Influence of Some Constituents of Serum on Gas Metabolism of Tissue in vitro.

III Report.

Influence of Sodium Lactate upon Tissue Respiration in vitro.

By

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Relation between the respiration and the formation or breakdown of lactic acid in animal bodies has been extensively studied in muscle tissue. But the experiment was formerly made on whole animal or organs.

Warburg's¹⁾²⁾³⁾ manometric method for surviving tissue slices has given us a more precise means for studying metabolic processes in tissues.

The method was first applied by Meyerhof⁴⁾⁵⁾ and his collaborators for investigating influence of lactic acid on tissue respiration. They found that addition of lactate to Ringer's solution caused increase of the respiration of various tissues such as muscle, liver, kidney and others. They considered further that the action of serum in increasing the tissue respiration was due to presence of lactate in the serum. Loebel⁶⁾ also confirmed the results of the above investigators in his experiment on brain tissue.

We confirmed in our first report⁷⁾ that the active factors which effected an increase of tissue respiration in serum had to be diffusible matters and then found glucose as one of the diffusible effective substances.

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1) Warburg, O.; Biochem. Z., 1923, **142**, 317.

2) Id.; ibid., 1924, **152**, 51.

3) Id.; ibid., 1925, **164**, 481.

4) Meyerhof, O., K. Lohmann and R. Meier; Biochem. Z., 1925, **157**, 459.

5) Meyerhof, O. and K. Lohmann; Biochem. Z., 1926, **171**, 381.

6) Loebel, R. Q.; Biochem. Z., 1925, **161**, 219.

7) Yamamoto, H.; Tohoku J. Exp. Med., 1938, **33**, 454.

As the second effective substance lactate was taken into consideration and was experimented on in reference to its influence on tissue respiration of kidney cortex.

Method.

The method for measurement of oxygen consumption and carbon dioxide output of the tissues was Warburg's second one.²⁾ Tissue used was cortical one of rabbit kidney. Rabbit was starved 24 hours before the experiment. The tissue was perfused with the normal Ringer's solution⁷⁾ (containing 0.2% glucose) before removal of the kidneys. The kidney was kept in the same Ringer's solution before and after having been dissected into thin slices. Gas for the experiments was 3.5 per cent carbon dioxide in oxygen. The pH of the solution suspending tissue slices in experimental vessels was 7.32 when saturated with this gas. Manometric readings were taken every 20 minutes during two hours and Q_o and Q_c were calculated for each 20 minutes.

Ringer's solutions containing 10, 20 and 60 mgm per cent sodium lactate (Katayama, chemical pure.) were prepared, and tissue respiration in these solutions were compared with that in the normal Ringer's solution.

The series of experiments were done simultaneously with the tissue slices from the same kidney. Ringer's solutions suspending tissue slices contained in one series the above cited concentrations of lactate and in the other series no lactate. The latter series served as a control experiment for the former.

Experiment.

1. *Effect of 10 mgm per cent sodium lactate in Ringer's solution upon tissue respiration of kidney cortex in vitro.*

Table 1 (Fig. 1) is the protocol of this experiment.

The means of the oxygen consumption Q_o and carbon dioxide output Q_c of Table 1 a for two hours were 18.3 and 15.9 cmm respectively, of which the means for the first hour were 20.3 and 17.6 cmm and those for the second 16.5 and 14.1 cmm.

The means of Q_o and Q_c of Table 1 b (control experiment for 1 a) for two hours were 14.9 and 12.6 cmm respectively, of which the means for the first hour were 16.0 and 13.6 cmm and those for the second 13.7 and 11.5 cmm.

The increase of Q_o by addition of 10 mgm per cent sodium lactate to Ringer's solution was 23% for two hours, that for the first and second hour was 27% and 20% respectively. The increase of Q_c by addition of lactate was 26% for two hours, that for the first and second hour was 29% and 23% respectively.

There was no appreciable difference between the R.Q. by addition of lactate.

2. *Effect of 20 mgm per cent sodium lactate in Ringer's solution upon tissue respiration of kidney cortex in vitro.*

Influence of sodium lactate of 20 mgm per cent was investigated.

