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THE COMBINATION OF CARBON DIOXIDE WITH MUSCLE: ITS HEAT OF NEUTRALIZATION AND ITS DISSOCIATION CURVE.

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The anaerobic formation of 1 g. of lactic acid in muscle is accompanied by a liberation of heat which is estimated by Meyerhof and Suranyi(1) as 380 to 390, say 385, calories. The origin of the whole of this heat is still not clear. According to these authors about 180 calories, according to Slater(2) 235 calories, are due to the formation of dilute dissolved lactic acid from hydrated glycogen, as calculated from the difference between the heats of combustion and from the heats of dilution and hydration. There remain 205 calories, or 150 calories, respectively, to be explained. Meyerhof(3, 4) has found the heat of acid dissociation of muscle proteins to be very high, namely about − 12,600 calories per g. molecule, so that if the whole of the lactic acid formed were neutralized by combination with the alkali protein of the muscle, there would be about 140 calories available, an amount practically sufficient to account for the remaining heat if we accept Slater's value for the heat of combustion of glycogen, some 65 calories too little if we accept Meyerhof's value. If, however, an appreciable fraction of the neutralization were at the expense of other buffers, for example K_2HPO_4, the heat of neutralization might be much less, namely for the fraction neutralized in this way about 1700 calories per g. molecule, *i.e.* 19 calories per g. of lactic acid (Meyerhof(3)). It is very necessary, therefore, to ascertain the nature of the reaction by which in fact lactic acid is neutralized in muscle.

Meyerhof and Lohmann(5) by alcoholic extraction of fatigued and unfatigued muscle arrived at the result that of all the base bound by lactic acid formed in muscle only one-half to two-thirds is derived from alkali protein, the remainder originating from other sources such

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as phosphate and carbonate. If this were so the neutralization of lactic acid by muscle should give rise only to 80 to 100 calories per g. of acid. It should be noted that bicarbonate cannot play any rôle in this respect unless time be given for the CO_2 formed on combination with acid to escape from the muscle, since the heat of neutralization of CO_2 by muscle is practically the same as that of lactic acid, and therefore if CO_2 be driven off from bicarbonate by lactic acid it merely combines with the alkali protein, thus liberating the same heat as if the lactic acid combined directly.

Since the nature of the alkali protein of the living muscle might very well be altered by chemical treatment and extraction, and since the major portion of the phosphate in muscle has been shown by Eggleton and Eggleton(6) to exist in organic combination and not simply in the inorganic form, the safest method of getting a true figure for the heat of neutralization of lactic acid in muscle is its direct determination. Meyerhof(3) investigated the heat liberated per g. of muscle when the upper legs of a frog were soaked in an approximately equal amount of Ringer's fluid containing valerianic acid in such a concentration as to give, after equi-partition, a concentration of 0·25 to 0·3 p.c. inside the muscle. He found values of about 0·3 calorie per g. of muscle, which would correspond roughly, in the case of lactic acid, to about 100 calories per g. of that acid neutralized. He did not determine, however, the final concentration of the valerianic acid inside the muscles, nor their final *p*H, and the time taken in each experiment for the acid to penetrate the whole muscle was very long—3 or 4 hours—thus rendering the heat measurements rather uncertain. Moreover, some control experiments in which the valerianic acid was changed for a neutral solution of sodium acetate in Ringer's fluid, gave heat about 0·1 calorie per g. of muscle as compared with about 0·3 under the action of the valerianic acid. Thus Meyerhof's experiments do not give any very precise idea of the absolute amount of heat liberated in muscle by the neutralization of lactic acid, though they do show that this is much higher than it would be were the buffers in muscle mainly phosphate and bicarbonate.

The present work represents a new attempt to determine directly the heat of neutralization of lactic acid by living muscle. Instead of a solution of valerianic acid, carbon dioxide gas was used, and instead of the upper legs of a frog the very small and thin sartorius mounted on a sensitive thermopile. The method, which has already been described by A. V. Hill ((7), p. 154), has the advantage, as compared with that of Meyerhof, that the diffusion of the gas may be complete in a few

minutes, owing to the thinness of the muscle, thus rendering the heat measurements more accurate. Moreover, the process is reversible: the carbon dioxide can be removed by a stream of nitrogen or oxygen and the muscles return to their original condition[1]. Hence in experiments on the heat of neutralization of carbon dioxide by muscle we are dealing with the action of living tissues in good condition. As found by Hill in the paper quoted, the carbon dioxide does not cause any appreciable change in the resting heat rate, so that in the deflection-time curve of the galvanometer the heat produced by the CO_2 can easily be separated from that due to resting processes occurring spontaneously in the muscle. The speed of diffusion of CO_2 is relatively high, and the measurement of the heat can be completed in a few minutes. Afterwards, if desired, the CO_2 can be removed by nitrogen and negative heat equal to the original positive heat will be found.

