THE EFFECT OF pH ON THE OXYGEN CONSUMPTION OF TISSUES.*

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INTRODUCTION.

In simple chemical oxidation such as the oxidation of pyrogallol or of a ferrous salt, the hydrogen ion concentration plays an important rôle. In general, as in the case of pyrogallol, a slight acidity depresses oxidation, and increasing alkalinity, even to a marked degree, greatly increases the rate of oxidation. It has been assumed that in general this applied also to biological oxidation, but there have been few observations upon this subject.

Recently Büchner and Grafe (1) concluded from their experiments, using the method of Warburg, that changes of pH had little effect on the oxygen consumption of finely cut tissue suspensions. These authors state that the oxygen consumption between pH 5 and 9 is practically constant and is diminished only at either extreme, gradually at first and then more markedly. These findings are rather surprising in view of our general knowledge of oxidation, but especially in the light of other investigations.

The work of Warburg (2) and McClendon and Mitchell (3) showed that in general an increase in hydroxyl ion concentration increased the oxygen consumption of sea urchin eggs and nucleated erythrocytes.

Bunzell (4) showed that the oxidase activity of plant material is markedly sensitive to changes in pH, and that from a complete inhibition at approximately pH 3, the activity increased up to pH 7. Unfortunately the alkaline side was not investigated.

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That the oxidation of fructose by molecular oxygen increased from pH 6.2 to 8.0 in a phosphate solution and increases in a glycocoll-NaOH buffer system up to pH 11.3 was recently shown by Warburg and Yabusoe (5).

Many of these observations are similar to the oxidation of pyrogallol in that the consumption of oxygen increases even after fairly marked alkalinity is reached. This is not always the case, as has been shown by Mathews and Walker (6) in a study of the oxidation of cysteine to cystine by molecular oxygen. These investigators found that this rate of oxidation had a fairly sharp optimum at an H ion normality of pH 8 and that slight changes in either direction greatly suppressed the rapidity of oxygen consumption. This is of special interest inasmuch as the -SH group undoubtedly plays an important part in biological oxidation. This point was further emphasized by the work of Hopkins (7) in the isolation from muscle of a thermostable compound, glutathione, with a maximum oxidative activity at approximately the pH of the blood (7.4 or slightly greater), and a marked inhibition at pH 6.8.

The optimum reaction for the oxidation of some substances may even be on the acid side of neutrality as recently has been shown by Fleisch (8). Using washed, minced muscle and citric acid, he showed that the optimum pH for the oxidation of the citric acid was about 6. At pH 7.6 the oxygen consumption was quite small in comparison.

Methods.

The tissues used in all the experiments were from rabbits killed instantaneously by a blow over the cervical spine. The animals were bled immediately from the carotid and jugular vessels as free of blood as possible. The tissues to be used were removed rapidly and chilled upon ice; the whole process before chilling was only of a few minutes duration. The tissues after being chilled were dissected as free as possible from connective tissue and ground up to a fine mince by means of a food chopper. Aliquot portions of the minced tissue were weighed out, about 15 cc. of Ringer's solution added, then 0.2 N acid or alkali, followed by dilution with Ringer's solution to the desired total volume.

The oxygen consumption was determined by means of the apparatus described in the preceding paper (9). Three flasks were mounted side by side in a frame to which a shaking motion was imparted. All determinations were made in a constant temperature water bath at 37.5°C.

The pH of the tissue suspensions was determined electrometrically immediately after the rate of oxygen consumption was determined. In general, the reaction of the tissue at the completion of the experiments was slightly more acid than the average during the determination. The magnitude of the changes of tissue pH after the addition of acids or alkalies at body temperature has been discussed in previous publications (10). Only on the alkaline side of neutrality is this change at all marked, but even this shift of pH during the short duration of our experiments would not alter appreciably the general results we obtained.

TABLE I.

Comparison of Rate of Oxygen Consumption of Heart Muscle, Skeletal Muscle, and Liver Tissue.