The means of the oxygen consumption Q_o and carbon dioxide output Q_c of Table 2 a were 18.9 and 15.7 cmm respectively for two hours, of which the means for the first hour were 19.9 and 16.9 cmm and those for the second 17.9 and 14.8 cmm.

The means of Q_o and Q_c of Table 2 b (control experiment for Table 2 a) were 17.6 and 15.5 cmm respectively for two hours, of which the means for the first hour were 20.3 and 18.3 cmm and those for the second 15.0 and 12.6 cmm.

The increase of Q_o in the presence of 20 mgm per cent sodium lactate in Ringer's solution was 7% for two hours; for the first hour a slight decrease was appreciable, however, and for the second hour the increase was 19%.

The increase of Q_c by addition of 20 mgm per cent lactate for two hours was 3%; for the first hour the decrease was appreciable (8%), but for the second hour the increase was found to be 17%.

In this case the effect of sodium lactate became with the lapse of time evident.

R. Q. were rather higher in the normal Ringer's solution for the first hour.

3. *Effect of 60 mgm per cent sodium lactate in Ringer's solution upon tissue respiration of kidney cortex in vitro.*

Sodium lactate was added in concentration of 60 mgm per cent to Ringer's solution and the tissue respiration in this solution was compared with that in the normal Ringer's solution.

Results were shown in Table 3 (Fig. 3).

TABLE
Effect of 10 mgm per cent sodium lactate in Ringer's
(a) Tissue respiration of rabbit kidney cortex in Ringer's

No.	Date	Qo						Qc		
		I 20	II 20	III 20	IV 20	V 20	VI 20	I 20	II 20	III 20
1	23/6	38.4	31.8	30.9	27.0	23.3	21.1	38.7	32.3	30.8
2	24/6	22.0	17.1	19.0	16.3	10.9	17.8	19.6	14.1	16.2
3	25/6	20.2	17.3	16.3	15.1	15.2	15.7	16.2	13.6	13.0
4	26/6	20.6	13.1	11.6	24.5	17.2	12.3	20.1	9.4	8.6
5	27/6	29.9	18.2	15.5	16.5	15.4	13.5	28.6	15.0	13.1
6	30/6	18.9	19.9	20.8	17.3	15.3	16.9	14.0	16.1	17.3
7	1/7	21.4	17.4	15.6	14.1	15.6	7.8	19.6	14.4	12.6
8	1/7	14.5	22.8	15.4	16.0	13.4	16.6	10.0	17.9	11.9
	Mean	23.2	19.7	18.1	18.4	15.8	15.2	20.9	16.6	15.4
		±1.74	±1.23	±1.30	±1.19	±0.71	±0.96	±2.05	±1.36	±1.43

(b) Tissue respiration of rabbit kidney cortex in

No.	Date	Qo						Qc		
		I 20	II 20	III 20	IV 20	V 20	VI 20	I 20	II 20	III 20
1	23/6	9.0	10.5	9.8	11.6	12.1	12.7	6.8	8.9	7.4
2	24/6	19.6	16.1	13.7	16.3	11.4	16.3	18.1	13.6	11.0
3	25/6	24.1	19.1	17.3	14.3	15.5	15.6	22.9	17.1	15.0
4	26/6	15.0	10.1	16.5	15.9	15.4	13.0	12.6	6.5	13.3
5	27/6	21.4	17.2	19.7	17.9	16.8	14.7	18.7	15.2	18.2
6	30/6	13.8	17.0	14.6	13.1	10.0	14.2	11.2	14.0	12.0
7	1/7	18.1	17.1	11.5	10.0	12.6	9.1	17.8	15.3	8.7
8	1/7	22.3	19.1	11.6	13.9	12.0	15.2	19.3	15.4	7.7
	Mean	17.9	15.8	14.3	14.1	13.2	13.9	15.9	13.3	11.7
		±1.27	±0.87	±0.86	±0.63	±0.64	±0.54	±1.37	±0.88	±0.95

TABLE
Effect of 20 mgm per cent sodium lactate in Ringer's
(a) Tissue respiration of rabbit kidney cortex in Ringer's

