THE RELATION OF OXYGEN TO THE SURVIVAL METABOLISM OF MUSCLE.

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(*From the Physiological Laboratory, Cambridge.*)

CONTENTS.

In 1795 von Humboldt showed that a frog's muscle when excised kept its irritability longer in oxygen than in air or hydrogen, and especially so at the higher temperatures. This statement was confirmed by Liebig[1] much later and was re-examined by Hermann during his well-known researches into the metabolism of muscle.

Hermann[2] found that oxygen when present in the surrounding atmosphere, pure or mixed, delayed the natural survival loss of irritability in the *gastrocnemius* muscle after excision, but hastened it, on the other hand, in the case of the *sartorius*. To explain this contrast between the behaviour of differently shaped muscles he suggested a double action of oxygen upon excised tissue; the first, a destructive action, analytic, attacking the layers of muscle from without inwards, the second a preservative process, opposed to, but more feeble than, the first, and accordingly only exercised upon the inner layers of the muscle which though reached by the oxygen were not, or not at once, the object of analytic attack. In the case of the thin *sartorius* muscle, with large surface relatively to its bulk, the superficial destructive action of oxygen counterbalanced, he believed, the preservative action and so led to a hastening of the death process. The preservative action, which was found to be slight and was considered only to occur in the

[1] *Arch. f. Anat. Phys. u. Wiss. Med.* 1850, p. 393.
[2] *Untersuch. ü. d. Stoffwechsel der Muskeln*, Hirschwald, Berlin, 1867.

inner layers of the more bulky muscle, Hermann explained as due either to a slight physiological absorption of oxygen, presumably in continuation of the natural process of respiration, or to the beneficial results of an improved elimination of CO_2, possibly through an unknown specific action of oxygen.

At the same time, Hermann found that an active *gastrocnemius* stimulated to contraction at frequent intervals in the presence of oxygen, lost, unlike the resting muscle, its irritability as soon as a muscle similarly treated in an indifferent atmosphere of hydrogen or nitrogen;—the preservative influence of oxygen, that is to say, was not demonstrable in the case of active muscle.

This account of the relations of oxygen to surviving muscle presents many difficulties. The action of oxygen upon superficial layers is said to be analytic and destructive, but its action upon the inner layers to be conservative. Such a reversal of effect can only be supposed to depend in some way upon the amount of dilution suffered by the oxygen in arriving at the inner layers, for the cells in all layers of a muscle must be considered chemically equivalent. Yet Hermann, like Liebig, showed that the effects of oxygen were manifested in the same quality, though quantitatively less, through descending degrees of its dilution with other gases. Thus atmospheric air was found by both to give the same qualitative results as oxygen. The observation again that the active *gastrocnemius*, unlike the resting one, received no maintenance of irritability from an oxygen supply is not in agreement with the doctrine that the acts of contraction and the acts of descending to *rigor mortis* involve similar substances in similar chemical processes.

In support of his account of the double action of oxygen on excised muscle, Hermann gave very few experimental data. Nothing was said of the time from excision at which his observations began, nor of the periods over which they extended, nor again of the temperatures at which they were made. There must be noted also the fact that these experiments, like his work in general upon muscle respiration, were carried out at a time when bacterial putrefaction was not recognized as a phenomenon independent of the chemical functions of muscle.

With a special view to determining in what mode the action of oxygen upon surviving muscle produces, as I have found[1], a large increase in the normal rate of CO_2 discharge after excision, I have repeated many of Hermann's observations upon the effects of the gas

[1] This *Journal*, XXVIII. p. 354. 1902.

upon the muscular functions. My results have shown no dissimilarity such as he described between the action of oxygen upon muscles of one shape or another, and no dissimilarity again in its action upon muscles in activity or at rest.

I. THE RELATION OF OXYGEN TO THE DEATH OF RESTING MUSCLE.

It has been shown in an earlier paper[1] that when rigor, with complete loss of irritability, is rapidly produced in an excised muscle by such agents as chloroform or lactic acid, or by any temperatures from 38° C. to 100° C. and above, or again by fatigue, a special discharge of CO_2 invariably accompanies the change of state produced in the muscle. When natural *rigor mortis*, or time-stiffening, on the other hand, occurs spontaneously in the muscle, its constituent events become, by slower procession, demonstrably separated in point of time. Thus in following the natural CO_2 discharge from a resting muscle I showed that the special CO_2 production associated with the processes of rigor—the processes, that is, which end in death-stiffening—occurs at a steadily maintained rate during the whole period of declining irritability and is not related in point of time to the final act of shortening itself[2]. It appeared that the maintained output of CO_2 marked the progress of a continuous chemical change which only at a late stage induced the opacity and stiffness of rigor.

More recently I have shown that the long 'plateau' of CO_2 production due to natural rigor occurring in atmospheric air, is at a minimum when oxygen is cut off from the muscle and is notably exaggerated in an atmosphere of pure oxygen[3]. Now if this increased CO_2 production due to oxygen marks an intensification or hastening of the natural survival changes, we should expect a hastening also of their climax which is marked by death-shortening. Oxygen, in such a case, could then be grouped with irritants, like chloroform, which simultaneously hasten the physical and chemical events of rigor. It would have in fact the action ascribed to it by Hermann of destructively attacking the muscle substance. But I have found, on the other hand, that the act of *rigor mortis* in excised muscle is delayed, apparently indefinitely, in an atmosphere of oxygen.

