial puncture the dog developed convulsions and died.

Dog 2.—Male. Control. Jan. 25, 1924. Weighed 9.0 kilos. Received no special preparation. The first blood obtained from the femoral vein showed: Lactic acid 11.9 mg. Sugar 85.4 mg. The first dose of strychnine was 2.2 mg. Total 4.1 mg. Hypersensitivity developed 9 minutes after the first dose. At the same time hyperpnea set in and a respiratory rate of 70 to 80 per minute was maintained until practically the end. During the first 19 minutes exercise was fair and venous blood obtained at the end of

1 Blood sugar determined by the method of Benedict.
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this period gave on analysis: Lactic acid 68.6 mg. Sugar 95.2 mg. For about 37 minutes following, exercise became more vigorous. Then it was poor for the next 27 minutes. In the 83rd minute of exertion, just before the animal died, blood was taken from the femoral artery. This sample was quite dark in color (estimated about 85 per cent saturated with oxygen). Its lactic acid content was 104.3 mg. Sugar 88.0 mg. After death rigor was evident in 25 minutes.

Dog 3.—Male. Control. Jan. 28, 1924. Weighed 6.5 kilos. Received no special preliminary treatment. The first blood was obtained from the femoral vein. Analysis: Lactic acid 41.3 mg. Sugar 118 mg. The initial dose of strychnine was 1.7 mg. Total 10.9 mg. A second sample of venous blood was taken after 53 minutes, when the animal had done an amount of work roughly equivalent to one of the less satisfactory phlorhizin-adrenalin dogs. Analysis: Lactic acid 51.8 mg. Sugar 108 mg. Subsequently, exercise was vigorous. Late in the experiment successively large injections of strychnine were given and 9 minutes before the end the animal went into generalized convulsions. These subsided for a minute during which a third sample of venous blood was drawn 125 minutes after exercise had begun. Analysis: Lactic acid 147.7 mg. Sugar 165 mg. Death followed in 7 minutes. Before 21 minutes had elapsed postmortem rigor was present.

As a rough control of the element of asphyxia a sample of arterial blood was drawn at the time of the second and third venipuncture, and a third sample in the period between. All were bright red.

Dog 4.—Female. Weighed 9.3 kilos. Fasted 8½ days. Phlorhizin for 6 days. The D:N ratios were 3.05 and 3.37 on the 4th and 5th days of phlorhizin. The work experiment was performed on the 6th day of phlorhizin, Jan. 21, 1924. The first blood was obtained from the femoral vein. Analysis: Lactic acid 35.7 mg. Sugar 48.8 mg. The initial dose of strychnine was about 2.2 mg. Total about 5.2 mg. For 12 minutes after hypersensitivity appeared, exercise was vigorous. Arterial blood was taken at the end of that time. Analysis: Lactic acid 40.6 mg. Sugar 108 mg. Subsequently, exercise was only fair. Arterial blood which was drawn in the 81st minute was quite dark. Analysis: Lactic acid 55.3 mg. Sugar 117 mg. Venous blood drawn 2 minutes later was analyzed. Lactic acid 68.6 mg. Sugar 115 mg. The urinary D:N ratios were: Before exercise, 3.48; after, 4.33. Total urinary acetone bodies were 13.6 mg. in 228 minutes before and 52 mg. in the 140 minutes which included the work period.

The rate of glycolysis was determined in the arterial blood sample drawn after 81 minutes of work. In 18 hours at 37.5°C. the blood sugar had dropped from 118 mg. to zero; while the lactic acid had risen from 55.3 to 140 mg.

Dog 5.—Female. Weighed 10.2 kilos. Fasted 7½ days. 8 doses of phlorhizin. D:N ratios: 4th day of phlorhizin, 3.00; 5th, 3.04; 6th (incomplete specimen), 3.03; 7th (incomplete specimen), 2.76. The exercise experiment was performed on the 8th day of phlorhizin, Feb. 6, 1924. Before strychnine, the venous blood sample gave on analysis: Lactic acid 27.3 mg. Sugar 43.5 mg.
First dose of strychnine 2.2 mg. Total 5.7 mg. After 16 minutes of vigorous exertion the animal had convulsions of only moderate intensity. Analysis of venous blood drawn 1 minute later: Lactic acid 75.6 mg. Sugar 62.5 mg. Arterial blood drawn at the same time seemed to have a slight oxygen unsaturation. The animal lived 22 minutes longer, but exercise was poor. Rigor was present 18 minutes after death.

Before exercise the urinary D:N ratio was 4.06; after exercise, 3.03.