No.	Date	Qo						Qc		
		I 20	II 20	III 20	IV 20	V 20	VI 20	I 20	II 20	III 20
1	12/6	31.3	35.4	29.1	22.2	20.9	18.9	30.2	35.6	27.1
2	13/6	20.9	20.2	17.4	16.1	14.7	15.4	16.7	16.0	14.0
3	13/6	16.3	22.1	13.4	16.5	21.0	19.1	11.9	18.1	12.2
4	17/6	13.1	25.3	17.1	14.1	12.4	14.3	10.6	20.1	14.1
5	18/6	14.1	20.3	16.0	17.1	12.7	16.8	11.6	15.7	12.4
6	19/6	31.6	21.2	10.0	23.8	33.0	28.5	26.4	18.9	6.4
7	20/6	25.2	11.4	17.7	18.0	18.8	14.3	19.0	7.4	13.0
8	20/6	13.9	18.0	17.4	15.1	13.5	12.8	14.0	17.6	16.2
	Mean	20.8	21.7	17.3	17.9	18.4	17.5	17.6	18.7	14.4
		±2.06	±1.40	±1.00	±0.83	±1.61	±1.11	±1.84	±1.49	±1.15

1.

solution upon tissue respiration of kidney cortex in vitro.

solution with 10 mgm per cent sodium lactate.

			R. Q.					
IV 20	V 20	VI 20	I 20	II 20	III 20	IV 20	V 20	VI 20
24.7	21.2	20.0	1.01	1.02	1.00	0.92	0.91	0.95
13.7	8.0	15.6	0.89	0.83	0.85	0.84	0.74	0.88
12.6	12.4	13.6	0.80	0.79	0.80	0.83	0.82	0.87
23.2	14.9	11.0	0.98	0.72	0.74	0.95	0.87	0.90
14.2	13.3	11.3	0.96	0.82	0.85	0.86	0.86	0.84
14.7	13.0	14.7	0.74	0.81	0.83	0.85	0.85	0.87
11.4	14.9	4.2	0.92	0.83	0.81	0.81	0.96	0.54
13.1	9.3	13.7	0.69	0.79	0.77	0.82	0.69	0.83
16.0	13.4	13.0	0.87	0.83	0.83	0.86	0.84	0.84
±1.29	±0.87	±1.00	±0.03	±0.02	±0.02	±0.01	±0.02	±0.02

the normal Ringer's solution.

			R. Q.					
IV 20	V 20	VI 20	I 20	II 20	III 20	IV 20	V 20	VI 20
9.3	9.5	10.3	0.76	0.85	0.76	0.80	0.79	0.81
13.6	8.8	13.8	0.92	0.85	0.81	0.83	0.77	0.85
12.3	13.6	13.7	0.95	0.90	0.87	0.86	0.88	0.88
13.0	12.9	10.7	0.84	0.64	0.81	0.82	0.84	0.82
16.8	14.5	13.4	0.87	0.88	0.92	0.94	0.86	0.91
11.1	8.5	12.0	0.81	0.82	0.82	0.85	0.85	0.85
7.3	11.1	6.7	0.98	0.89	0.76	0.73	0.88	0.74
11.3	9.0	12.9	0.87	0.81	0.66	0.81	0.75	0.85
11.8	11.0	11.7	0.88	0.83	0.80	0.83	0.83	0.84
±0.67	±0.65	±0.59	±0.02	±0.02	±0.02	±0.01	±0.01	±0.01

2.

solution upon tissue respiration of kidney cortex in vitro.

solution with 20 mgm per cent sodium lactate.