When an atmosphere of pure CO_2 is introduced around a muscle there is a rise of temperature due partly to the heat of solution of the gas and partly to the heat of combination of carbonic acid with the alkali available. The latter heat is the sum (*a*) of the negative heat of dissociation of carbonic acid into its ions, which is equal to -2800 calories per g. molecule (Landolt and Börnstein's Tables), and (*b*) of the positive heat of unionization of the buffering acid or acids involved. Thus, for our present purpose we need to know (1) the total heat, (2) the solubility of CO_2 in muscle, and (3) the CO_2 combining capacity of muscle at the temperature and the CO_2 partial pressure of the experiment. The total heat is measured. For the solubility we will assume the figure given by Fenn(9), according to whom a muscle dissolves an amount of CO_2 equal to that dissolved by 80 p.c. of its weight of water. The heat of solution of CO_2 at constant pressure is given by Brown and Hill(10). The experimental determination of the amount of combined CO_2 is described in a later part of this paper. Since in the heat experiments an atmosphere of nitrogen was changed for one of CO_2, the combined CO_2 was determined in both conditions and the difference calculated. The average amount of CO_2 combined in 1 g. of muscle at 740 mm. Hg partial pressure of CO_2 (which represents the average CO_2 partial pressure in the heat experiments) and at an average temperature of 19° C. was found to be 0·47 c.c. In nitrogen the CO_2 combined in the muscle was found to average about 0·02 c.c. per g. The difference, therefore—

[1] After removing the CO_2 the muscles are inexcitable but regain their excitability on soaking in Ringer's solution; the excitability is apparently of the reversible type described by Dulière and Horton(8).

0·45 c.c.—represents the extra amount of CO_2 combined when nitrogen was changed for CO_2.

Experimental. For the heat measurements the myothermic apparatus recently described by A. V. Hill(7) was employed. This apparatus consists of a sensitive thermopile, kept at a temperature constant within 0·001° C., connected to a moving coil galvanometer. It allows a fairly accurate measurement of the heat liberated at rest by a small and thin sartorius. During any process examined the extra heat liberated is given by the area of the deflection-time curve above its resting zero line. English and Dutch *Rana esc.* and *Rana temp.* were used without distinction. They were kept previously at 2° C. for some days. In nearly all the experiments a pair of sartorii was employed. After careful dissection they were mounted on the thermopile and kept in Ringer's solution at *p*H about 7·4, containing phosphate to the amount of 8 mg. p.c. P. The chamber containing thermopile, muscle and Ringer's solution was put into the bath at constant temperature and oxygen was bubbled through the solution in order to maintain the muscles in a good resting condition and to quicken the process of equalizing the temperature of the thermopile with that of the bath. After an hour or two the solution was sucked out and the stream of oxygen changed for one of oxygen-free nitrogen. After 40 to 45 minutes, when the muscles were entirely free from oxygen and the galvanometer had settled down to a constant position representing the resting heat-rate, the experiment was started. If resting muscles were the object of study the carbon dioxide gas was let in at this moment; if fatigued muscles were to be investigated the muscles were stimulated in nitrogen by single maximal break shocks, 42 per minute, and the heat liberated by stimulation recorded and calculated, as described by Hill. From this heat the amount of lactic acid produced per g. of muscle was estimated, assuming that 1 g. of lactic acid corresponds to 385 calories. After fatigue the carbon dioxide was run in as in the previous case.

In most experiments the carbon dioxide, which was carefully prepared free from oxygen, was introduced into the muscle chamber very slowly. In five experiments, however, it was introduced rapidly in order to change as quickly as possible the atmosphere in the chamber and so to allow the gas to act upon the muscles at its maximum partial pressure from the beginning of the diffusion. Most of the experiments were performed in April and May: five, however, in November gave practically the same results.

There is an immediate deflection when the first traces of carbon

dioxide come into the chamber, and this continues until an equilibrium is attained between the muscles and the gas. The experiment occupies 10 to 20 minutes. The area of the deflection-time curve was measured above a line joining the initial to the final steady position of the galvanometer, and the heat production was calculated from the area of this curve according to the method described by Hill(7).

In Table I are shown the results of experiments on resting muscle. The variations between different experiments are obviously comparatively small, allowing us safely to assume an average value of 0·380 calorie per g. of muscle.

TABLE I. Total heat produced by carbon dioxide in resting muscle.

Temperature	Heat by CO_2 per g. of muscle (in calories)	Remarks
18° C.	·360	
17·7° C.	·398	April and May
17·2° C.	·397	
20·0° C.	·407	
20·4° C.	·342	
,,	·406	
,,	·359	A. V. Hill ((7), p. 154), March
,,	·430	
18·7° C.	·373	November
19·5° C.	·321	
19·5° C.	·376	
Mean 19·3° C.	Mean ·379	

Now at 19° C. and 740 mm. Hg partial pressure (atmospheric pressure less water vapour pressure) the amount of CO_2 dissolved in 1 g. of muscle is 0·89 (*i.e.* the solubility coefficient of CO_2 in water) multiplied by 0·8 (*i.e.* Fenn's factor) multiplied by 740/760: result 0·695 c.c. The heat of solution of CO_2 at constant pressure, calculated by interpolation for 19·3° C. from the data in the paper by Brown and Hill(10), is 4820 calories per g. molecule. For 1 g. of muscle, therefore, the heat of solution of the carbon dioxide simply dissolved is $0·695 \times 4820/22,400 = 0·15$ calorie. The remaining $0·38 - 0·15 = 0·23$ calorie represents the heat of solution plus the heat of ionization plus the heat of neutralization of the 0·45 c.c. of carbon dioxide combined, or 11,450 calories per g. molecule. The heat of solution is 4820 calories and the heat of ionization is − 2800 calories, so that the heat of neutralization is $11,450 - 4820 + 2800 = 9400$ calories, say. This is 3/4 of the value given by Meyerhof, namely 12,600 calories, for the heat of neutralization by alkali-protein and seems to indicate that the resting muscle neutralizes acid to the extent of 3/4 by the unionization of its alkali-protein buffers, and only to the extent of 1/4 by other buffers. These other buffers are presumably

phosphates, since it is obvious that, in the present experiments with CO_2 and in any experiments in which CO_2 is not able to escape, bicarbonate cannot play any part. It should be noted that one atmosphere of CO_2 makes the muscle approximately as acid as the lactic acid maximum produced by fatigue (see below), so that presumably about 3/4 of the lactic acid liberated by stimulation to extreme fatigue is neutralized in the same way by the use of proteins rather than phosphate. Thus the neutralization of lactic acid in muscle is accompanied by the liberation of about 105 calories per g., *i.e.* a somewhat higher figure than that given by Meyerhof and Suranyi(1), according to whom it is about 80 calories.