The muscles used have been the *gastrocnemius*, the *sartorius* and the conjoined *gracilis* and *semi-membranosus* of the frog. The muscle

[1] This *Journal*, XXIII. p. 10. 1898.
[2] *loc. cit.* pp. 39—42.
[3] This *Journal*, XXVIII. p. 354. 1902.

for experiment was arranged in the tubular glass chamber shown in Fig. 1. The bony origin of the muscle was fixed in a loop of silver wire whose end passed through the india-rubber closing the lower end of the chamber. A light steel rod hooked into the tendon of insertion connected the muscle directly with a recording lever above it, through an opening in the india-rubber closing the upper end of the chamber. This opening was blocked to the passage of gases by a small plug of vaseline, which allowed the necessary vertical play of the connecting rod through it without losing its continuity. Two tubes, lateral and inferior, allowed the passage of a given gas through the chamber. When suitable connexions were made to the steel connecting rod above and to the silver wire attachment below, as shown in the figure, electric stimulation could be applied directly to the muscle, and the response recorded upon a drum by the lever, without any uncontrolled escape of gas from the chamber. By these means the irritability of the muscle to a given shock could be tested from time to time, or the response to stimuli recorded under different conditions, as in Part III.

In all the experiments the chamber with its connecting rod and lever was used in duplicate, and simultaneous records were always taken upon the same drum to give a comparison between one muscle submitted to a given atmosphere and its pair from the same animal, for control, under conditions differing only in respect of its atmosphere.

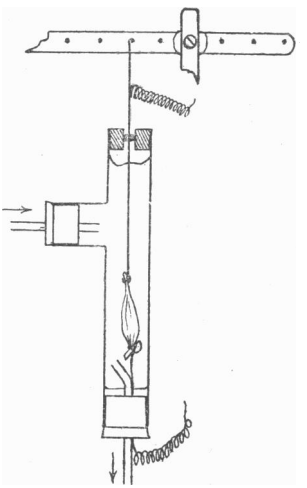

Fig. 1.
Air-tight muscle chamber.

The gases used in the experiments to be described here were oxygen, and nitrogen, and various mixtures of them, including air. The oxygen was supplied from a cylinder; the nitrogen was made from a strongly alkaline solution of urea by the action of sodium hypobromite, and both alike were freed from last traces of CO_2 by passage through a strong solution of potash. In the experiments lasting for more than half an hour or so, the gas in any given case was supplied continuously to the chamber at a slow rate, to escape by the lower exit tube through two or three millimetres of water. By this constant renewal of the gas within

the chamber the possible effects of the CO_2 produced by the muscle were eliminated. The continuous supply was effected by the slow replacement of gas in a simple reservoir by water coming to it from an aspirator upon the plan of Mariotte's bottle. The gas, leaving its reservoir, passed through the muscle chamber at a rate of nearly 10 c.c. per hour. The saturation with water-vapour of the incoming gas allowed the longest experiments to be made without any apparent drying of the muscle.

The results obtained with different muscles may be given separately.

Gastrocnemius. This is the example given by Hermann of a bulky muscle. He found, like Humboldt, and Liebig, that its irritability was maintained longer in the presence of oxygen, and his explanation of the fact, given in terms of a double action of oxygen upon muscle, has been stated already. My results fully confirm this maintenance of irritability by oxygen. Without exception, in a large number of experiments, I have found the irritability of a muscle in nitrogen or in air to disappear several hours, in some cases as much as 50 hours, before that of the corresponding muscle surrounded by oxygen. In view of this agreement the details of experiment appear unnecessary, but further illustration of the maintenance of irritability will be given under Part III.

Turning now to the incidence of death-shortening, a point not specially investigated by Hermann, it may be said at once that the shortening of *rigor mortis*, like its accompaniments of stiffness and opacity, is delayed by the presence of oxygen, as in air, and appears never to be exhibited at all in an atmosphere of pure oxygen.

When graphic records were taken, by the means described, of the variations in length of two *gastrocnemii*, one in air, the other in oxygen, it was found that the 'control' muscle in air, after the usual slight preliminary relaxation, entered upon the obvious contraction of rigor in general near the 24th hour from excision, but sooner or later according to the temperature and other circumstances. The muscle in oxygen, on the other hand, showed in no instance any death-shortening at all. Sooner or later (rarely before the 3rd day, at temperatures near 18° C., and under cleanly conditions), bacterial putrefaction sets in and ends such an experiment. But in no case has shortening been found in the muscle under oxygen before this onset of putrefaction and its resultant lengthening.

I have not attempted by the use of more elaborate aseptic pre-

cautions to extend this negative result over a more lengthened period than up to the end of four days from excision—a period during which in two experiments no shortening occurred in the oxygen atmosphere; for the effect of oxygen in abolishing the incidence of rigor is more strikingly shown when means are taken to hasten the natural time of the onset of death-stiffening. Such a hastening of *rigor mortis* may be produced in many degrees by strychnine poisoning.