			R. Q.					
IV 20	V 20	VI 20	I 20	II 20	III 20	IV 20	V 20	VI 20
19.7	19.9	16.3	0.96	1.01	0.93	0.89	0.95	0.86
12.4	11.5	12.6	0.80	0.79	0.80	0.77	0.78	0.82
13.2	19.7	16.9	0.73	0.82	0.91	0.80	0.94	0.88
11.6	9.5	11.5	0.81	0.79	0.82	0.82	0.77	0.80
13.5	9.4	14.3	0.82	0.77	0.78	0.79	0.74	0.85
20.0	30.4	27.7	0.84	0.89	0.64	0.84	0.92	0.97
13.8	15.2	11.4	0.75	0.65	0.74	0.77	0.81	0.80
13.5	11.3	11.0	0.01	0.98	0.93	0.90	0.84	0.86
14.7	14.6	15.2	0.84	0.84	0.82	0.82	0.84	0.86
±0.82	±1.74	±1.22	±0.02	±0.03	±0.03	±0.01	±0.02	±0.01

(b) Tissue respiration of rabbit kidney cortex in

No.	Date	Qo						Qc		
		I 20	II 20	III 20	IV 20	V 20	VI 20	I 20	II 20	III 20
1	12/6	24.4	20.5	18.8	18.8	16.6	10.1	22.3	18.8	16.4
2	13/6	30.6	16.8	14.3	18.1	18.2	13.2	27.0	13.5	11.3
3	13/6	22.9	19.0	15.3	15.0	13.9	15.2	21.0	17.5	13.2
4	17/6	18.7	13.1	13.7	9.9	9.8	11.6	18.0	10.8	11.6
5	18/6	14.1	23.8	17.0	17.4	18.3	16.5	12.2	22.1	14.9
6	19/6	25.2	18.8	16.3	16.4	15.4	14.7	22.5	17.4	14.1
7	20/6	38.0	30.6	26.7	24.5	18.3	18.1	36.5	28.9	24.8
8	20/6	21.3	16.1	8.1	9.1	7.3	13.8	21.7	15.9	7.6
	Mean	24.4	19.8	16.3	16.2	14.7	14.2	22.7	18.1	14.2
		±1.65	±1.23	±1.09	±1.15	±1.05	±0.63	±1.46	±1.23	±1.07

TABLE

Effect of 60 mgm per cent sodium lactate in Ringer's

(a) Tissue respiration of rabbit kidney cortex in Ringer's

No.	Date	Qo						Qc		
		I 20	II 20	III 20	IV 20	V 20	VI 20	I 20	II 20	III 20
1	4/10	25.5	26.0	21.9	16.5	14.9	14.0	22.2	22.4	18.2
2	7/10	33.1	27.4	22.2	16.7	13.1	14.5	31.8	23.5	19.3
3	8/10	19.0	17.2	17.6	15.0	18.4	15.6	15.4	12.6	13.6
4	9/10	27.6	15.9	22.6	18.1	13.2	15.5	27.0	13.1	17.8
5	10/10	20.0	18.4	20.1	13.2	11.0	16.4	15.3	13.4	15.7
6	10/10	16.8	14.3	20.4	18.6	13.5	18.9	12.0	9.3	14.3
7	21/10	19.0	20.9	20.6	16.7	15.5	17.6	14.1	16.3	15.7
	Mean	23.0	20.0	20.8	16.4	14.2	16.1	19.7	15.8	16.4
		±1.70	±1.41	±0.43	±0.45	±0.60	±0.46	±2.16	±1.46	±0.61

(b) Tissue respiration of rabbit kidney cortex in

No.	Date	Qo						Qc		
		I 20	II 20	III 20	IV 20	V 20	VI 20	I 20	II 20	III 20
1	4/10	18.8	20.9	20.6	16.8	17.3	14.6	15.5	17.5	17.4
2	7/10	12.9	14.9	13.7	18.6	16.3	14.7	11.2	12.7	11.4
3	8/10	19.1	25.5	19.9	14.3	15.8	15.2	16.7	23.5	17.2
4	9/10	14.6	19.4	13.9	21.7	12.5	16.5	13.0	18.4	10.1
5	10/10	24.2	18.5	20.2	20.3	15.2	18.0	22.4	16.1	17.6
6	10/10	21.0	17.6	18.0	19.0	16.7	14.2	18.2	15.7	15.2
7	21/10	21.2	16.1	15.8	13.9	13.0	10.3	18.6	13.0	12.9
	Mean	18.8	19.0	17.4	17.8	15.3	14.8	16.5	16.7	14.5
		±1.01	±0.87	±0.88	±0.83	±0.50	±0.53	±0.97	±0.92	±0.91

the normal Ringer's solution.