From this appropriate determination of the part played by phosphate in the neutralization of carbon dioxide it is possible roughly to calculate its concentration in muscle. If 1/4 of the total combined carbon dioxide, *i.e.* 0·11 c.c. per g. of muscle, combines with phosphate, then 0·15 mg. of phosphate changes from K_2HPO_4 into KH_2PO_4. Now when a muscle in equilibrium with nitrogen is brought into equilibrium with carbon dioxide at a partial pressure of 740 mm. Hg, its *p*H decreases from about 7·2 to about 6. The ratio K_2HPO_4/KH_2PO_4 at *p*H 7·2 is 7/3, at *p*H 6 it is 1/9 (Sörensen's data quoted by Clark(11)). In 1 g. of muscle therefore, according to our calculation, 0·15 mg. of P is transformed from K_2HPO_4 to KH_2PO_4 by a process in which 7/10 of the total P in the form of K_2HPO_4 becomes 1/10. Thus 6/10 of the total P = 0·15 mg., and the total P = 25 mg. per 100 g. This calculation of course is only approximate, but it is interesting to obtain a figure of the same order of magnitude as is found by chemical estimation of the inorganic phosphate.

The investigation of the heat of combination of carbon dioxide in muscle has been extended to muscles containing various amounts of lactic acid as a result of previous stimulation under anaerobic conditions. Two series of experiments were performed, one on the heat by carbon dioxide, with the technique already described, another on the amount of carbon dioxide combined. In both cases the muscles after stimulation in nitrogen for various lengths of time were brought into equilibrium with an atmosphere of CO_2 at a partial pressure of about 740 mm. Hg.

Table II shows the results of the heat experiments. In column 3 instead of the concentration of lactic acid its heat of neutralization is given, calculated by multiplying the observed heat of stimulation by 105/385, which, according to the above results, represents the ratio of the heat of neutralization to the total heat accompanying the anaerobic formation of 1 g. of lactic acid in muscle. Column 6 gives the heat of combination of carbon dioxide per g. of muscle, calculated by subtracting from the total heat by carbon dioxide, the part due to the simple solution of this gas. The latter depends on the temperature; as this varied in the different experiments it seemed safer to make a separate correction for each case, instead of taking the average temperature and

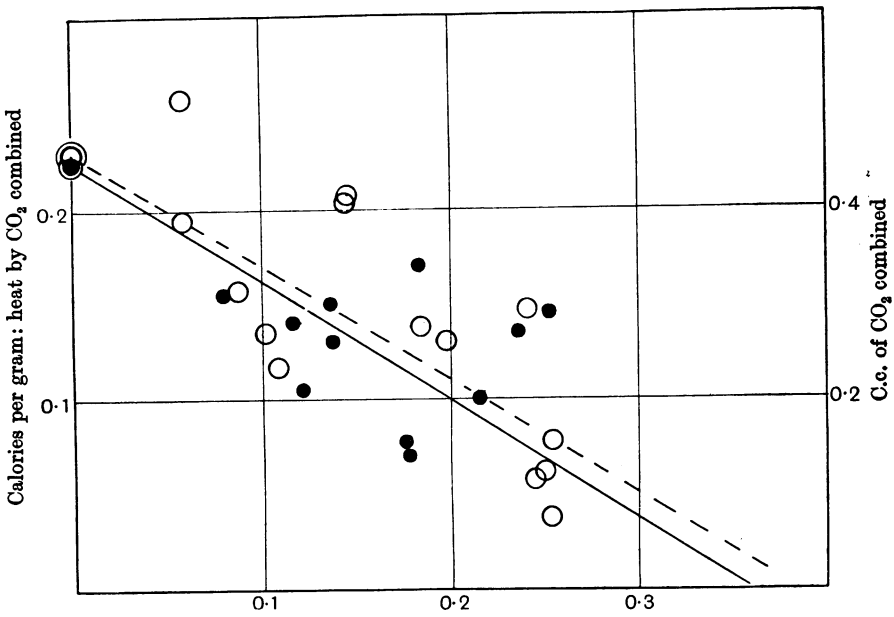

Fig. 1. Hollow circles and broken line: relation between total heat by combined CO_2, when muscles were subjected to 1 atmosphere of that gas, and heat by neutralization of lactic acid in previous anaerobic stimulation (heat calculated from heat observed during stimulation).

Full circles and continuous line: relation between amount of combined CO_2 and heat by neutralization of lactic acid in previous anaerobic stimulation (heat calculated from observed Σ T1 and the "isometric coefficient").

TABLE II. Heat of combination of carbon dioxide with fatigued muscle.

Partial pressure of CO_2 about 740 mm. Hg: muscles stimulated in nitrogen by single shocks, 42 per minute, to the degree indicated by the second column.