Time.	Heart muscle, 2.9 gm. + 10 cc. Ringer's solution. pH 6.71.	Skeletal muscle, 30 gm. + 30 cc. Ringer's solution. pH 6.68.	Liver tissue, 30 gm. + 30 cc. Ringer's solution. pH 6.66.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
15	48	35	94
50	109	98	185
70	135	142	220
90	165	162	250
Per gm. 1st hr. . .	44.2	4.1	7.2

RESULTS.

A comparison of the rates of oxygen consumption of heart and skeletal muscle and liver tissue from the same rabbit is presented in Table I.

The rate of oxygen consumption of minced heart muscle suspended in Ringer's solution was extremely great, 44.2 c. mm. per gm. the 1st hour, while that of skeletal muscle was only about one-tenth as great, 4.1 c.mm. per gm. the 1st hour. The oxygen consumption of liver tissue 7.2 c.mm. per gm. the 1st hour, was intermediate between that of heart and skeletal muscle. No attempt was made to adjust the reaction of the tissue suspensions

TABLE II.

Rates of Oxygen Consumption of Liver Tissue at Various pH Values.

Experiment 1.

Time.	20 gm. liver tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	5 cc. NaOH. pH 7.27.	10 cc. NaOH. pH 8.96.	15 cc. NaOH. pH 9.65.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
15	160	90	35
30	220	143	55
50	285	186	70
83	345	225	92
Per gm. 1st hr. . .	15.5	10.3	4.0

Experiment 2.

Time.	20 gm. liver tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	3 cc. NaOH. pH 6.90.	4 cc. NaOH. pH 7.05.	5 cc. NaOH. pH 7.30.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
15	50	42	72
30	90	120	160
60	162	210	222
90	220	285	298
Per gm. 1st hr. . .	8.1	10.5	11.1

Experiment 3.

Time.	20 gm. liver tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	4 cc. NaOH. pH 7.18.	6 cc. NaOH. pH 7.56.	7 cc. NaOH. pH 7.83.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
22	130	121	98
37	190	176	136
50	220	208	195
60	242	230	220
84	270	250	237
Per gm. 1st hr. . .	12.1	11.5	11.0

TABLE II—*Concluded.*

Experiment 4.

Time.	15 gm. liver tissue + 0.2 N NaOH, made up to 30 cc. with Ringer's solution.		
	2.25 cc. NaOH. pH 7.08.	3.00 cc. NaOH. pH 7.46.	3.75 cc. NaOH. pH 7.75.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
7	10	32	20
15	32	80	50
25	65	100	85
34	92	142	112
50	160	200	173
60	190	214	195
Per gm. 1st hr. . .	12.6	14.3	12.7

Experiment 5.

Time.	10 gm. liver tissue + 0.2 N HCl, made up to 25 cc. with Ringer's solution.		
	3.5 cc. HCl. pH 5.5.	1.5 cc. HCl. pH 5.87.	0.0 cc. HCl. pH 6.66.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
18	16	37	45
23	19	48	54
37	27	65	75
60	35	72	90
Per gm. 1st hr. . .	3.5	7.2	9.0

in this experiment and the comparisons are based upon the pH values that are obtained when tissues are removed from the animal organism; that is, approximately 6.4 to 6.7 (10). The rate of oxygen consumption in all tissues studied gradually decreased with time at 37.5°C. This was especially apparent during the 2nd hour of the experiment.

Since it was impossible to get more than a few gm. of heart muscle from a rabbit, all further studies were made upon skeletal muscle and liver tissue.

Table II presents the data of a series of experiments showing the effects of change in reaction on the rate of oxygen consumption of liver tissue. In the first four experiments, various amounts of