In the experiment of which the graphic record is given in Fig. 2, a frog (with brain destroyed) was thrown into severe general tetanus by the injection of strychnine. The spasms were cut short by pithing after three minutes. Preparations of the *gastrocnemii* were set up, one in oxygen, the other in air. As the figure shows, the 'control' muscle in air entered upon well-marked rigor soon after the 6th hour—some 20 hours earlier than the normal time for the unfatigued muscle. The muscle in oxygen showed no contraction up to the end of 48 hours. At that time no putrefaction was detected in either preparation, and both were inexcitable by any stimulus. Though I have not yet found death-shortening to occur in any muscle exposed to pure oxygen, the irritability of such a muscle is seldom maintained beyond the end of the second day.

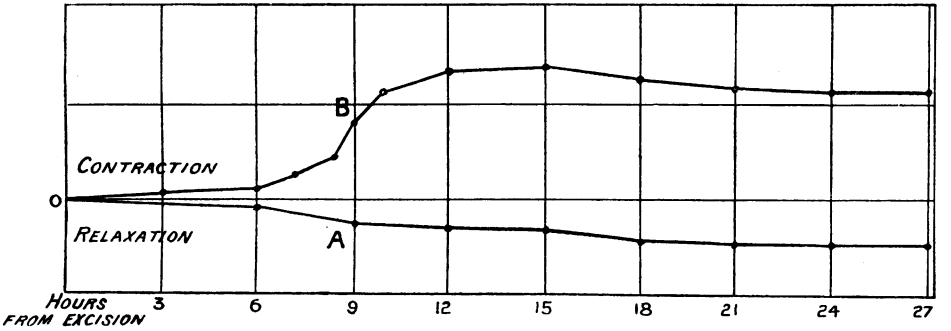

Fig. 2. *Changes in length of a pair of excised gastrocnemii, after fatigue.* The ordinates are measured directly from the record upon the drum. The levers magnified 6½ times. Load 3 grammes. Temp. 23° C.

A. Exposed to oxygen. *B.* Exposed to air.

Exactly corresponding results have been obtained in cases where *rigor mortis* has been hastened by direct electrical stimulation of the muscles instead of by strychnine spasms. Corresponding results were obtained also when, in place of air, nitrogen only was supplied to the 'control' muscle. In this series of experiments with fatigued

muscles the difference between air and nitrogen was hardly noticeable, the influence of atmospheric oxygen effecting only a slight delay in the advance of rigor.

In another experiment, shown in Fig. 3, the strychnine spasms were allowed to continue actively for 5 minutes. The subsequent record of the two muscles, arranged as before for comparison, is very significant. In both alike a slight preliminary relaxation was followed by a very early shortening at the third hour. From this point onwards the muscle *B*, in air, continued to contract. At the ninth hour, on the other hand, *A*, in oxygen, showed a reversal of the changes within it. Its lever redescended to the base line and beyond it again in relaxation. At the 27th hour, with no trace of putrefaction in either, muscle *B* was stiff,

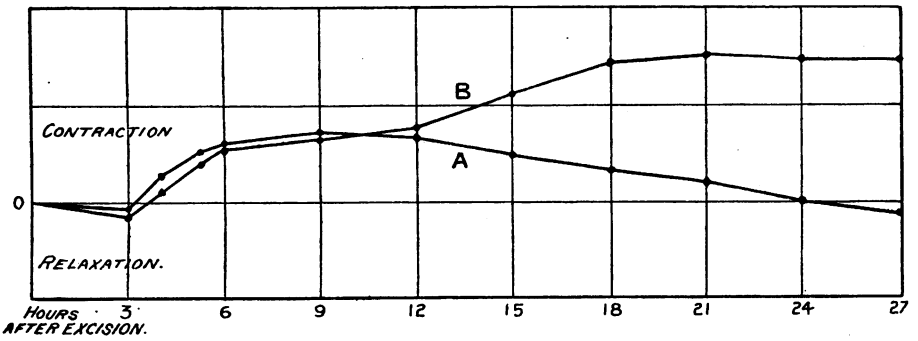

Fig. 3. *Changes in length of a pair of excised gastrocnemii, after fatigue.* Ordinates and magnification as in Fig. 2. Load 3 grammes. Temp. 16° C.

A. Exposed to oxygen. *B*. Exposed to air.

opaque, shortened and quite inexcitable by any stimulus. The muscle *A*, in oxygen, was flaccid, relaxed, and in appearance as though freshly excised. It gave good contraction with break induced shocks just perceptible by the tongue (secondary coil at 14 cm.). In the record given here there appears a marked differentiation between an earlier spasmic shortening phase, comparable with the 'idio-muscular' contraction, and the later coagulatory phase of final *rigor mortis*—the latter being exhibited only in the case of the muscle *B*.

The conjoined semi-membranosus and gracilis. The results with this muscle-preparation were in complete agreement with those described for the *gastrocnemius*. In a typical experiment lasting for 42 hours from excision of the resting muscles, at a temperature of 18° C., the preparation in oxygen at the end of that time was uncontracted, flaccid and

translucent, contracting well in response to strong break induced shocks. At the same time the control preparation in air was stiff, shortened, opaque and unexcitable. It had shown its earliest shortening at the 24th hour.

