THE EXCRETION OF LACTIC ACID IN THE URINE AFTER MUSCULAR EXERCISE.*

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The classic work of Fletcher and Hopkins (1906–07) demonstrated conclusively that lactic acid is formed in muscle during contraction. Since that time lactic acid production has been proved to be of fundamental importance in muscle physiology (Hill and Meyerhof, 1923; Embden, Griesbach, and Schmitz, 1914–15). Hill's studies on oxygen utilization led him to conclude that large quantities of lactic acid are formed in muscle during strenuous exercise. Free diffusion into the blood may result in concentrations of over 100 mg. of lactic acid per 100 cc. of blood, about five times the normal value (Hill and Lupton, 1922–23; Barr, Himwich, and Green, 1923). With such abnormal quantities in the blood it is natural to assume that lactic acid will pass through the kidney and be excreted in the urine. Ryffel (1909–10) and Feldman and Hill (1911) found from 60 to 450 mg. excreted in 30 minutes after short periods of running. If Hill and Lupton (1922–23) are correct when they calculate that from 20 to 90 gm. of lactic acid may be formed in the body during a few minutes of strenuous exercise, the amounts found in the urine seem remarkably small. Various facts, however, suggest explanations for such a relationship. Relatively inactive muscles are able to absorb lactic acid from the blood in which the concentration has been raised by exercise (Barr and Himwich, 1923). Other tissues of the body may perhaps function in the same way. After short periods of strenuous exercise, lactic acid disappears

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rapidly owing to oxidation and synthesis so the time in which excretion can take place is short. The ability of the body to utilize large quantities of lactic acid is shown by the experiments of Nencki and Sieber (1882) who fed 20 gm. of sodium lactate a day for 4 days to a diabetic without being able to detect a trace of lactic acid in the urine and by the experiments of Haggard and Henderson (1920) who injected large quantities of lactic acid without seriously diminishing the alkaline reserve of the blood.

It should be emphasized that Hill's work shows that exercise sufficiently mild to permit continuous performance for a considerable time (*i.e.* ½ to 1 hour) does not lead to a continuous piling up of lactic acid. The severity of the sustained effort seems to be such that a "steady state" is reached where lactic acid is disposed of as fast as it is formed. This may account for the fact that only small quantities of lactic acid have been found in the urine after long periods of exercise. Jerusalem (1908) found 33 mg. in 250 cc. of urine after mountain climbing and Campbell and Webster (1922) found 61 mg. in urine after 5 hours of hard work.

EXPERIMENTAL.

In order to obtain additional data concerning the excretion of lactic acid in urine after strenuous exercise several experiments were carried out on young men using 2 to 3 minute periods of exercise (stair running). The urine was collected in 10 or 15 minute periods to permit the rate of excretion to be followed in some detail. The experiments were usually carried out in the morning, after breakfast, at which both subjects drank coffee. After a suitable fore period, the subject ran up and down one flight of stairs as rapidly as possible, moving his arms vigorously with each step. The collection of urine was continued in most experiments for 50 to 60 minutes.

After the urines were measured they were analyzed for lactic acid by the method of Clausen (1922). It is recognized that other materials may be determined as well as lactic acid, but as the urines were free from protein, acetone, and acetoacetic acid, there seems to be little reason for doubting that the increased values observed after exercise were due to the excretion of lactic acid itself. The isolation of 400 mg. of zinc lactate from a portion

of the urine from one experiment demonstrates the presence of a considerable quantity of lactic acid. From 0.5 to 2 cc. of urine were extracted with ether and the analysis carried out as out-

TABLE I.

Lactic acid in urine. Subject W. Mar. 31, 1924. Stair running, 2 min.

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	Remarks.
a.m.	*cc.*	*mg.*	*mg. per 100 cc.*	
9.00– 9.15	16.3	2.6	16	
9.15– 9.30	23.0	200	870	Exercise 9.15–9.17 a.m.
9.30–10.00	26.5	276	1040	

Total lactic acid excreted, due to exercise, 470 mg.

TABLE II.

Lactic and phosphoric acids in urine. 10 min. periods. Subject W. Apr. 14, 1924. Stair running, 2 min. (8 round trips).