Temperature (°C.)	Heat by stimulation (calories per g. of muscle)	Heat by neutralization of lactic acid (calories per g. of muscle)	Heat by total CO_2 (calories per g. of muscle)	Correction for dissolved CO_2 (calories per g. of muscle)	Corrected heat by CO_2 combined (calories per g. of muscle)
17·6	·530	·145	·365	·158	·207
18·6	·214	·058	·348	·155	·196
17·2	·318	·087	·318	·161	·157
17·0	·720	·197	·292	·162	·130
17·5	·397	·108	·376	·158	·117
17·0	·921	·250	·224	·162	·062
17·7	·928	·253	·196	·158	·038
18·0	·372	·101	·290	·155	·135
18·0	·525	·144	·358	·155	·203
19·2	·930	·254	·226	·148	·078
19·7	·214	·058	·405	·147	·258
20·0	·885	·241	·293	·146	·147
19·4	·673	·184	·285	·147	·138
19·2	·897	·245	·205	·147	·058

making an average correction as was done for the experiments on resting muscle. In Fig. 1 the heat by carbon dioxide is plotted as hollow circles against the heat of neutralization of the lactic acid, and the broken line is drawn through the points so plotted.

In Table III are given the results of the experiments on the amount of carbon dioxide combined with muscles in various stages of fatigue. The heat was not measured in these experiments, but from the total tension which was observed in the series of twitches the amount of lactic acid formed was calculated from the "isometric coefficient of lactic acid" (see Hill(7), p. 169). In column 6 the heat of neutralization of the lactic acid so measured has been assumed to be equal to the concentration of this acid expressed in grams per gram of muscle, multiplied by 105 (see above).

TABLE III. Amount of carbon dioxide combined with fatigued muscle.

Partial pressure of CO_2 about 740 mm. Hg: muscles stimulated in nitrogen by very short tetanic stimuli, 42 per minute, the total tension being given in the second column. The lactic acid produced is calculated from the formula 1 g. cm. of tension-length $= 10^8$ g. of lactic acid.

Temperature (°C.)	Total tension developed (g.)	Muscles: Length (cm.)	Muscles: Weight (g.)	Lactic acid produced per 100 g. of muscle (g.)	Heat of neutralization of lactic acid (cal. per g. of muscle)	Amount of CO_2 combined in 1 g. of muscle (c.c.)
19·0	8,120	3·7	·27	·11	·116	·28
19·0	11,400	3·9	·215	·205	·215	·20
19·0	5,900	3·4	·130	·129	·136	·26
19·0	12,900	3·4	·180	·240	·252	·29
19·2	7,800	3·3	·150	·170	·178	·14
19·1	4,400	3·1	·175	·075	·079	·31
20·0	11,100	3·2	·210	·168	·176	·155
22·0	5,100	2·8	·125	·115	·121	·210
20·5	4,550	2·7	·095	·130	·136	·300
21·0	14,200	3·2	·203	·225	·236	·27
21·5	7,600	2·9	·125	·174	·183	·34

Fig. 1 shows the relation between amount of combined carbon dioxide (full circles) and heat by neutralization of lactic acid, and the continuous line is drawn through the points. A comparison of the points and the two lines in Fig. 1 shows that for a given increase by stimulation in the concentration of lactic acid in muscle, the decrease of the heat by carbon dioxide is, within the limits of experimental error, proportional to the decrease of the amount of combined carbon dioxide; so that the heat by combination of 1 g. molecule of carbon dioxide is the same in a muscle at rest as in a muscle previously stimulated to different degrees of fatigue.

The total heat liberated in the formation of 1 g. of lactic acid in

muscle, viz. 385 calories, is somewhat greater, on any reckoning, than the heat available in the chemical processes known to occur. Accepting Slater's value for the heat of combustion of glycogen, 340 calories should be liberated when 1 g. of lactic acid is formed from glycogen and neutralized: accepting Meyerhof's value, 285 calories. Some chemical process is still required to fill the gap.

THE CO_2 DISSOCIATION CURVE OF FROGS' MUSCLES.

As stated above, in calculating the heat liberated, per gram molecule, by CO_2 reacting with muscle, it is necessary to ascertain the amount of combined CO_2 in the resting tissue (*a*) kept in an atmosphere of nitrogen for some specified time, and (*b*) in equilibrium with an atmosphere of moist CO_2. The method of Warburg was employed, acid being tipped on to the muscle after equilibration, without other change in the conditions. A Barcroft differential manometer apparatus was used, the bottles being slightly modified to allow the muscles to stick on to the wall in a good extended position and so to reach equilibrium more quickly. A side tube with tap permitted the required gas to flow through the bottle, and thence to pass out through the three-way tap of the manometer without contact with the capillary tube of the latter. In the control bottle only a few drops of distilled water were placed, that is to say the arrangement was used differentially only so far as temperature changes were concerned. Since the method gave good and consistent results the experiments were extended to a wide range of CO_2 pressures, allowing the muscles to come into equilibrium with a series of known mixtures of nitrogen and CO_2, the combined CO_2 being determined as before by turning it out with acid. In this way a "CO_2 dissociation curve" of frogs' muscle was constructed.