			R. Q.					
IV 20	V 20	VI 20	I 20	II 20	III 20	IV 20	V 20	VI 20
16.6	14.1	8.2	0.91	0.92	0.87	0.88	0.85	0.81
14.8	15.2	7.6	0.88	0.80	0.79	0.82	0.84	0.58
13.0	12.2	13.3	0.92	0.92	0.86	0.87	0.88	0.88
8.1	7.7	9.6	0.96	0.82	0.85	0.82	0.79	0.83
15.5	16.7	15.1	0.87	0.93	0.88	0.89	0.91	0.92
13.9	13.4	13.6	0.89	0.93	0.87	0.85	0.87	0.93
22.2	15.9	16.6	0.96	0.95	0.93	0.90	0.87	0.92
7.4	5.9	7.2	0.82	0.99	0.94	0.81	0.81	0.52
13.9	12.6	11.4	0.90	0.91	0.87	0.86	0.85	0.80
±1.07	±0.97	±1.04	±0.01	±0.02	±0.01	±0.01	±0.01	±0.04

3.

solution upon tissue respiration of kidney cortex in vitro.

solution with 60 mgm per cent sodium lactate.

			R. Q.					
IV 20	V 20	VI 20	I 20	II 20	III 20	IV 20	V 20	VI 20
12.1	10.8	10.4	0.87	0.86	0.83	0.73	0.73	0.74
13.5	10.0	11.9	0.93	0.86	0.87	0.81	0.77	0.82
11.8	15.3	13.1	0.81	0.73	0.77	0.79	0.83	0.84
14.7	9.7	12.4	0.98	0.83	0.79	0.81	0.74	0.80
9.2	7.5	13.2	0.77	0.73	0.78	0.70	0.68	0.80
14.5	10.3	15.8	0.72	0.65	0.70	0.78	0.76	0.84
12.6	11.7	14.6	0.74	0.78	0.76	0.75	0.75	0.83
12.6	10.8	13.1	0.83	0.78	0.79	0.77	0.75	0.81
±0.47	±0.55	±0.44	±0.03	±0.02	±0.01	±0.01	±0.01	±0.01

the normal Ringer's solution.

			R. Q.					
IV 20	V 20	VI 20	I 20	II 20	III 20	IV 20	V 20	VI 20
13.5	14.8	11.9	0.82	0.84	0.84	0.80	0.86	0.82
16.3	15.6	13.4	0.87	0.85	0.83	0.88	0.96	0.91
12.0	13.3	13.2	0.87	0.92	0.86	0.84	0.84	0.87
18.8	9.6	13.0	0.89	0.95	0.73	0.87	0.77	0.79
18.2	13.0	15.5	0.93	0.87	0.87	0.90	0.86	0.86
16.0	14.6	11.6	0.87	0.89	0.84	0.84	0.87	0.82
11.2	10.7	8.2	0.88	0.81	0.82	0.81	0.82	0.80
15.1	13.1	12.4	0.88	0.88	0.83	0.85	0.85	0.84
±0.86	±0.59	±0.54	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01

Fig. 1. Comparison of the tissue respiration of rabbit kidney cortex in Ringer's solution with 10 mgm per cent sodium lactate and in the normal Ringer's solution.

Fig. 2. Comparison of the tissue respiration of rabbit kidney cortex in Ringer's solution with 20 mgm per cent sodium lactate and in the normal Ringer's solution.

Fig. 3. Comparison of the tissue respiration of rabbit kidney cortex in Ringer's solution with 60 mgm per cent sodium lactate and in the normal Ringer's solution.

The means of the oxygen consumption Q_o and carbon dioxide output Q_c of Table 3 a for two hours were 18.4 and 14.7 cmm respectively, of which the means for the first hour were 21.3 and 17.3 cmm and those for the second 15.6 and 12.2 cmm.

The means of Q_o and Q_c of Table 3 b (control experiment for Table 3 a) for two hours were 17.2 and 14.7 cmm respectively, of which the means for the first hour were 18.4 and 15.9 cmm and those for the second 15.6 and 13.5 cmm.