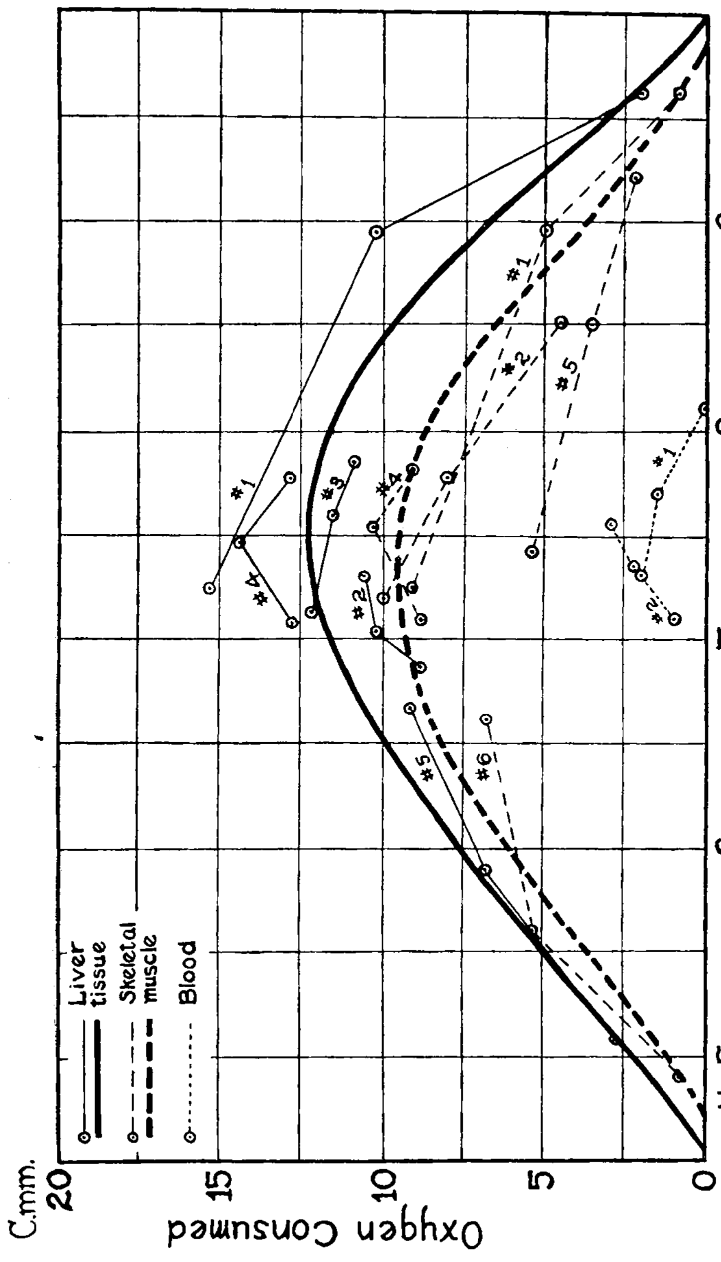

FIG. 1. Showing the c. mm. of oxygen consumed by 1 gm. of tissue or 1 cc. of blood during the 1st hour at various pH values.

TABLE III.

Rates of Oxygen Consumption of Skeletal Muscle Tissue at Various pH Values.

Experiment 1.

Time.	20 gm. muscle tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	5 cc. NaOH. pH 7.29.	10 cc. NaOH. pH 8.95.	15 cc. NaOH. pH 9.64.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
15	72	30	5
45	135	80	15
55	162	94	28
75	188	115	40
Per gm. 1st hr...	8.4	5.0	1.5

Experiment 2.

Time.	20 gm. muscle tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	5 cc. NaOH. pH 7.22.	7.5 cc. NaOH. pH 7.78.	10 cc. NaOH. pH 8.50.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
20	52	30	10
33	115	52	30
43	142	110	70
50	170	1 5	85
60	200	145	93
Per gm. 1st hr...	10.0	7.7	4.6

Experiment 3. Same rabbit as in Experiment 2 but muscle kept on ice for 24 hrs.

Time.	20 gm. muscle tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	6 cc. NaOH. pH 7.48.	8 cc. NaOH. pH 8.11.	12 cc. NaOH. pH 9.22.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
15	30	0	0
45	69	20	0
90	110	35	22
Per gm. 1st hr...	3.7	1.5	0.7

TABLE III—*Concluded.*

Experiment 4.