The experiments were begun in May, 1928, and it was not realized until later that Fenn(9) had been engaged in a similar investigation. Since his method is different, and not entirely free from objection, the present experiments were continued. In principle Fenn's method consists in the sudden replacement of the gas (*e.g.* a CO_2 mixture) with which the tissue has come into equilibrium by another gas (*e.g.* oxygen), followed by the measurement of the CO_2 given out or taken up before a new equilibrium is reached. The replacement of the one gas by the other necessarily occupies a few seconds, and in this time it can be calculated from the diffusion equations (see *e.g.* Hill(12), p. 70) that a considerable fraction, perhaps as large as 15 p.c., of the CO_2 has escaped. Diffusion is extremely rapid in the first moments after such a sudden change in

conditions. In the method of turning out the combined CO_2 by acid no such change of conditions is necessary, and no error of this kind arises[1].

The partial pressures of CO_2 in Fenn's experiments did not exceed 250 mm. Hg. It is interesting to follow the curve to pressures greater than this, for two reasons: (*a*) by extending the range it is possible to determine its later slope more accurately, and (*b*) the higher pressures give us important information of the buffering capacity of muscles in the neighbourhood of *p*H 6·0, *i.e.* of isolated muscles stimulated to exhaustion, or of intact muscles in the living body after extremely violent exercise of short duration where the oxygen debt is high and the amount of lactic acid large. The complete range, therefore, up to one atmosphere of CO_2, has been studied in the present experiments.

Another difference between the present experiments and Fenn's is that the former employed mixtures of CO_2 and nitrogen, the latter mixtures of CO_2 and oxygen. The present experiments in pure nitrogen agree with a few of Fenn's in that gas, in showing only 2 to 3 volumes p.c. of combined CO_2: whereas for pure oxygen Fenn gives a figure of 8 to 10 volumes p.c., which in Fig. 3 below is seen to correspond to a partial pressure of CO_2 in nitrogen of 10 to 15 mm. Hg.

The difference is probably due to two factors: (*a*) the lactic acid formation in nitrogen, tending to displace CO_2 from bicarbonate, and (*b*) the formation of fresh CO_2 in oxygen. Taking the rate of lactic acid formation at 16° C. to be 0·2 mg. per g. per hour, and assuming that one molecule of lactic acid displaces one molecule of CO_2, this would mean a loss of 5 volumes p.c. of CO_2 per hour in a muscle standing in nitrogen. In the present experiments an interval of 45 to 50 minutes was allowed for equilibrium to be attained, so that the total CO_2 combining capacity might be expected to be diminished by 3·7–4·2 volumes p.c. A very rough calculation can be given of the amount of diffusible CO_2 contained in a resting frog's muscle in oxygen at 20° C. owing to the new formation. In a "steady state" (see Hill(12), p. 51), if α c.c. per g. per minute be the rate of formation of CO_2 in a muscle b cm. thick exposed to oxygen on one side only, and if k be the diffusion constant of CO_2, the amount contained, per c.c. of muscle, will be $\alpha b^2/3k$. Taking α as 7×10^{-4}, b as 0·1 cm., and k as $5{\cdot}4 \times 10^{-4}$ (calculated from Krogh(13)), this is 0·43 volume p.c. of diffusible physically dissolved CO_2, which would have an average partial pressure of about 4 mm. Hg. Thus there is an appreciable partial pressure of CO_2 in a muscle resting in pure

[1] Some experiments were performed by Fenn with a direct method, but he does not say how far these results agree with those made with his indirect method.

oxygen, due to its new formation: this pressure, owing to the shape of the dissociation curve, should be sufficient to account for the quantities found by Fenn. These two factors together are probably adequate to explain the difference between Fenn's experiments and those described here: both are affected by an error, and it is difficult to see how this can be completely avoided. The error due to the new formation of CO_2 by a muscle in oxygen is the more serious. One obvious precaution is to employ as thin muscles as possible in order either (*a*) to quicken equilibration in nitrogen, or (*b*) to lower the content of CO_2, due to new formation, in oxygen.

Experimental. As in the above experiments on the heat production, thin sartorius muscles only were employed, a pair for each experiment, weighing on the average 100 to 150 mg. the pair. They were dissected very carefully, cut as near as possible to their connections with the bone, and soaked for one hour in oxygenated Ringer's solution, containing 8 mg. p.c. P, at *p*H 7·3.

The muscles, although perhaps slightly injured owing to their removal from the bones, seemed always in a good resting condition, and never showed any shrinking or spontaneous twitches. Some of them, tested after soaking, were found to be normally responsive to electrical stimuli. The temperature of the room was 16° to 19° C. After an hour in Ringer's solution they were gently and quickly freed from adherent solution by touching with filter paper, weighed on a torsion balance, and placed in the muscle chamber of a Barcroft differential manometer, in a little side pocket *a* (see Fig. 2). At the bottom of the chamber 0·3 c.c. of a 20 p.c. solution of tartaric acid was run in: the chamber was fixed in position and evacuated by a water aspirator, in order to remove from the tissue and the acid solution all the carbon dioxide and oxygen present; a mixture of nitrogen and carbon dioxide in the proportions required was passed through, entering from the side tube *b* and leaving through the tap tube *c*—the gas was allowed to run slowly for 35 to 40 minutes, during which 1 litre of it passed through the bottle; then taps 1 and 2 were closed, putting the chamber into communication with the manometer, and both the bottles of the apparatus were placed in a water bath at 17° to 19° C. By shaking the apparatus and observing the reading of the manometer it was easy to make sure of the moment when the diffusion process had been completed and the acid solution and the muscle had reached equilibrium with the gases in the bottle. The acid was then poured on the muscles by gently tilting the apparatus, and the amount of carbon dioxide driven off determined

and calculated for N.T.P. The time required to drive out the combined carbon dioxide was 30 to 40 minutes, so that it was thought unnecessary