As the above figures show, the difference caused by addition of 60 mgm per cent sodium lactate to Ringer's solution was quite small and within the limit of experimental errors.

R. Q. were relatively higher in the normal Ringer's solution than in the lactate-Ringer's solution.

Concluding Remarks.

Since Meyerhof and others^{4,5)} published their reports on the relation between lactate and tissue respiration, several investigators have studied on this subject.

Gerard⁸⁾ observed that sodium lactate, 0.1 per cent, had no effect on tissue respiration of nerve, though stronger solution caused some decrease. The sodium lactate concentration of 0.1 per cent is higher than the highest concentration of 60 mgm per cent in our case, with which we found only a slight change of the tissue respiration of kidney cortex. Loebel and Hickling, however,⁹⁾ in their studies on excised testicular tissue of rat observed that with m/40 (about 0.28 per cent) sodium lactate in the medium the oxygen consumption was greater and remained at almost the same level for several hours and that the R. Q. showed a definite rise. Richardson and his coworkers¹⁰⁾ found that the sections of dog kidney were influenced by sodium lactate (0.01 mol) resulting in raising the oxygen consumption and respiratory quotient. The increase of respiration amounted to 44%. Dickens and Simer¹¹⁾ found in their experiments on liver tissue a definitely high R. Q. in addition to an increased respiration in the presence of lactate (m/45). They¹²⁾ confirmed again later that the concentration of lactate in the serum was sufficient to cause a marked consumption of this substance by kidney tissue in serum. Kisch¹³⁾ also found an increased oxygen consumption of kidney and liver tissues by addition of m/50-m/5000 sodium lactate. The increase amounted to 10-50% in this case. He extended his studies on various other tissues and obtained more remarkable influences of a similar nature. Shaffer and others¹⁴⁾ observed that sodium lactate (100 mgm per cent) added to Ringer's solution produced no appreciable effect on nerve tissue respiration.

Some of the above cited investigators have shown that the tissue respiration was increased with relatively higher concentration (m/40) of sodium lactate in Ringer's solution. Our own results summarized in table 1, 2 and 3 show, however, that the increased extent of respiration was the most remarkable when sodium lactate concentration was 10 mgm per cent. With concentration of 20 mgm per cent the increase was less, and with that of 60 mgm per cent there was some appreciable

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8) Gerard, R. W.; *Am. J. Physiol.*, 1927, **82**, 381.

9) Loebel, R. O. and R. A. Hickling; *ibid.*, 1927, **81**, 494.

10) Richardson, H. B., E. Shorr and R. O. Loebel; *J. Biol. Chem.*, 1930, **86**, 551.

11) Dickens, F. and F. Šimer; *Biochem. J.*, 1930, **24**, 1301.

12) *Id.*; *ibid.*, 1931, **25**, 11.

13) Kisch, B.; *Biochem. Z.*, 1932, **253**, 346.

14) Shaffer, M., T. H. Chang and R. W. Gerard; *Am. J. Physiol.*, 1935, **111**, 697.

accelerating effect on the tissue respiration at the beginning of the experimental period. Against the remarks of other investigators respiratory quotient was not raised by addition of sodium lactate in our case.

At any rate, as the addition of 10 mgm per cent sodium lactate to Ringer's solution caused a remarkable and 20 mgm per cent a somewhat less remarkable increase of the rate of the tissue respiration in vitro (these concentrations being considered to be physiological¹⁶⁾), we are sure that the lactate is one of the factors which cause higher rate of tissue respiration in serum than in Ringer's solution.

Summary.

By Warburg's second method²⁾ with our modification effect of sodium lactate on tissue respiration of rabbit kidney cortex was investigated.

Sodium lactate was added to Ringer's solution in concentrations of 10, 20 and 60 mgm per cent.

With 10 mgm per cent the greatest increase was observed which amounted to 20–30% of the normal value.

With 20 mgm per cent the increase was a little less and with 60 mgm per cent we could find some accelerating effect at the beginning of the experimental period.

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16) Oinuma, S.; Japanese Anatomical and Physiological Constants and Data (Jap.), 1934, Okayama, 136.