Sartorius. It was in the case of this thin delicate muscle that Hermann found no preservative action of oxygen was demonstrable. He believed such action, if it occurred, to be more than counterbalanced by the superficial destructive action of the gas.

I am not able to confirm this. Like all the other muscles examined, the *sartorius* has never shown any approach of *rigor mortis* when exposed to pure oxygen. Indeed I have so far not succeeded in obtaining a record of its death-shortening in air, but have only found it to occur in nitrogen, in the complete absence of oxygen.

In a series of six experiments, of which three were concerned with resting pairs of muscles, the other three with muscle fatigued by strychnine tetanus, both muscles after excision, one in oxygen, the 'control' in air, alike showed an elongation and an ascent of the lever above the original base line. The elongation was always more marked in the case of the *sartorius* in oxygen, the muscle in air showing an arrest of the early elongation or a return towards the base line, but not a clear contraction. The longer preservation of irritability in the muscle exposed to oxygen was always found, and this point will be noticed again below. I am inclined to attribute this apparent absence of *rigor mortis* in the *sartorius* exposed to air, to the very thinness of the muscle of which Hermann speaks; for the atmospheric oxygen may in this case perhaps reach all parts of the muscle effectively enough to have an action like that of pure oxygen upon the more bulky *gastrocnemius.*

When the comparison was made between oxygen and nitrogen, the *sartorius* gave the same results as the other muscle-preparations. A typical record is shown in Fig. 4. In this case, strychnine was given as before, and an intermittent tetanus of 5 minutes was allowed. At the 15th hour the muscle *B* in nitrogen began to shorten; *A*, in oxygen, continuously lengthened. At 28 hours after excision, though both were inexcitable, *A* appeared as when freshly excised, while *B* was stiff and less translucent.

It may be noted here that a *sartorius* muscle sometimes enters into well-marked *rigor mortis* if it be enclosed within a tube of small volume (less than 10 c.c.) containing oxygen, in which the gas remains unchanged. Under these conditions I have found no constant difference

in the time of rigor onset between two muscles enclosed in small unchanged atmospheres of oxygen and nitrogen respectively. In one case at least I have found the *sartorius* in oxygen rigid and unirritable

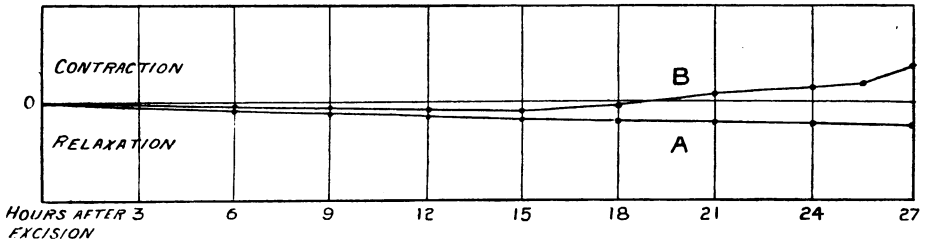

Fig. 4. *Changes in length of a pair of excised sartorii, after fatigue.* Ordinates and magnification as in Fig. 2. Load 1½ grammes. Temp. 18° C.

A. Exposed to oxygen. *B.* Exposed to nitrogen.

when its pair in nitrogen was still excitable. It is possible that such a result may be secondary to the increased formation of CO_2 by the muscle contained in oxygen. The fact at all events suggests an explanation of Hermann's results, for the muscles he observed were contained in small tubes confining unchanged atmospheres.

The results of all the foregoing observations can be expressed by saying that an abundant supply of oxygen markedly delays, though it does not prevent, the loss of irritability in a surviving muscle, and, further, that it is able to delay indefinitely, or to abolish, the visible phenomena of natural *rigor mortis*, even when special means are taken to hasten their appearance.

No evidence has been gained to show that the effect of oxygen upon the *gastrocnemius* is twofold, as Hermann suggested. The chief support for his view, he found in the divergence of his results in the cases of the thick and thin muscles respectively. The idea seems in part also to have been suggested by the observation of a stiff, clotted, even putrid *gastrocnemius* showing upon section apparently normal inner layers[1]. I have sometimes found upon section that an inner core of the *gastrocnemius* remains irritable when the outer layers give no response, but this condition I believe has generally depended upon improper conditions which have allowed a superficial drying of the muscle.

[1] *loc. cit.* p. 51.

The indefinite delay or prevention of visible *rigor mortis* by oxygen stands in sharp contrast with the exaggeration caused at the same time by oxygen in the survival discharge of CO_2,—a discharge which, there is reason to consider, occurs normally as an accompaniment and a sign of the chemical events which culminate in rigor. It is impossible under these facts to regard the increase of CO_2 due to oxygen as the result of a stimulant action comparable with that, for instance, of chloroform, which hastens not only the discharge of CO_2 but also the onset of rigor. It appears rather that the oxygen in producing an increased output of CO_2 has an action to be described perhaps as extractive, and the facts taken together indicate that the greater the completeness with which the survival processes within the muscle result in the formation and output of free CO_2, the slower is the decay of irritability and the more remote the final act of shortening.