Dog 6.—Female. Weighed 6.1 kilos. Fasted 5½ days. Given phlorhizin six times in that period. D:N ratios were: 4th day, 3.14; 5th day, 2.73. The work experiment was performed on the 6th day of phlorhizin, Feb. 11, 1924. Analysis of arterial blood before: Lactic acid 42.0 mg. Sugar 47.6 mg.

Initial dose of strychnine 1.9 mg. Total 4.1 mg. The muscular contractions were vigorous and after 19 minutes the dog seemed ready to go into a convulsion. Arterial blood was then drawn. Analysis: Lactic acid 68.6 mg. Sugar 69 mg.

O2 saturation was 55.6 per cent (determined by Van Slyke-Stadie technique for O2 capacity and content (8)). Exercise became fair and in the 51st minute a third arterial blood was drawn. It was bright red. Analysis: Lactic acid 114.1 mg. Sugar 123 mg.

The animal died 28 minutes later. Postmortem rigor appeared in 15 minutes. The urinary D:N ratio was 3.08 before exercise; and 3.35 after; in a specimen which represented little more than bladder washings.

Dog 7.—Male. Weighed 10.0 kilos. Fasted 1½ days. 2 doses of phlorhizin, 7 doses of adrenaline, and 1 of suprarenalin (Metz) were given during that time. The animal reacted with shivering, nausea, and vomiting. Then the exercise experiment was performed (June 6, 1924). Blood was obtained by puncture without previous exposure of the femoral artery. Analysis: Lactic acid 15.4 mg. Sugar 83.3 mg. Total acetone bodies 25.0 mg. The initial dose of strychnine was 3.3 mg. Total 16.7 mg. No hypersensitivity was present until 10.3 mg. had been given. Exercise was very satisfactory. After about 10 minutes of work arterial blood was drawn. Analysis: Lactic acid 60.2 mg. Sugar 88.2 mg. Total ketone 20.0 mg. Exercise continued vigorous. About 20 minutes from the start the dog had a tonic spasm and breathing ceased. Blood was drawn from the heart, which was still beating. Analysis: Lactic acid 56.0 mg. For a considerable portion of the experimental period this dog had shown marked hyperpnea.

Dog 8.—Female. Weighed 7.5 kilos. Fasted 2 days. 9 doses of adrenaline given in 27 hours. Phlorhizin given twice in the same period. Urinary D:N ratios in consecutive short period specimens were: 4.96, 3.04, 2.73, 2.55, and 2.42. The exercise experiment was performed June 12, 1924. Analysis of arterial blood before: Lactic acid 25.2 mg. Sugar 90 mg. Total ketone bodies 9.8 mg.

The initial dose of strychnine was 2.2 mg. Total 8.9 mg. After 20 minutes of fair exercise another arterial sample was obtained. Analysis: Lactic acid 29.4 mg. Sugar 94 mg. Total ketone 1.6 mg.

Before exercise the urinary D:N ratio was 2.32; after exercise 2.41. The
total ketone excretion in 78 minutes preceding exercise was 10.2 mg., and in 115 minutes including the work period it was 40.0 mg.

Dog 9.—Female. Weighed 14 kilos. Fasted 9 days. Phlorhizin nine times. Stole food on 5th and 6th days. The urinary D:N ratio for the 12 hour night period of the 7th day was 3.4. The following 24 hour specimen gave a D:N ratio of 3.02. Following adrenalin every 3 hours the ratios of consecutive short period samples became 3.76, 6.95, 9.85, 10.1, 7.04, 5.02, 3.41, 3.11, and 3.28. The work experiment was done on the 9th day of phlorhizin, June 22, 1923. Before exercise an arterial sample was obtained by puncture without previous exposure of the vessels. Analysis: Lactic acid 30.8 mg. Sugar 75.0 mg. Total ketone 21.3 mg. After 24 minutes of vigorous work a second arterial sample was drawn. Analysis: Lactic acid 32.9 mg. Sugar 83.3 mg. Total ketone 17.0 mg. Soon after, large successive doses of strychnine were given intramuscularly. In the 39th minute of work the dog went into a tonic spasm; respiration began to fail and a highly unsaturated sample of blood was obtained. It may have come from artery or vein. Analysis: Lactic acid 77.7 mg.

The urinary D:N ratio before work was 3.52; after, 3.72. The total acetone excretion for 125 minutes preceding strychnine was 42.0 mg., in 55 minutes including the work period it was 11.0 mg.