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	PO_4	Remarks.
a.m.	*cc.*	*mg.*	*mg. per 100 cc.*	*cc. 0.1M*	
9.17– 9.27	17.8	1.9*	11	1.15	Drank 200 cc. water.
9.27– 9.37	28.0	2.4*	9	1.36	Room temperature20°C.
9.37– 9.47	29.0	1.9*	7	1.12	
9.47– 9.57	18.7	126	670	1.68	Exercise 9.47–9.49 a.m.
9.57–10.07	34.0	426	1250	2.94	
10.07–10.17	15.5	145	930	2.14	
10.17–10:27	9.1	29	320	1.06	
10.27–10.37	11.8			0.88	
10.37–10.47	9.5	1.7*	18	0.57	
10.47–10.57	11.5	1.6*	14	0.43	

Total lactic acid excreted due to exercise 720 mg.

All specimens gave negative tests for protein, acetoacetic acid, and acetone.

* Single analysis.

lined by Clausen. Distillation with sulfuric acid instead of permanganate was used. We found that considerable experience was necessary before suitable analyses could be obtained on pure zinc lactate solutions. The part of the procedure which was most

difficult to control was the oxidation and distillation into the sodium bisulfite solution, using a vigorous current of air. Most

TABLE III.

Lactic acid in urine. 15 min. periods. Subject Li. Apr. 3, 1924. Stair running, 3 min. (10 round trips).

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	Remarks.
a.m.	*cc.*	*mg.*	*mg. per 100 cc.*	
9.30– 9.45	13.0	1.4	11	
9.45–10.00	15.0	1.7	11	9.50 a.m. drank 200 cc. water.
10.00–10.15	18.5	129	700	Exercise 10.00–10.03 a.m.
10.15–10.30	17.5	56	320	
10.30–10.45	34.5	3.5	11	
10.45–11.00	31.5	1.8	6	
11.00–11.15	32.5	2.3	7	

Total lactic acid excreted due to exercise 180 mg.

All specimens gave negative tests for protein, acetoacetic acid, and acetone.

TABLE IV.

Lactic acid in urine. 10 min. periods. Subject Li. May 26, 1924. Stair running, 2 min.

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	Remarks.
p.m.	*cc.*	*mg.*	*mg. per 100 cc.*	
2.00–2.10	9.0	1.3	14	
2.10–2.20	10.0	1.6	16	
2.20–2.30	7.0	47	670	Exercise 2.20–2.22 p.m.
2.30–2.40	8.3	86	1040	
2.40–2.50	5.0	12	240	
2.50–3.00	3.6	1.2	33	
3.00–3.10	6.8	1.8	26	
3.10–3.20	6.0	1.8	30	

Total lactic acid excreted due to exercise 140 mg. Negative tests for protein except trace in first specimen after exercise; this and the following specimen gave negative tests for acetoacetic acid.

of the trouble was obviated by introducing a thermometer into the distilling tube so that the bulb was beneath the surface of the

liquid. If the temperature of the boiling liquid was maintained between 145° and 150° during the distillation a quantitative recovery of the aldehyde could be made. The temperature of the boiling liquid was from 5° to 10° lower than that of the metal bath outside. Water was regularly added by way of the capillary intake tube to keep the concentration of H_2SO_4 approximately 1:1 and thereby eliminate charring. The distillation was continued for 1 hour. The blank for 0.01 N iodine was 0.07 cc. and for 0.001 N iodine, 0.7 cc. The following qualitative tests were made on many of the specimens of urine: protein, heat, and acetic acid; acetoacetic acid, ferric chloride; acetone, Rothera's test.

DISCUSSION.

The normal urines when analyzed by Clausen's method gave results ranging from 1 to 2.5 mg. of lactic acid excreted in 10 minutes. These figures agree with those of Ishihara (1913), Dapper (1913), and Clausen (1922). As lactic acid has never been isolated from normal urine it is doubtful whether these analyses represent lactic acid. After exercise the excretion rose suddenly, reaching a maximum in the second 10 minute period. During this period the excretion ranged in different experiments from 85 to 630 mg. After 20 minutes the excretion fell rapidly and at the end of 30 to 50 minutes lactic acid had probably disappeared from the urine as normal figures were again obtained. Allowing for normal figures we have calculated that a total of 140 to 1370 mg. of lactic acid were excreted after these short periods of strenuous exercise. In all of the experiments Subject W. excreted more lactic acid than Subject Li. after the same amount of work.