II. THE CO_2 DISCHARGE FROM ACTIVE MUSCLE IN THE PRESENCE OF PURE OXYGEN.

In an earlier paper[1] I showed that in the case of a frog's muscle surviving in air, a period of active contraction was not marked by an increase of the normal rate of CO_2 discharge unless the activity was so pushed by the stimulation as to induce premature rigor. On the general grounds of the probable similarity between the chemical events of contraction and those ending in *rigor mortis* it is natural to enquire whether a liberal supply of oxygen, without which the CO_2 yield due to the rigor processes has been found deficient, can reveal similarly in the form of CO_2 the results of contraction.

A simple form of experiment chamber like that figured in the former paper[2] was arranged in duplicate to contain two 'crossed' pairs of *gastrocnemii*. To one chamber air was supplied throughout, to the other pure oxygen was sent soon after the beginning of the experiment by use of an apparatus previously figured[3]. Comparative estimations were made, at intervals, of the CO_2 leaving the chambers, by means of the Blackman apparatus. The results of a typical experiment are given graphically in Fig. 5. As in similar diagrams already published, this, drawn on the same scale and plan, gives all the information of a series of detailed protocols. The ordinates are proportionate to rates of CO_2 output in cubic centimetres per half-hour, the abscissæ to time intervals;

[1] This *Journal*, XXIII. p. 10. 1898.
[2] *loc. cit.* p. 74.
[3] This *Journal*, XXVIII. p. 356. 1902.

so that the enclosed areas represent the actual volumes of CO_2 determined by the titrations.

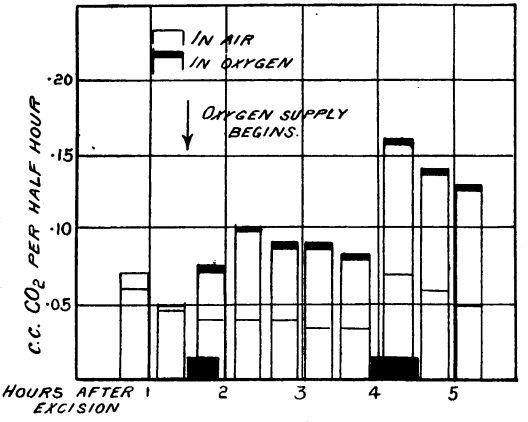

Fig. 5. *Course of survival discharge of* CO_2 *from two 'crossed' pairs of gastrocnemii.* Contraction periods are shaded. Temp. 17° C.

Experiment (Fig. 5). Two pairs of *gastrocnemii* were excised at 1.30 p.m., and arranged as 'crossed' pairs in experiment chambers *A* and *B*. Through the chambers, closed and air-tight, the air current was started at 1.55 p.m. (rate 120 c.c. per hour). Temp. 17° C. Periods of estimation are shown in Fig. 5. From 3.0 p.m. onwards an oxygen current, in place of air, was maintained throughout chamber *A*, at constant rate (120 c.c. per hour).

First period of stimulation, lasted from 3.5 p.m.—3.25 p.m. Both pairs of muscles were connected in series in the secondary circuit, and maximal induced break shocks were given every 10 sec.—1 Daniell cell, the secondary coil at 4 cm. Both pairs contracted well and equally in response, up to the end of the period. The load was 15 grammes for both pairs. The volume of each empty chamber was 10 c.c.

Second period of stimulation, from 5.25—5.55 p.m. The muscles were severely tetanised in this period by the interrupted current (secondary coil at 4 cm.—0 cm.) at intervals of five seconds, until after eight minutes they ceased to lift the load. Pair *B* in air lost its irritability just appreciably before pair *A* in oxygen. The current was applied at the same intervals for the rest of the period.

It will be seen that the curve of CO_2 discharge from the muscles *B*, in air—the lower curve in Fig. 5—showed no disturbance during the first period of active contraction. During the second period, however, in which the stimulation was pushed so as to induce rigor, the curve rose as the CO_2 output was increased. Such a course of respiration has been already described as typical under similar conditions for a muscle in air[1].

[1] *loc. cit.* pp. 68—76.

The curve of discharge for the muscles *A*, on the other hand, followed a different course. In the first two periods of estimation it is nearly superimposed upon the other, showing a similar initial output. The oxygen supply began at 1½ hours from excision, 5 minutes before the first stimulation. In the presence of oxygen the muscles in this case exhibit a rise in the rate of CO_2 discharge for the active period—a rise which cannot be the effect of the supply of oxygen acting alone, since the elevation in the curve of discharge is too abrupt to mark the simple exposure to oxygen of a resting muscle during an early, as opposed to a late, survival period[1], and since also the rate soon declines again. After the second period, that of severe stimulation, the discharge rate again rises and again declines, the curve through the change remaining roughly parallel to that given by the control muscles and at a higher level throughout.

When the 'control' muscle is supplied not with air but with pure nitrogen and its CO_2 product in activity compared with that of its pair in oxygen, corresponding results are obtained, more marked in degree. A typical experiment of this kind may be described.