Dog 10.—Female. Weighed 7.8 kilos. Fasted 7½ days. Received five daily injections of phlorhizin. On the last day received adrenalin subcutaneously for 9 doses. The urinary D:N ratios were: 3rd day, 2.92; 4th day, 3.85. Short period specimens during adrenalinization gave consecutively: 4.41, 2.59, and 2.40. The exercise experiment was performed on the 5th day of phlorhizin, Jan. 9, 1924. An arterial blood was obtained before. Analysis: Lactic acid 48.3 mg. Sugar 67.1 mg. The initial dose of strychnine was 2.2 mg. Total 5.2 mg. After 73 minutes of poor exercise venous blood was drawn. Analysis: Lactic acid 66.5 mg. Sugar 66.6 mg. Exercise became worse as the experiment proceeded. About 10 minutes before the last blood was withdrawn the fore and hind limbs became perfectly flaccid. The last blood was obtained from the artery 92 minutes after work had begun. It was bright red. Analysis: Lactic acid 88.2 mg. Sugar 53.0 mg. Rigor had not developed in the hind legs 20 to 30 minutes after death.

The urinary D:N ratio before was 3.69; after, 3.73. The total acetone bodies excreted during 132 minutes before exercise was 153 mg.; in the next 141 minutes which included the work period, 58 mg. were excreted.

Dog 11.—Female. Weighed 8.6 kilos. Fasted 8½ days. Received phlorhizin for 6 days. The urinary D:N ratios were: 4th day, 3.14; 5th day, 3.23. On the next day adrenalin was given every 3 hours and the D:N ratios on two specimens were 3.18 and 2.90. The exercise experiment was performed on the 6th day of phlorhizin, Jan. 16, 1924. The blood was venous. Analysis: Lactic acid 28.4 mg. Sugar 41.6 mg. The initial dose of strychnine was 2.2 mg. Total 5.6 mg. For 17 minutes exercise was poor. Analysis of arterial blood drawn at that time: Lactic acid 28.0 mg. Sugar 48.7 mg. Then, by direct stimulation of all four extremities and by opposing the
contractions with the hands a fair amount of work was performed. Venous blood was drawn 46 minutes after work had begun. Analysis: Lactic acid 29.4 mg. Sugar 42.5 mg.

Exercise lapsed; became poor. A final arterial blood was obtained 83 minutes from the start. Analysis: Lactic acid 28.0 mg. Sugar 43.4 mg.

The urinary D:N ratios were: Before, 2.74; after, 3.16. Total urinary acetone bodies during 186 minutes which included the work period were 221 mg.

Dog 12.—Female. Weighed 10 kilos. Fasted 8 days. Given six injections of phlorhizin. On the last day adrenalin was injected intramuscularly every 3 hours. The reaction was not noticeably more severe than that following subcutaneous administration. The urinary D:N ratios

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<td>1</td>
<td>Before.</td>
<td>Vein (?)</td>
<td>31.3</td>
<td>120*</td>
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<td>25 and 27 min.</td>
<td>Artery.</td>
<td>73.5</td>
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<td>Before.</td>
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<td>11.9</td>
<td>85.4</td>
<td>Fair for 19 min. Vigorous to 57th min. Then poor to the 83rd min.</td>
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<td>83 &quot;</td>
<td>Artery.</td>
<td>104.3</td>
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<td>118</td>
<td>Poor for 53 min. Then vigorous to 124th min.</td>
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<td>53 min.</td>
<td>&quot;</td>
<td>51.8</td>
<td>108</td>
<td>Generalized convulsion 124th min.</td>
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<td>125 &quot;</td>
<td>&quot;</td>
<td>147.7</td>
<td>165</td>
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*Blood sugar determined by the Benedict method. Folin-Wu method employed in other experiments.

were: 3rd day, 3.64; 4th day, 3.80. Following adrenalin the ratios were: 7.11, 5.65, 3.79, 2.07, and 3.05. The exercise experiment was performed on the 6th day of phlorhizin, Dec. 7, 1923. Blood before was venous. Analysis: Lactic acid 55.6 mg. Sugar 51.2 mg. Exercise was poor for about 75 minutes after hypersensitivity appeared and then failed completely. Arterial blood was drawn about 77 minutes after work. Analysis: Lactic acid 53.5 mg. Sugar 62.5 mg.

The urinary D:N ratio before exercise was 3.07; after, 3.53.

In Table I will be found a summary of the lactic acid and glucose content of the blood, before and after exertion in control.
animals. In these normal dogs fair or vigorous muscular contractions were obtained in each case. Convulsions preceded death in two animals. Blood lactic acid mounted progressively during the course of the exercise. The smallest total increase was 39.2 mg. The greatest was 106.4 mg. The sugar content of the blood increased markedly in two experiments; and to a slight extent in the third. In Dog 2 hyperpnea was extreme, and, in addition, the final blood sample showed obvious oxygen unsaturation.