A few experiments were carried out in which a second period of exercise was undertaken 30 or 40 minutes after the first to determine whether the lactic acid excretion would be influenced by the previous exercise. The variations in the urine and the total quantity of lactic acid excreted were similar to those found after a single period of exercise. The maximum quantity of lactic acid excreted in the two periods of one experiment was 2.16 gm.

The concentration of lactic acid in the normal urines ranged from 6 to 21 mg. per 100 cc. After exercise the maximum con-

centration varied from 700 to 1840 mg. per 100 cc. In some of these urines the concentration of lactate may have been higher than any other solid constituent of the urine.

The concentration of lactic acid in the urine as well as the quantities excreted reached maxima, in most instances, during the second 10 minute period and then decreased rapidly, reaching normal values in from 30 to 50 minutes after exercise. The rise and the return to normal proceed at about the same rate as that

TABLE V.

Lactic and phosphoric acids in urine. 10 min. periods. Subject Li. Apr. 30, 1924. Stair running, two periods of 2 min. each (8 round trips each time).

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	PO_4	Remarks.
a.m.	*cc.*	*mg.*	*mg. per 100 cc.*	*cc. 0.1 M.*	
8.30– 8.40	10.0	1.3	13	1.56	Drank 200 cc. water.
8.40– 8.50	6.5	1.0	15	0.81	
8.50– 9.00	12.5	103	830	1.44	Exercise 8.50–8.52 a.m.
9.00– 9.10	15.2	131	860	2.04	Slight perspiration.
9.10– 9.20	7.7	26	340	1.03	
9.20– 9.30	12.1	76	630	1.16	Exercise 9.20–9.22 a.m.
9.30– 9.40	8.1	72	890	1.34	Moderate perspiration.
9.40– 9.50	8.5	15	176	0.46	Drank 100 cc. water.
9.50–10.00	8.5	2.1	25	0.46	
10.00–10.10	11.3	1.4	12	0.39	
10.10–10.20	13.3	1.3	10	0.36	
10.20–10.30	17.0	1.2	7	0.27	

Total lactic acid due to exercise: first period, 250 mg.; second period, 160 mg. Single analyses only.

observed for other urinary constituents reported in the previous paper (Wilson, Long, Thompson, and Thurlow, 1925). In two experiments, the phosphate excretion was again followed. Hill, Long, and Lupton (1924) have found that the concentration of lactic acid in the blood rises rapidly with exercise and reaches a maximum probably in 5 minutes or less after its cessation. Their data indicate, however, that the return to normal is gradual and not complete for 1 to 1½ hours. Whether the difference in time

necessary to reach normal levels in blood and urine is due to individual variations or to imperfections inherent in the quantitative method used (Long, 1923–24) or to a definite threshold for lactic acid excretion is impossible to state at present.

The elimination of 1 to 2 gm. of lactic acid involves the removal from the body of 11 to 22 mM of base because lactic acid is practically completely combined with base at the reaction of the urine. This is an appreciable quantity of base to be furnished in addition

TABLE VI.

Lactic acid in urine. 10 min. periods. Subject Li. June 26, 1924. Stair running, two periods of 2 min. each (8 round trips each time).

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	Remarks.
a.m.	*cc.*	*mg.*	*mg. per 100 cc.*	
8.00– 8.10	6.5	1.2	18	Drank 300 cc. water.
8.10– 8.20	7.5	1.6	21	Room temperature 21.7°C.
8.20– 8.30	9.0	94	1040	Exercise 8.20–8.22 a.m.
8.30– 8.40	10.7	128	1200	Considerable perspiration.
8.40– 8.50	6.0	53	880	
8.50– 9.00	10.7	155	1450	Exercise 8.50–8.52 a.m.
9.00– 9.10	10.7	197	1840	Considerable perspiration.
9.10– 9.20	7.2	92	1280	
9.20– 9.30	5.2	10	192	
9.30– 9.40	6.4	2.5	39	
9.40– 9.50	5.9	1.4	24	
9.50–10.00	8.8	2.1	24	

Total lactic acid due to exercise: first period, 270 mg.; second period, 450 mg. Single analyses only.

to the quantity necessary to form the salts of hydrochloric, sulfuric, and phosphoric acids. Replacement of lost base is most simply accomplished by an increased CH of the urine which permits the excretion of phosphate with the minimum amount of base, and by the increased elimination of ammonia. Such an adjustment was probably at work in the control experiment of Subject W. on May 30 (reported in the previous paper), carried out in the afternoon after a double exercise experiment in the morning. The pH of the urine was 5.3 whereas in all other

experiments in which the same individual was the subject the pH was found to range between 6.2 and 7.4 at the same time of day. In the morning 2.16 gm. of lactic acid had been excreted with the removal of 24 mM of base.