Time.	20 gm. muscle tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	4 cc. NaOH. pH 7.15.	6 cc. NaOH. pH 7.52.	7 cc. NaOH. pH 7.80.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
13	45	55	50
20	70	105	76
40	117	175	135
52	145	198	165
68	170	215	185
Per gm. 1st hr...	8.3	10.4	8.9

Experiment 5.

Time.	20 gm. muscle tissue + 0.2 N NaOH, made up to 40 cc. with Ringer's solution.		
	5 cc. NaOH. pH 7.46.	7.5 cc. NaOH. pH 8.50.	10 cc. NaOH. pH 9.25.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
9	50	8	0
30	97	18	15
48	117	40	30
61	122	60	48
80	140	70	69
100	160	83	78
Per gm. 1st hr...	6.0	3.0	2.4

Experiment 6.

Time.	20 gm. muscle tissue + 0.2 N HCl, made up to 40 cc. with Ringer's solution.		
	0.0 cc. HCl. pH 6.61.	3.0 cc. HCl. pH 5.58.	7.0 cc. HCl. pH 4.48.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
27	55	47	5
47	112	72	12
60	142	112	15
Per gm. 1st hr...	7.1	5.6	0.8

0.2 N NaOH were added so as to cover the pH range from 6.9 to 9.65. In Experiment 5, 0.2 N HCl was added to the liver suspension so as to extend the pH range further toward the acid side. At the extreme acid and alkaline limits, pH 5.5 and 9.65, oxygen consumption was greatly depressed. The maximum rates of oxygen consumption of liver tissue were obtained in those experiments in which the pH was adjusted approximately 7.3 to 7.6. In Fig. 1 (1) each experiment on liver tissue is represented by the light continuous lines and the general effect of pH on the oxygen consumption is approximated by the heavy curve.

TABLE IV.

Rates of Oxygen Consumption of Whole Blood, Freshly Drawn from Normal Individuals and Adjusted to Various pH Values.

Experiment 1. Blood from R. J. R.

Time.	15 cc. blood + 0.2 N HCl and made up to 30 cc. with Ringer's solution and aerated free of CO_2.		
	0.0 cc. HCl. pH 8.06.	1.5 cc. HCl. pH 7.71.	3.0 cc. HCl. pH 7.32.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
15	0	0	10
30	0	14	22
60	0	25	30
Per cc. 1st hr....	0	1.7	2.0

Experiment 2. Blood from J. C.

Time.	30 cc. blood + 0.2 N HCl and made up to 60 cc. with Ringer's solution and aerated free of CO_2.		
	4 cc. HCl. pH 7.51.	6 cc. HCl. pH 6.34.	7 cc. HCl. pH 7.12.
min.	*c. mm. oxygen*	*c. mm. oxygen*	*c. mm. oxygen*
10	20	17	15
15	33	26	22
37	72	55	38
60	90	64	50
120	165	105	92
Per cc. 1st hr....	3.0	2.1	1.3

Table III gives the data for similar experiments using skeletal muscle. Experiment 3 is a duplicate of Experiment 2, except that the minced muscle was kept on ice for 24 hours. Unfortunately the pH values were not quite the same, but it is apparent that the muscle tissue had lost a good deal of its power to consume oxygen. For the sake of comparison with liver tissue each individual experiment on skeletal muscle together with an ideal curve is represented by broken lines in Fig. 1. It will be noted that for any given pH, muscle tissue consumes on the average less oxygen than liver tissue, but that the general effect of change of pH is approximately the same for the two tissues.

The experiments both on muscle and liver tissue from different animals but otherwise under identical conditions, showed a considerable variation in ability to utilize oxygen. It is difficult to tell whether this variation was due to inherent differences in the vital tissues, such as food content or enzymatic conditions, or to subsequent changes either during or after death of the animals. The latter probably is more reasonable, for such factors as shock during death and differences in the completeness of the removal of the blood from the tissues could easily influence the rapidity of oxidation.

Table IV gives the data of two experiments on the effect of changes in pH of whole blood from normal individuals upon its ability to utilize oxygen. The experiments are plotted in Fig. 1 (dotted lines) and show the small consumption of oxygen as compared with the other tissues. The effect of changes of pH on the oxygen metabolism of the blood was roughly comparable to that of liver and muscle tissue, but enough points were not available to establish clearly a complete curve.