Table II gives a summary of the results on phlorhizinized dogs. Two of these received phlorhizin for 6 days, and one for 8 days. In Dog 4 the D:N ratios before exercise were very close to the theoretic. The D:N ratios in Dogs 5 and 6 are somewhat low. In the absence of ideal D:N ratios it may be pointed out that the experiments of Lusk (9) show that after 2 days of phlorhizin the non-protein respiratory quotients indicate no carbohydrate combustion. Dogs with D:N ratios as much above the ideal as ours are below yield respiratory quotients indicative of complete diabetes. Finally, Dogs 4, 10, and 11 gave almost perfect D:N ratios after treatment with the same lot of phlorhizin.

In this group of experiments muscular contractions were
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scarcely less vigorous than in the normal. Blood lactic acid rose progressively during exercise. The smallest total increase was 32.9 mg. The greatest was 72.1 mg. The resting blood sugar was low and during exercise showed an increase in each

TABLE III.
Phlorhizin-Adrenalin Series.

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<td>mg. per 100 cc.</td>
<td>mg. per 100 cc.</td>
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<td>7</td>
<td>Before.</td>
<td>Artery.</td>
<td>15.1</td>
<td>83.3*</td>
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<td>60.2</td>
<td>88.2*</td>
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<td>About 20 min.</td>
<td>Right heart.</td>
<td>56.0</td>
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<td>8</td>
<td>Before.</td>
<td>Artery.</td>
<td>25.2</td>
<td>90*</td>
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<td>94*</td>
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<td>30.8</td>
<td>75.0*</td>
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<td>24 min.</td>
<td>&quot; (?),</td>
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<td>41</td>
<td>&quot;</td>
<td>77.7</td>
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<td>10</td>
<td>Before.</td>
<td>Vein.</td>
<td>48.3</td>
<td>67.1</td>
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<td>73 min.</td>
<td>Artery.</td>
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<td>66.6</td>
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<td>92</td>
<td>&quot;</td>
<td>88.2</td>
<td>53.0</td>
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<td>Before.</td>
<td>Vein.</td>
<td>28.4</td>
<td>41.6</td>
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<td>48.7</td>
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<td>Vein.</td>
<td>29.4</td>
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<td>95</td>
<td>Artery.</td>
<td>28.0</td>
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<td>12</td>
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<td>Vein.</td>
<td>55.6</td>
<td>51.2</td>
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<td>About 75 min.</td>
<td>Artery.</td>
<td>53.5</td>
<td>62.5</td>
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*Blood sugar determined by the Benedict method. Folin-Wu method employed in other experiments.

successive blood sample. The difference between the first and final blood sugar value was even greater than in the normal. As to asphyxia, it may have been a factor in the third and fourth
blood samples taken from Dog 4. Also, the rate of glycolysis in this dog deserves mention. In 18 hours at 37.5°C, sugar was completely gone and lactic acid had mounted in proportion.

Table III summarizes the results obtained after the administration of adrenalin to phlorhizinized dogs. The period of fasting and phlorhizin administration varied greatly in different animals. Adrenalin was given in nine doses at 3 hour intervals; the last dose being given 3 to 6 hours before the commencement of the work experiment. The general condition of all the animals after receiving adrenalin was very poor.

Of the two dogs whose combined phlorhizin and adrenalin treatment lasted less than 36 hours, one—Dog 7—exercised vigorously and increased the blood lactic acid by 40.6 mg. during work. The second—Dog 8—did a fair amount of work, but showed no appreciable increase in lactic acid.

Of those animals which received adrenalin after long preparation with phlorhizin, only one—Dog 9—was able to perform vigorous work. Lactic acid increased 46.9 mg. over the initial value. This animal had stolen food 3 days before the work experiment; but adrenalin had only been started after two successive D:N ratios had shown that no excess sugar was being excreted. At most, the food might have added to her glycogen store. It could not have improved her power to burn glucose.

Dog 11 did a fair amount of work without any lactic acid accumulation in three samples drawn at various times during exercise. The other two long term phlorhizin-adrenalin dogs did little work, and showed no significant change in lactic acid.

In all six cases of this series the resting blood sugar was low. Exercise caused no constant or significant change. Marked oxygen unsaturation was noted in the last blood sample in Dogs 7 and 9. In Dogs 7, 8, and 9 the total blood ketones were determined. They fell somewhat during exercise, but the number of observations is too few to have much weight. The low D:N ratios after repeated injections of adrenalin in phlorhizinized dogs have been noted by Sansum and Woodyatt.