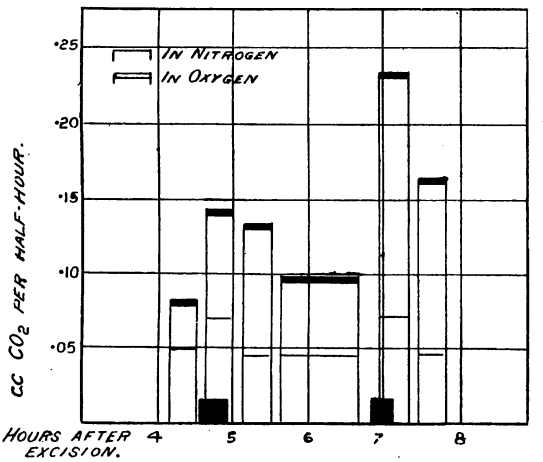

Fig. 6. *Course of survival discharge of* CO_2 *from two muscle-chains of five gastrocnemii each ('crossed' pairs).* Contraction periods are shaded. Temp. 19° C.

Experiment (Fig. 6). Five pairs of *gastrocnemii* were excised at 11.15 a.m., and were arranged in 'crossed' pairs to give two similar muscle-chains. Each bore a load of 20 grammes. The chains were arranged to hang freely in two vertical narrow tubular chambers—similar to those in the last experiment. The air current was started at

[1] Cf. this *Journal*, XXVIII. p. 358. 1902. Figs. 2 and 3.

11.40 a.m. Both chambers were immersed through the whole experiment in water maintained at 19° C. The volume of each, empty, was 20 c.c.

The periods of estimation are shown in Fig. 6, excepting a preliminary estimation for the period 12.30—12.50 p.m. which showed identical rates of output for the two muscle-chains in air. At 1.5 p.m. oxygen was sent to chain *A*, nitrogen only to chain *B*. The arrangements for delivering the nitrogen were the same as those already described for the delivery of oxygen in a former paper[1]. The first estimation in Fig. 6 shows the discharge of CO_2 to be not quite twice as much from *A* as from *B* between 3.12 and 3.32 p.m.

First period of stimulation. This lasted from 3.35—3.55 p.m. The muscle-chains were connected upon the same secondary circuit and strong break shocks were sent to both at the rate of one per second. The break shocks were just not maximal. Both muscle-chains contracted well and apparently equally through the whole period, but the relaxation of chain *B*, in nitrogen, became perceptibly less prompt in the latter half of the period.

Second period of stimulation, from 5.53—6.3 p.m. The muscles were thrown into severe tetanus by an interrupted current, the short circuit key in the secondary circuit being held open for one second every three seconds. The secondary coil was at 0 cm. Both chains gave contractions up to the end of the period, but the chain *B* in nitrogen lifted its load markedly less than chain *A* during the latter half of the period and at the end of the period was nearly brought to a standstill.

Control estimations made after removal of the muscle-chains showed that no CO_2 could be detected in either the nitrogen or the oxygen currents.

In this experiment the stimulations given for both periods were such as to produce great activity, though the first unlike the second ended short of giving pronounced fatigue effects in either muscle-chain. After each active period the chain in nitrogen showed a slight increase in its CO_2 production, the yield rising from ·05 c.c. to ·07 c.c. and from ·045 c.c. to ·07 c.c. CO_2 per half-hour for each stimulation respectively. In each case the yield returned promptly to its former level. The muscle-chain in oxygen on the other hand showed an increase of CO_2 output from ·08 c.c. to ·14 c.c. per half-hour after the first active period and from ·095 c.c. to ·23 c.c. per half-hour after the later period of greater activity.

In all the experiments of this kind it has been found that by the application of less severe or less rapid stimulation the CO_2 output of the muscle-chain in nitrogen is in general left unaffected (as Fig. 5 shows in the case of the muscle in air), whereas the output of the muscle-chain in oxygen shows an invariable increase always proportionate, at least roughly, to the degree of severity of the stimulation. The result of all the experiments has been to show that the chemical processes involved in muscular contraction, which in the presence of an abundant supply of oxygen, lead to an increased output of CO_2 directly related in amount to the degree of

[1] This *Journal*, XXVIII. p. 356.

muscular activity, do not, in a deficiency of oxygen, as when only air or nitrogen are present, result in such an increase, or are represented, not adequately or proportionately, by only a slight temporary increase in the rate of CO_2 output.

When giving an account in an earlier paper of the observations which showed that active contractions of a surviving muscle in air, within limits determined probably by the onset of rigor, do not increase the CO_2 discharge, I concluded that, "the act of contraction which has been shown upon so many grounds to resemble in its chemical accompaniments the process of *rigor mortis*, must be regarded as differing from that in this respect,—that while in both processes complex molecules are replaced by simpler ones, the splitting decomposition in the case of rigor goes at least one stage further than that associated with contraction, and has for one of its products free CO_2.[1]" More recently I have observed that even the processes of rigor which result in the discharge of CO_2 are partially incomplete in the absence of oxygen[2]. In the same way it now appears that it is upon the presence or absence of oxygen in sufficient abundance that the discharge of free CO_2 as an immediate result of contraction depends.

That such a relation as this between the survival respiration of active muscle and a contemporary supply of oxygen has not been shown before depends probably upon the long influence of the classic experiments of Matteuci and of Hermann. Matteuci[3], who found that a muscle after several hours' complete deprivation of oxygen still yielded CO_2 upon contraction, concluded that the yield was entirely independent of any nearly contemporary oxidative process and was due wholly to the break-down of some previously oxidised muscle-substance. Under his experimental conditions it is very probable that the contractions under discussion rapidly induced rigor. The results of Hermann have been noticed already.