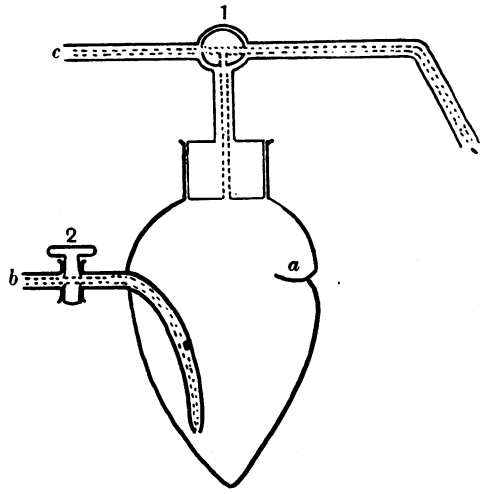

Fig. 2. Muscle chamber of Barcroft differential apparatus: *a*, side pocket for muscle; *b*, side tube for admitting gas; *c*, tap tube, for exit of gas.

to grind up the tissue as Fenn did. For the calibration of the apparatus a method suggested by Professor Barcroft was employed: this consists in introducing into one of the two bottles (in the present case the bottle which served as a muscle chamber) a known volume of air, by means of a mercury pump kept at the same temperature as the apparatus. The displacement in the level of the clove oil in the capillary tube of the latter was found to be directly proportional to the volume of air introduced. This method, compared with that of mixing inside the bottle a solution of Na_2CO_3 with one of acid, has the advantage of being much quicker and of eliminating a possible cause of error due to the presence of bicarbonate in the solution of Na_2CO_3.

The mixture of nitrogen and carbon dioxide was prepared in a bottle above acidulated water in diffusion equilibrium with the gas, by exhausting the bottle and then filling it up with the two gases in the ratio required. Water coming from a second bottle in communication with the first one and in equilibrium with the same partial pressure of carbon dioxide was used to force the gas through the muscle chamber. Two samples of the mixture, one at the beginning and one at the end of the procedure of passing the gas through the muscle chamber, were taken and analysed for carbon dioxide by the Haldane gas analysis apparatus.

In order to know exactly the amount of carbon dioxide combined it was necessary to find out whether all the gas coming out of the tissue was derived from bicarbonate, or if part of it represented CO_2 physically dissolved in the muscles and driven out by their acidification, in other words, to ascertain whether saturation with acid diminished the solubility of carbon dioxide in muscle. Six control experiments therefore were performed, in which the muscles in the chamber (Fig. 2) were replaced by their weight of distilled water. When the acid solution and the water had come into equilibrium with the carbon dioxide in the chamber (740 mm. Hg partial pressure) the acid was mixed with the water and a displacement observed in the manometer, indicating a diminution of 5 p.c. in the solubility of carbon dioxide in water. In all the experiments on muscles, therefore, from the apparent quantity of carbon dioxide combined, 5 p.c. of the amount of that gas physically dissolved at that partial pressure was calculated and subtracted.

When muscles were to be investigated after fatigue, they were taken from the body with their connections to the pelvic bones, connected to an isometric lever in the ordinary way and immersed in the phosphate Ringer's solution. After two hours the Ringer's solution was sucked out and nitrogen passed through the chamber for 40 to 45 minutes; then the muscles were stimulated in nitrogen by very short tetanic stimuli (42 per minute) for different lengths of time and until fatigue had developed. Carbon dioxide was at this moment substituted for nitrogen, and after 15 minutes the muscles were rapidly cut off from the bones, weighed and put into the Barcroft's bottle full of CO_2, the remaining procedure being just the same as for the resting muscles. The rapidity of these last manipulations was necessary in order, so far as possible, to prevent the tissues from coming into contact with the oxygen of the air with consequent aerobic disappearance of some of the lactic acid formed during stimulation.

Some experiments in rigor were performed; the sartorii were dissected, soaked in Ringer's solution for one hour, then weighed and placed in the Barcroft's bottle as before. Nitrogen was passed for 45 minutes to allow all the oxygen to be turned out, the bottle was then closed and placed in a water bath at 40° C. for one hour, until full rigor had developed. Then carbon dioxide was introduced as described above. By this procedure any contact with oxygen of the muscles in rigor was avoided.

The results of the experiments on fatigued muscles have been reported above; on rigor muscles three satisfactory experiments were

made at a partial pressure of about 745 mm. Hg, giving a concentration of combined carbon dioxide respectively of 15, 11 and 13 volumes, average 13 volumes per 100 g. of muscle.

On resting muscle at the same partial pressure (745 mm.) a large number of experiments were performed owing to the importance of this figure for the calculation of the heat by carbon dioxide as discussed above. In Table IV only some of them are given, *i.e.* those which have

TABLE IV. Data for construction of CO_2 dissociation curve of muscle.