**DISCUSSION.**

The outstanding fact in the preceding experiments is that lactic acid may be formed in an animal which is unable to oxidize
carbohydrate. It also appears in a completely phlorhizinized animal whose glycogen stores have been reduced to a minimum. In the three dogs which were fasted and made diabetic with phlorhizin the results are in agreement, and, in each observation, there is undoubted evidence of lactic acid formation. The results in the group where adrenalin treatment was added, are not so uniform. It is clearly demonstrated in Experiments 7, 9, and 10 that after such treatment lactic acid may be formed. In the other three, no accumulation of lactic acid was noted. These latter results do not rule out the possibility of lactic acid formation, since accumulation in the blood depends upon two factors. First, sufficient must be produced to accumulate in the muscle and, then escape into the circulation; and second, the rate of diffusion of lactic acid into the blood must be greater than the rate of removal from the blood. In two of the dogs on which negative results were obtained there was not enough exercise. Contractions were feeble and no lactic acid accumulation was expected. In Dog 9 the muscular contractions were also weak, but it was possible during one period to elicit, by direct stimulation, an amount of work which was deemed sufficient to cause accumulation. Three blood samples taken at various stages showed no deviation from the resting level. This result is puzzling and is the only one which might be fairly interpreted as a possible inability on the part of the animal to form lactic acid.

The significance of lactic acid formation in these experiments is not entirely clear but has sufficient bearing on several important questions to justify discussion.

**Relation of Lactic Acid to the Combustion of Carbohydrate.**

Lactic acid appears in many biological processes. It is formed during muscular contraction in normal animals. It has been found to accumulate in several pathological conditions (10) and following the administration of many drugs (11). It is formed in perfusing the livers of normal animals. In shed blood it appears during glycolysis, and in muscle, during post-mortem rigor. The suggestion has been made that it is a normal intermediary in the oxidation of carbohydrate, and as an extension of this hypothesis, it has been suggested that in phlorhizin diabetes the organism cannot oxidize carbohydrate because it cannot
form lactic acid (12). Four of the six observations of Embden and Isaac (13) with liver perfusion in phlorhizinized animals indicated that the excised liver had lost its normal ability to change glucose to lactic acid. Lépine and Boulud (14) found that glycolysis was diminished in phlorhizin diabetes. However, our single glycolysis experiment (Dog 4) gives no indication of an invariable difficulty in the formation of lactic acid from glucose in the test-tube. There is, moreover, in the literature, a considerable amount of evidence, which seems to support the validity of our results. Milne and Peters (15) found no diminution of glycolysis in the blood of one phlorhizinized, and many depancreatized dogs. Embden, Schmitz, and Meincke (16) demonstrated that after 4 days of phlorhizin lactic acid was present in dog's muscle. On standing, as much lactic acid was formed as by the muscle of normal, well fed animals. Even after strychnine convulsions in phlorhizinized dogs one-half of the normal amount of lactic acid appeared.

These results indicate that in phlorhizin diabetes lactic acid may be formed from carbohydrate. The experiments of Mandel and Lusk (17) and of Embden, Schmitz, and Meincke (16) show that the reverse process, glucose formation from lactic acid, may take place. In phlorhizin diabetes, therefore, the defect is not in the reaction glucose $\rightarrow$ lactic acid. The question of the actual site of the defect cannot be answered by these experiments.

There is a further question whether lactic acid is under any circumstances an intermediary in the oxidation of carbohydrate, and the evidence upon this point is scanty. There are in vitro experiments (18), which show that lactic acid is formed during the decomposition of glucose in strongly alkaline solution. Subsequent addition of hydrogen peroxide leads to the combustion of the other products, but not of the lactic acid. If hydrogen peroxide and alkali are added together to a glucose solution, oxidation occurs without the appearance of lactic acid. Even if such results be applicable to the problem of in vivo oxidation, they lend no support to the view that lactic acid is an intermediary.

By injecting large doses of insulin in normal dogs, Briggs, Koechig, Doisy, and Weber (19) obtained a great lowering of the blood sugar and an increase in lactic acid. These results have
been interpreted to indicate that the defect in diabetes lies in
the formation of lactic acid from glucose. More recently, Toenniessen (20) reported that the addition of insulin causes an increased
formation of acetaldehyde in blood, and also, in the combined
pulps of muscle and pancreas to which lactic acid has been added.
It has not been demonstrated that the acetaldehyde increased
at the expense of lactic acid. The experiments of Noble and
Macleod (21) may have some bearing on this question. They
found that injection of racemic sodium lactate did not relieve
the symptoms of insulin shock in two dogs. If lactic acid is
an intermediary in the normal combustion of carbohydrate it is
not easy to explain why it fails to exert as beneficial an action
as glucose. This evidence makes it seem not unlikely that the
oxidative and anoxidative breakdown of carbohydrate may
proceed along separate paths. If that should prove to be the
case, the defect in phlorhizin diabetes would lie in the oxidative
breakdown. The anoxidative breakdown of carbohydrate to
the lactic acid would not be involved.