That the liberation of free CO_2 in the act of contraction is not wholly the result, when it occurs, of earlier processes of oxidation, but depends also upon the adequate contemporary supply of oxygen, is a relation already indicated by a large class of experiments. It is very significant that the yield of CO_2 by muscles through which an artificial circulation is conducted has during periods of active contraction been variously described as increased (Chauvean and Kauffman[4]) as some-

[1] *loc. cit.* p. 83. [2] This *Journal*, XXVIII. p. 356. 1902.

[3] *Ann. de Chim. et de Physique*, 3e Série, XLVII. p. 129. 1856.

[4] *Comptes Rendus de l'Acad. Franç.* 1887.

times increased (Ludwig and pupils[1], von Frey and Gruber[2]), and as unaffected (Minot[3]). These discrepancies receive an explanation on the natural supposition that the conditions for oxidation are not in general adequate under the circumstances of artificial circulation, and least adequate, when, as by Minot, serum alone was used for the circulating fluid.

When the undisturbed muscle within the body actively contracts, all observers have agreed that additional CO_2 is delivered from the lungs. But here again it may be noticed that under conditions in which the oxidative functions of the blood have been disturbed, even this additional CO_2 yield from the lungs may be found impaired. Thus Meyer[4] in arsenic and phosphorus poisoning found a lowering of CO_2 production accompanied by an accumulation of lactic acid within the body, and Araki[5] showed that in poisoning by carbonic oxide, when oxidative processes are hindered, lactic acid appeared in the urine[6]. Observations of this kind, when taken together with the phenomena of survival respiration in the presence or absence of oxygen, indicate that the splitting processes of muscular metabolism do not come to completion without an adequate contemporary supply of oxygen, but yield products belonging to a stage of dissociation earlier than that of CO_2 production.

III. The influence of oxygen upon fatigue.

Since reason has been given for believing that the dissociative processes of muscular contraction are more complete in the presence of abundant oxygen, it may be expected that the final products left within the muscle after contraction will be different and have different toxic or other effects upon the muscle functions according to the amount of oxygen available at the time.

In the case of mammalian muscles through which an artificial circulation was maintained, Ludwig and Schmidt[7] showed that oxygen even in small quantities had a very marked action in preserving irritability, and corresponding results have been obtained by numerous

[1] *Ludwig's Arbeiten*, Leipzig, 1868.
[2] *Archiv f. Anat. u. Phys.* 1885.
[3] *Ludwig's Arbeiten*, Leipzig, 1877.
[4] *Arch. f. exp. Path. u. Pharmakol.* XVII. p. 304. 1883.
[5] *Zeitschr. f. phys. Chem.* XV.—XVII., XIX. 1891—94.
[6] See also *Schafer's Textbook*, I. p. 894.
[7] *Ludwig's Arbeiten*, Leipzig, 1869.

other observers. The case of an excised muscle deprived of circulation, however, is a different one, and it has already been noted that Hermann[1] could detect no preservative action of oxygen for excised amphibian muscle, contracting at short intervals. I have re-examined this point and have found evidence of a very significant relation between the onset of fatigue in an active excised muscle and its oxygen supply.

In all the experiments to be given, the apparatus described on p. 477 was used. The muscle chamber (Fig. 1) was very convenient in use, and the most active excursions of the connecting rod and lever did not disturb the little plug of vaseline which closed the upper aperture of the chamber. The muscles used were the *gastrocnemius* and the *sartorius* of the frog.

Gastrocnemius. A pair of muscles was arranged in the duplicate chambers for direct stimulation. One chamber was filled with oxygen, the other, for control, with nitrogen: in most experiments and in all lasting over half-an-hour a slow circulation of these gases was maintained (see p. 477). The loads and the length of lever in each case having been equalised, curves of fatigue were obtained by taking isotonic records on a slow drum of the responses to maximal induced break-shocks sent at short regular intervals through both muscles—the muscles being placed in series upon the same secondary circuit. The record of a typical experiment is given in Fig. 7. In this case, as in the similar experiments below, break-shocks only were applied to the muscle by the use of a revolving automatic key.

As the figure shows, both muscles contracted similarly at first. The approach of fatigue is marked earlier, however, in the case of *B* by a diminution of the contraction height and by the incompleteness of successive relaxations. In 4 minutes *B* ceased to give any response to the stimulus applied to both. After 5 minutes, with *B* at a standstill while *A* was still contracting, the stimulation was discontinued for 5 minutes. When the stimulation began again as before, the recovery due to rest was well marked in the case of *A*, and only transitory in the case of *B*. After the third rest interval shown on the record, *B* gave only the smallest noticeable response.