CO_2 partial pressure (mm. Hg)	CO_2 combined c.c. per 100 g. of muscle	CO_2 partial pressure (mm. Hg)	CO_2 combined c.c. per 100 g. of muscle	CO_2 partial pressure (mm. Hg)	CO_2 combined c.c. per 100 g. of muscle
0	2·7	134	29	500	33
23	10·3	144	26·5	520	43
34	14	150	32	745	51
36	16·5	170	32	745	44
38	17	180	10	745	53·2
45	9	180	29	745	48·7
61	20	181	41	745	47·2
65	20	300	36	745	40·2
106	24	300	38	745	49·2
110	26	320	33	—	—

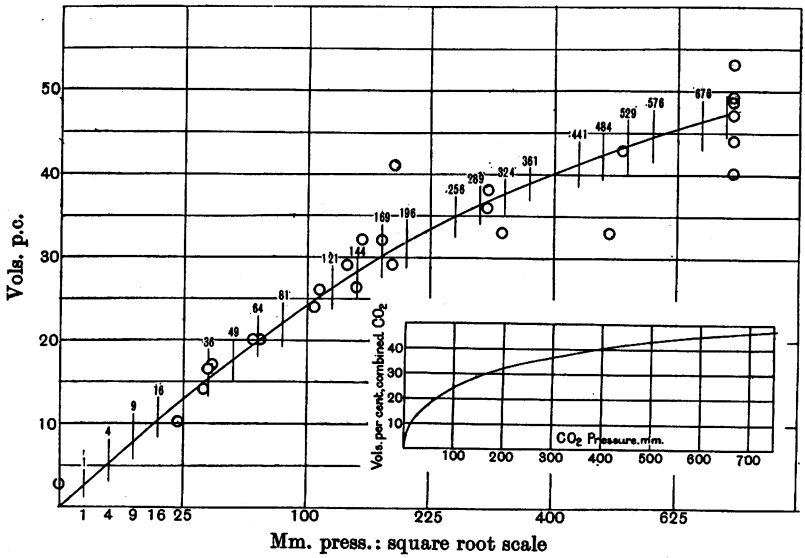

Fig. 3. CO_2 dissociation curve of muscle, from the data of Table IV: plotted on a square root scale of CO_2 partial pressure, to avoid steepness and congestion near the origin. Inset, the same plotted in the ordinary way. Muscles 45 to 50 minutes without oxygen. For fresh muscles multiply ordinates by 1·08.

been employed in Fig. 3 for plotting the dissociation curve. The temperatures of the water bath varied in the different experiments from 17° to 19° C., and an average of 18° has been assumed throughout the calculation.

The CO_2 dissociation curve, plotted in the ordinary way as in the inset of Fig. 3, rises steeply at the beginning, then gradually less steeply, finally becoming nearly horizontal. As already stated by Fenn, the curve runs much lower than that for blood; thus at a partial pressure of 50 mm. Hg of CO_2 it reaches only 18 c.c. per 100 g. of muscle, whereas in the blood of the frog it is as high as 65 to 70 volumes per 100 c.c. (see Wastl and Seliskar(14)). At 20 mm. Hg, which according to Fenn represents the partial pressure of carbon dioxide in frog's blood under normal conditions, an increase of pressure of 1 mm. will increase the amount of combined carbon dioxide by about 0·8 c.c. per 100 c.c. in the case of blood, and by only about 0·3 c.c. per 100 g. in the case of muscle. This figure confirms Fenn's results, and is in keeping with the experiments on cats by Shaw(15); it contrasts, however, with the findings of Brocklehurst and Y. Henderson(16), who attribute to human muscle a power to combine with carbon dioxide on raising the pressure of that gas in the lungs, which is very much smaller than one-third of the power of blood. It may be added here that if muscle had in fact, as they maintain, a very low capacity to combine with carbon dioxide, its hydrogen-ion concentration would be much higher than it actually is, as is easily seen by considering the Henderson-Hasselbalch equation.

The CO_2 dissociation curve is roughly of a parabolic form, and when it is plotted in the ordinary way to include the whole range up to one atmosphere pressure, the first part of the curve is so steep that it is scarcely possible to read off values in this important region. It is easier, moreover, to interpolate between points which lie approximately on a straight line. For both reasons the data of Table IV have been plotted in Fig. 3 as follows: the volumes p.c. of combined CO_2 vertically against the square root of the partial pressure of CO_2 horizontally. Theoretically the curve must pass through the origin, and we should interpret the observed point at zero pressure on the vertical axis as being due to the fact that it is impossible actually, in the time allowed, to attain zero pressure inside the muscle: the partial pressure corresponding to this volume of combined CO_2 is apparently about 1 mm.

From the curve of Fig. 3, which can be drawn with reasonable certainty between the observed points, we can read off the volume of

combined CO_2 for any partial pressure of CO_2. From this, employing the Henderson-Hasselbalch equation,

$$pH = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]},$$

and assuming that the dissolved CO_2 at 18° C. is given by the usual solubility coefficient multiplied by Fenn's factor of 0·8, we may calculate the pH of the muscle corresponding to any partial pressure of CO_2. We will assume a pK' at 18° C. of 6·198, as given for human blood plasma by Cullen, Keeler and Robinson(17) and used by Fenn. The relation between pH and CO_2 pressure is shown in Fig. 4, the latter being, as