DISCUSSION.

That tissues continue to utilize oxygen for some time after their removal from the body has been proved abundantly and known for a long time. Careful quantitative studies under controlled conditions have, however, been few. An interesting question is the relationship of oxygen utilization in minced tissue suspensions to that of the intact organ within the animal. It is, of course, apparent that the two conditions are not comparable and that widely divergent figures should be expected. In the first place, in the mincing of the tissues, structures are destroyed. The character of tissues and cells plays an important part in intracellular reactions. This is well known and is thoroughly discussed and reviewed by Lillie (11). In the second place divergent results might be expected because of a different physiological environment, the absence of nervous control and tonus, limitation of food supply, and, perhaps of greatest importance, the change in oxygen tension. Normally the oxygen tension in tissues is very low, perhaps only a few mm., while in our experiments with atmospheric air, it, of course, was very much greater. However, our experiments with minced tissue in suspension agree in general with those on the intact organs by earlier workers. In particular the experiments of Barcroft and his associates (12) and Evans (13) are of interest for they demonstrated that the intact cat's liver used from 3.0 to 10.8 c. mm. of oxygen per gm. per hour while fasting and from 14.4 to 30.0 c. mm. per hour when the animal was previously fed. The average oxygen consumption of minced liver in our experiments at pH 7.4 was 10 to 15 c. mm. per gm. per hour, the animals having had no food for about 15 hours. In regard to the heart no comparisons can be made, for the heart-lung preparation studies of Evans were made on the contracting heart. The normally beating heart, according to Evans, consumed from 25.8 to 51.0 c.mm. of oxygen per hour. In our experiments at a pH of 6.7, the unadjusted pH of minced heart muscle, the oxygen consumption was 44.2 c.mm. per hour. This high rate of utilization is probably due to shreds of cardiac musculature still maintaining in part their property of rhythmic automaticity together with the increased oxidation due to the increased oxygen tension.

Our experiments also showed that liver tissue consumes more oxygen than skeletal muscle tissue, this probably is due to the greater storage of food together with the diversified metabolic activities of the former organ.

In respect to the effect of pH on the oxygen consumption of tissues, our results differ from those of Büchner and Grafe (1) who found little effect between pH 5 and 9. In all our experiments, including those on blood, the maximum rate of oxygen consumption was in the vicinity of the normal reaction of the blood, pH 7.4 to 7.5. These changes can best be seen from

Fig. 1. This optimum reaction is in harmony with the findings of Hopkins (7) in regard to glutathione, a substance that undoubtedly plays an important rôle in tissue oxidation.

It must be borne in mind that the depression of the oxidation on the alkaline side may not necessarily be due to an increase of pH within the cell. Although NaOH was added, in the presence of the CO_2 of the tissue this was changed to $NaHCO_3$, thereby increasing the amount of CO_2 present and thus probably increasing the diffusion of CO_2 into the cell. This view of increased cellular acidity resulting from the administration of $NaHCO_3$ was first advanced by Gesell (14).

It is also possible that the increased alkalinity does not directly effect the oxidative process in the tissue, but that the consumption of oxygen is hindered by altering the physical condition of the suspension. That such a physical change results upon addition of alkali to a tissue suspension is well known. In our experiments is was apparent because the suspensions upon addition of alkali appeared more viscid. However, it is reasonable to assume from the data given, that variation in either direction from the normal pH of the tissue of the living organism results in a depression of the oxidative process.

CONCLUSIONS.

The rate of oxygen consumption of the minced tissue suspensions studied is a function of the hydrogen ion concentration. The optimum pH was found to be in the vicinity of the normal reaction of the blood, pH 7.4 to 7.5. At pH values less than 4.5 and greater than 10, oxygen consumption is nearly completely depressed.

Different tissues are affected in a similar manner by changes in pH, but at the same pH, various tissues have different rates of oxidative activity. Heart muscle is most active, liver tissue much less, and skeletal muscle still less.

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