**Chemical Mechanism of Muscular Contraction.**

The question arises whether the accumulation of lactic acid,
observed by us, was associated with the process of muscular
contraction. A very strong argument in favor of this idea is
that the increase seems to coincide with the violence and dura-
tion of exercise. Several other factors which might conceiv-
ably lead to lactic acid formation should not be dismissed without
discussion. Such factors are: asphyxia, hyperpnea, strychnine,
and, in some experiments, adrenalin. It may have been noted
that in two experiments the dog had stopped breathing at the
time that the lactic acid accumulation was noted. Macleod
(22) has noted that during rest the prolonged asphyxiation of
etherized dogs causes an increase of lactic acid in the blood.
In our animals the period of asphyxia did not last more than 1
to 3 minutes. Two unpublished observations on intense asphyxia
of 3 minutes duration in a resting phlorhizinized dog yielded
no increase in lactic acid. During exercise, however, a brief
period of asphyxia might cause an abnormally great accumulation
in the blood, for Fletcher and Hopkins (23) have shown that the
absence of oxygen during contraction causes a lactic acid accumulation in the muscle. In this case, however, the contractile process causes the production of lactic acid and asphyxia merely prevents its removal. Therefore, in two experiments asphyxia may have been a factor in the accumulation. In the other observations it was absent.

The factor of hyperpnea may next be considered. In a single normal animal—Dog 2—great hyperpnea was noted. In many of the animals the breathing was irregular, and, occasionally, there were short periods of overventilation. Since none of our phlorhizinized dogs reacted to strychnine with overbreathing it seems unnecessary to dwell on this factor.

In these experiments muscular contractions were obtained by producing a state of strychnine hypersensitivity. In view of the number of drugs which cause an increased excretion of lactic acid in the urine it might be suggested that strychnine itself causes a lactic acid acidosis independent of muscular contraction.

There are two reasons for not accepting this explanation for the total increase in lactic acid. While in the normal dogs it is conceivable that an obscure action of strychnine contributed to the accumulation of lactic acid, the exercise was violent and must have caused an overflow of lactic acid into the blood. The final figures were of the order that one expects with such grades of exertion under physiologic conditions. The accumulation of lactic acid was scarcely more than was found in the phlorhizinized animals and corresponded to the somewhat greater degree of exertion. The results give no indication that there was in the normal animals a summation of a "direct" strychnine effect and of the effect of muscular exertion. In the second place, there is little warrant for assuming that a phlorhizinized animal would lose the power to form lactic acid from glycogen in the process of muscular contraction, while preserving the ability to produce lactic acid in some other possible response to strychnine.

Unpublished experiments of Loebel and Tolstoi show that 1 hour after the injection of adrenalin, there is in man a moderate lactic acid accumulation in the blood. Where adrenalin was given in the experiments reported in the present paper the exercise did not follow until 3 or 6 hours after the last dose. Adrenalin
may have affected the resting values, but it is not likely that it would have caused the increases which occurred following the strychnine administration.

There seems little reason to doubt that the lactic acid accumulation which is seen in these experiments arose from the contraction of muscle. This is important for it indicates that the usual lactic acid mechanism is responsible for muscular contraction in completely phlorhizinized animals, and even in those which are completely phlorhizinized and have minimal glycogen stores.

Source of the Lactic Acid.

From consideration of chemical structure and from a mass of in vitro and in vivo evidence it is well established that under ordinary conditions lactic acid may arise from carbohydrate or from the carbohydrate fraction of the protein molecule. There is little evidence to indicate that it arises from the breakdown of fat. We purpose to consider whether in our animals the well established sources of carbohydrate could supply the lactic acid, or whether it is necessary to assume that any of the lactic acid proceeded from fat.

In the dogs treated with phlorhizin alone it seems entirely possible that the lactic acid may have arisen from a store of glycogen (24, 25).

The experiments of Sansum and Woodyatt make it seem unlikely that glycogen could have been the source of lactic acid in those dogs to which adrenalin was administered. Even after adrenalinization, however, it is possible that a carbohydrate precursor of lactic acid may still be present in the muscle, for Embden and Isaac (13) have shown that in dogs, despite prolonged fasting and treatment with phlorhizin and strychnine, the muscles retain a considerable store of substance—other than glycogen—which will produce lactic acid. Furthermore, in our experiments after the most thorough treatment, the blood sugar never fell below a level of about 40 mg. per 100 cc. A certain level of blood sugar is maintained. From analogy it does not seem unlikely that in the muscles a minimal store of lactic acid precursor is maintained to the last moments of life.