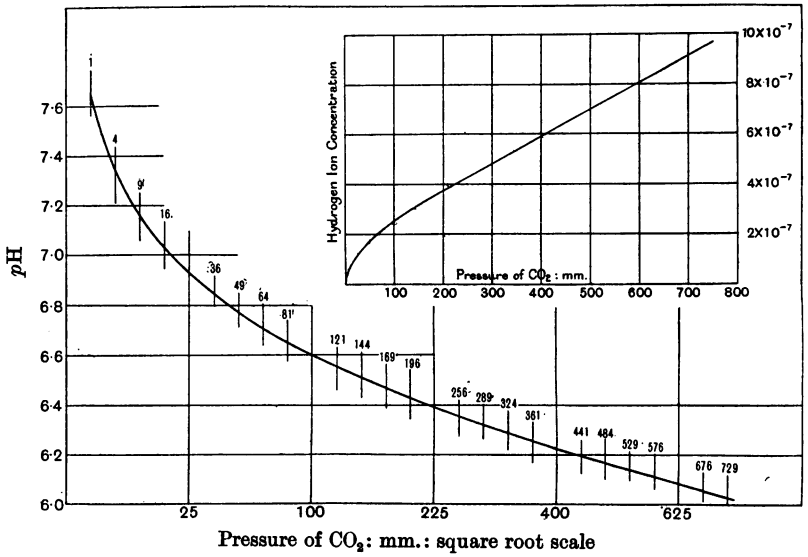

Fig. 4. The pH of muscle, calculated by the Henderson-Hasselbalch equation from the data of Fig. 3, shown as a function of the partial pressure of CO_2. CO_2 pressure on a square root scale, to avoid congestion at low pressures. Inset, the hydrogen-ion concentration (cH) as a function of CO_2 partial pressure. Muscles 45 to 50 minutes without oxygen. For fresh muscles add 0·034 to the pH, and divide the hydrogen-ion concentration by 1·08.

in Fig. 3, on a square root scale, in order to avoid undue steepness at the beginning of the curve. Inset is the relation between hydrogen-ion concentration (cH) and partial pressure of CO_2, in ordinary coordinates. We see that a muscle in an atmosphere of CO_2 has a pH of about 6, which corresponds to the lowest value which it can reach when it is removed from the body and stimulated to extreme fatigue: see Meyerhof and Lohmann(5). At a partial pressure of CO_2 of 20 mm. Hg which, according

to Fenn, represents the partial pressure of the gas in the blood of the intact living frog, the *p*H is 6·9: Fenn gives 7·2. According to Fig. 4 the CO_2 pressure required to give a *p*H of 7·0 is 18·5 mm. For a given CO_2 pressure the lower CO_2 combining capacity of muscle implies that it is appreciably more acid than blood.

The relation between *c*H and CO_2 pressure, as shown in the inset of Fig. 4, is almost exactly linear over the chief part of the range: at low pressures, however, as in blood, it bends down (see Barcroft, Bock, Hill, Parsons, Parsons and Shoji(18)). Moreover, as in blood, it can be shown that a linear relation exists between the volume of CO_2 taken up and the hydrogen-ion concentration. It is obvious, indeed, that with a CO_2 dissociation curve so similar in shape to that of blood, similar relations must, in general, exist.

It should be noted that these calculated *p*H's, and indeed all the data of Figs. 3 and 4, refer to the case of muscles kept without oxygen for 45 to 50 minutes, in which therefore lactic acid has accumulated to a small degree as described above. The true *p*H's of resting muscles are somewhat higher than those given here. If we assume that the total CO_2 capacity has been diminished by 4 volumes p.c. as the result of this lactic acid formation, and that this total capacity (see inset of Fig. 3) is about 50 volumes p.c., we may calculate roughly that in the fresh resting muscle the volume of combined CO_2 is 1·08 times as great as here given at every partial pressure of CO_2. The ordinates, therefore, of the curves of Fig. 3 should be multiplied by 1·08 throughout to represent the case of fresh resting muscle, and the hydrogen-ion concentrations calculated from them divided by 1·08. Thus in Fig. 4 the *p*H's given should be increased by log 1·08 = 0·034, if we wish to allow for lactic acid formation during the interval required for equilibration. The effect is not large, but just appreciable.

Summary.

The part played by buffers in the liberation of the heat which accompanies the new formation of lactic acid in muscle has been reinvestigated by a direct method, *i.e.* by determining the heat of combination of H_2CO_3 with living frog's muscle.

It has been found that in resting as in fatigued muscle the neutralization of 1 g. molecule of H_2CO_3 gives rise to 9400 calories. On the assumption that the heat of dissociation of muscle proteins is − 12,600 calories, as given by Meyerhof, the above value indicates that 3/4 of the neutralization is effected by muscle-proteins, and only 1/4 by other

buffers such as phosphates. 105 calories, therefore, of the 385 calories (according to Meyerhof) which accompany the formation in muscle of 1 g. of lactic acid, are to be accounted for by the process of neutralization of the acid itself. This figure is somewhat higher than that arrived at by an indirect method of Meyerhof and Suranyi, *i.e.* 80 calories per g. of lactic acid.

The CO_2 dissociation curve of a frog's sartorius, kept in an atmosphere of nitrogen and CO_2, at partial pressures of the latter ranging from 0 to 745 mm. Hg, has been constructed. The curve as a whole is about one-third as high as that given for frog's blood by Wastl and Seliskar, which implies that the inside of the muscle is appreciably more acid than blood at the same partial pressure of CO_2. At 20 mm., which is stated by Fenn to be the partial pressure in the living frog, the *p*H of muscles is about 6·9.

The relation between *c*H and pCO_2 has been plotted and found to be a linear one over the major part of a wide range of *c*H, extending from 1×10^{-7} (*i.e.* about the *c*H of a resting frog's muscle in the normal living body) to $9{\cdot}7 \times 10^{-7}$, the *c*H of muscle completely fatigued.

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