In looking over the literature, we failed to find that the lactic acid obtained from urine after exercise had been identified as dextro- or sarcolactic acid. It is known to be formed in muscle during exercise and to be present in urine in various pathological

TABLE VII.

Lactic acid in urine. 10 min. periods. Subject W. May 30, 1924. Stair running, two periods of 2 min. each (8 round trips each time).

Time.	Urine volume.	Lactic acid.	Lactic acid concentration.	Remarks.
a.m.	*cc.*	*mg.*	*mg. per 100 cc.*	
9.10– 9.20	20.2	1.6	8	Drank 100 cc. water.
9.20– 9.30	13.8	1.4	10	
9.30– 9.40	23.0	197	860	Exercise 9.30–9.32 a.m.
9.40– 9.50	48.5	632	1300	Slight nausea and dizziness with perspiration.
9.50–10.00	25.8	410	1590	
10.00–10.10	13.5	139	1030	
10.10–10.20	14.3	147	1030	Exercise 10.10–10.20 a.m.
10.20–10.30	30.0	396	1320	
10.30–10.40	17.0	166	980	
10.40–10.50	9.8	71	720	
10.50–11.00	10.0	18*	180	
11.00–11.10	10.0	2.8*	28	

Total lactic acid due to exercise: first period, 1370 mg.; second period, 790 mg.

* Single analysis.

conditions, but the few investigators who have isolated lactic acid (usually as the zinc salt) from urine after exercise or after strychnine convulsions have failed to prove that they had the dextro form. The active form may be identified by the analysis of the hydrated crystals (zinc dextro-lactate crystallizes with 2 molecules of water of crystallization and inactive zinc lactate with 3) or by determining the specific rotation. As the water content of the crystals is said to be variable (Fletcher and Hopkins,

1906–07), many investigators have been satisfied to analyze the dried crystals for zinc to identify their preparations. Spiro (1877–78) isolated zinc lactate from tetanized animals and obtained a small quantity of impure material from the urine of men after mild exercise. Colasanti and Moscatelli (1887) isolated zinc lactate from the urine of soldiers after a forced march. In neither investigation were the data sufficient to demonstrate that the zinc salt of dextro-lactic acid was obtained.

After determining the lactic acid in urine following exercise, the remaining urine from one experiment was extracted, as in the quantitative procedure, and zinc lactate prepared by heating the ether extract with water and zinc carbonate. After evaporating off the ether and filtering, the solution was concentrated to a small volume and allowed to stand. The zinc lactate obtained was recrystallized once from water and analyzed.

Water found, 13.2 per cent. Theoretical for zinc sarcolactate, 12.9 per cent. Theoretical for inactive zinc lactate, 18.2 per cent.

0.3602 gm. of dried salt (representing 0.4153 gm. of hydrated salt) was dissolved in water and made up to 10 cc. Using a 1 dm. tube, $\alpha = -0.31°$ at about 20°. $[\alpha]_D = -7.5°$ for hydrated salt. For a 4.18 per cent solution (Hoppe-Seyler and Araki, 1895), $[\alpha]_D = -7.51°$.

Zinc found, 26.1 per cent of anhydrous salt. Theoretical for zinc sarcolactate, 26.8 per cent.

A few mg. of the dried salt gave a strongly positive reaction with Hopkins' thiophene test.

The isolation of zinc sarcolactate demonstrates that it is the dextro- or sacrolactic acid which is present in urine after strenuous exercise.

SUMMARY.

The excretion of lactic acid in urine after 2 to 3 minutes of strenuous exercise was studied in 10 and 15 minute periods. The excretion reached maxima varying from 86 to 630 mg. during the second 10 minute period after exercise. Thereafter, the elimination diminished and normal values were reached in 30 to 50 minutes.

The total quantity of lactic acid excreted after one period of

exercise varied from 140 to 1370 mg. A second period of exercise following shortly after the first yielded similar quantities.

The lactic acid was shown to be the dextro form.

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