Another possibility is that even though the muscle's original
store of carbohydrate was completely exhausted because of adrenalin administration, a new supply might be elaborated from protein. This might be utilized to form a new store of glycogen before the experiment, or the glucose derived from protein broken down during the experiment might go through a lactic acid stage. There is some indirect evidence which possibly favors the view that during rest a portion of the glucose from protein is stored. As noted by Sansum and Woodyatt, and by us, the D:N ratios in certain experiments assume a definitely lower level after the administration of adrenalin. This may be explained by assuming that part of the carbohydrate fraction of the protein molecule is retained to form a new glycogen store.

It seems sufficiently clear that as long as an animal lives there is a possible carbohydrate source of lactic acid in the body, and it is, therefore, by no means necessary to assume that the lactic acid proceeds from any other source.

The possibility that carbohydrate or lactic acid may be formed from fat cannot be neglected. It is a well known fact that fat may be formed from carbohydrate and a priori it might seem equally easy for the body to accomplish the opposite transformation of fat into lactic acid or lactic acid precursor. For this, however, no direct evidence has been adduced. The exact and important experiments of Krogh and Lindhard (26) are interpreted as indirect evidence that such a transformation does occur. With a high fat, low carbohydrate diet, work was accomplished less efficiently than with a low fat, high carbohydrate diet. The diminished efficiency and certain changes in the respiratory quotient during exertion led them to advance the hypothesis that fat was converted into carbohydrate when the carbohydrate in the food was limited. The chief obstacle to the acceptance of this idea has been the work of Stiles and Lusk (27) who found that D:N ratios remain quite constant in phlorhizinized animals during rest and exercise. This constancy was taken as an indication that the only source of urinary glucose in fasting phlorhizin

\(^2\)Similar changes in the non-protein R. Q. as calculated in the ordinary way have been recently observed by Boothby and Sandiford, but they did not note any diminution of efficiency following high fat, low carbohydrate diets (personal communication).
dogs was protein. If, as suggested by Krogh, fat may be converted to carbohydrate, and if this mechanism is not affected by phlorhizin, urinary glucose would be furnished from fat as well as protein, and the D:N ratios would not be fixed.

This objection may be answered by assuming that carbohydrate formed from fat is immediately oxidized. In that case there would be no alteration of the D:N ratios or of the respiratory quotients. This hypothesis carries the rather dangerous assumption that in a state of complete diabetes carbohydrate or a carbohydrate-like substance formed from fat is readily oxidizable at a time when the ordinary forms of carbohydrate cannot be utilized. Also the view that fat is oxidized as carbohydrate creates serious difficulties when one attempts to explain why ketone bodies arise from fat and not from carbohydrate unless one is prepared to make two assumptions; viz., that the conversion of fat to carbohydrate occurs only during exercise, and that ketone production does not result when fat is oxidized during work. There are certain experimental data which indicate that the second assumption runs counter to the facts.

**Sources of Energy in Muscular Exercise.**

While it may be freely admitted that the possibility of lactic acid formation from fat has not been excluded, this assumption is not necessary to explain the facts. Whatever the chemical transformations may be, the respiratory quotients indicate that, in diabetes, the chief, or in some circumstances, perhaps the only, source of energy from oxidation is fat. At this point much confusion has arisen in the literature.

On the one hand, experiments performed on isolated muscle by Fletcher and Hopkins, Hill, Meyerhof, and Embden, indicate that the only chemical mechanism for muscular contraction is the formation of lactic acid from carbohydrate. This has led to a wide-spread belief that carbohydrate furnishes the only source of energy for exertion. This belief has been strengthened by some experiments of Meyerhof (28) in which he found a respiratory quotient of 1.00 during the contraction of isolated muscle. On the other hand, there are many experiments on the respiratory metabolism of the intact animal during exertion which indicate
that the energy for muscular exercise comes from various foodstuffs. To be sure, exercise in a normal, well fed man is accompanied by a quotient which approaches unity (29). When high fat diets (26, 30) or high protein diets (30) are given, however, the respiratory quotients indicate that the energy is derived from the foodstuffs which preponderate in the diet. In prolonged fasting (30) and in severe diabetes (31, 32) the energy may be derived almost entirely from fat. For example, in the experiments of Anderson and Lusk, a dog which had fasted 13 days and then ran 10 miles for more than 3 hours, showed during the last hour a non-protein respiratory quotient of 0.713 or the theoretic value for human fat as given by Zuntz.

These two classes of evidence: one, concerned with the experiments on isolated muscle; the other, with the respiratory metabolism of intact animals, are not necessarily contradictory. Muscular contraction consists of two phases: a contractile phase, which is accompanied by the conversion of some form of carbohydrate into lactic acid, and a recovery phase during which oxidation occurs while part or all of the lactic acid is re-synthesized to glycogen. The chemical reaction of the contractile phase, while accompanied by the liberation of a measurable amount of energy, requires no oxygen for its accomplishment. It produces no CO₂. It has no effect on the respiratory quotient. In the steady state of exercise the quotient is a reflection only of the oxidative processes which occur during the recovery phase.

That the energy during the contractile phase is derived from the formation and neutralization of lactic acid is well established. The experiments described in this paper indicate that even under the most extreme conditions of phlorhizin diabetes and of glycogen depletion these reactions may account for all the energy transformations during this phase. It is equally well established that during the recovery phase, the energy arises from oxidation; but the foodstuffs which are oxidized to furnish this energy have not been definitely determined. Fletcher and Hopkins considered that the energy came from the oxidation of lactic acid, since they found in muscles, which recovered from fatigue in an atmosphere of nitrogen, a much greater accumulation of lactic acid than in muscles which recovered in an atmosphere of oxygen. Their interpretation was generally accepted until
Hill and Meyerhof demonstrated that the heat given off during the recovery phase was much less than would result from the oxidation of all the lactic acid which had been produced. They found that the major portion of this compound was reconverted to a precursor during the recovery phase. This later work offers an alternative explanation for the accumulation of lactic acid under anaerobic conditions. The reconversion of lactic acid to glycogen is an endothermic reaction; and will not occur unless energy is supplied from the external surroundings. In an atmosphere of oxygen, this is furnished by oxidations. In an atmosphere of nitrogen there are no oxidations, and, therefore, no available energy for the reconversion of lactic acid. The latter product accumulates, not necessarily because of a specific failure to oxidize lactic acid, but because of the general failure to oxidize any substance which may supply the energy needed for the reconversion. Whether, under certain conditions, a part of the lactic acid serves as the substance whose oxidation furnishes the requisite energy, is not at all clear. The high quotients obtained by Meyerhof upon excised frog's muscle, may support that interpretation. In the living animal, however, the respiratory quotients offer convincing evidence that according to the various conditions of the experiment, the energy may be derived from carbohydrate alone, from fat alone, or from a combination of the two in any proportion. In severe or complete diabetes, fat must furnish the chief, if not the only, source of energy during the recovery phase.

**SUMMARY AND CONCLUSIONS.**

1. The effect of strychnine hypersensitivity and convulsions has been studied in three groups of animals: (a) normal dogs, (b) dogs rendered completely diabetic with phlorhizin, and (c) dogs with complete phlorhizin diabetes to whom adrenalin was given in order to reduce the glycogen stores to their lowest level.

2. The exertion which is induced during strychnine hypersensitivity is accompanied by an accumulation of lactic acid in the blood which may be almost as great in the phlorhizinized as in the normal dog.

3. Even when the glycogen reserves of the body have been re-
duced to a very low level by the prolonged administration of phlorhizin and adrenalin, lactic acid may accumulate in the blood during the muscular exertion elicited in a period of strychnine sensitivity.

4. Lactic acid may be formed in an animal which has lost the power to oxidize carbohydrate. This is evidence that the defect in phlorhizin diabetes is not in the formation of lactic acid from carbohydrate. The failure to burn carbohydrate at a time when the animal can form lactic acid may be interpreted in two ways: (a) lactic acid is an intermediary in carbohydrate combustion. The defect in phlorhizin diabetes lies in its oxidation. Or (b) lactic acid is not an intermediary in carbohydrate combustion. It is only formed in the anoxidative breakdown of carbohydrate. This process is quite separate and distinct from the oxidation of carbohydrate.

5. In phlorhizinized, as well as in normal dogs, the accumulation of lactic acid is almost certainly associated with the muscular exertion. This is evidence that even in animals unable to oxidize carbohydrate the normal mechanism for muscular contraction may be employed.

6. The probable source of lactic acid in these experiments has been discussed. Even after treatment with adrenalin the body retains substances, like protein, which are known to yield lactic acid. While fat cannot be excluded as a source of lactic acid this assumption is not necessary to explain the facts.

7. The sources of energy in muscular exercise have been discussed and the following working hypothesis has been advanced: (a) That during the contractile phase of muscular activity energy is available because of the chemical reactions which result in the formation and neutralization of lactic acid. These reactions occur even during complete phlorhizin diabetes and glycogen depletion. (b) That during the recovery phase the lactic acid is reconverted to glycogen by means of energy derived from the processes of oxidation. The respiratory quotients indicate that according to the conditions of the experiment the energy may come from carbohydrate alone, from fat alone, or from any combination of the two. In phlorhizin diabetes fat must furnish the chief, if not the only, source of energy during the recovery phase.
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STUDIES OF THE EFFECT OF EXERCISE IN DIABETES: II. LACTIC ACID FORMATION IN PHLORHIZIN DIABETES

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