Such a result has been invariable in all the experiments. This delay of the fatigue phenomena produced by oxygen is found, but in a lessened degree, as the oxygen is progressively diluted. Comparisons

[1] *loc. cit.*

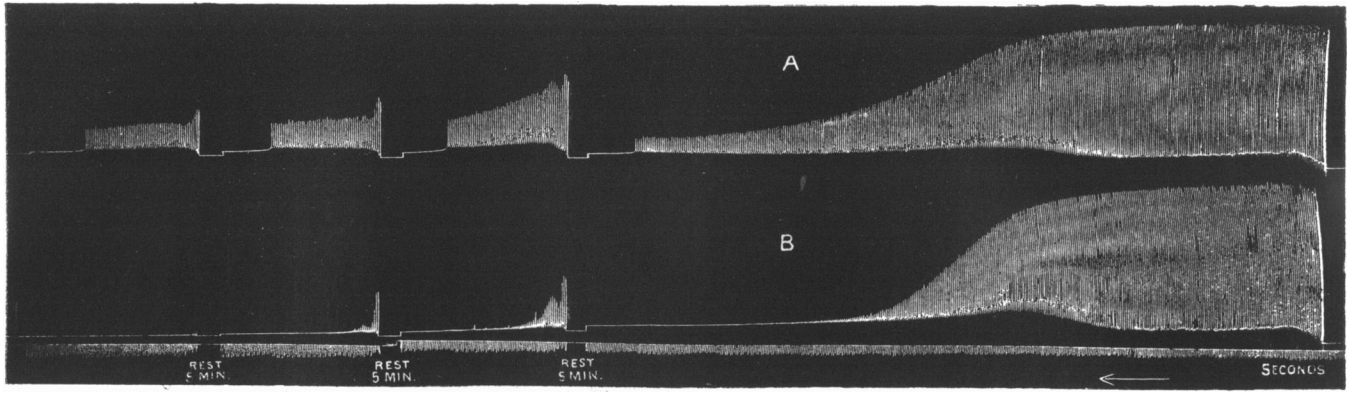

Fig. 7. *Fatigue in a pair of gastrocnemii.*
Max. break-shocks nearly one per second. Load 10 grammes. Temp. 18° C. *A.* Exposed to oxygen. *B.* Exposed to nitrogen.

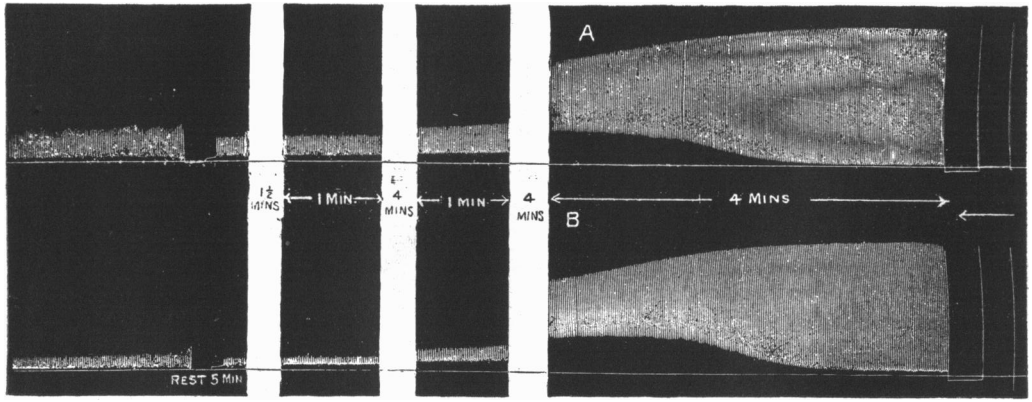

Fig. 8. *Fatigue in a pair of gastrocnemii.* Max. break-shocks one per sec. Load 10 grammes. Temp. 19° C. All the lacunæ in the record are within the first continuous period of intermittent stimulation lasting 15¾ minutes.
A. Exposed to oxygen. *B.* Exposed to air.

for instance between muscles in oxygen and air, in air and in nitrogen gave concordant but well-marked results. In Fig. 8 is given the abbreviated record of such an experiment.

Before claiming the delay of fatigue always found in the case of the muscle exposed to oxygen, as due solely to the influence of oxygen in such comparative experiments as those quoted, it must be shown that the nitrogen atmosphere is not directly inimical to the muscle substance within survival periods at least as long as those covered by the experiments. In Fig. 9 the contractions of two *gastrocnemii* in oxygen and nitrogen respectively are shown, given in response to a maximal break induced shock, of the same strength throughout, sent to both muscles, upon the same secondary circuit, every five minutes. The number of contractions performed does not produce appreciable fatigue in either muscle, it will be seen, for $2\frac{1}{2}$ hours, and we may conclude that simple exposure to the nitrogen atmosphere has not within that time directly impaired the muscle functions. It may be noticed that a little later than this the muscle in nitrogen ceased to respond and entered upon *rigor mortis*.

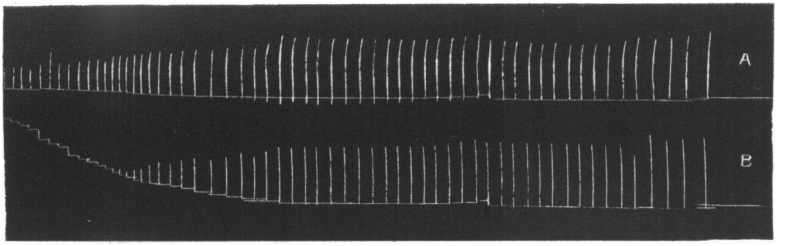

Fig. 9. *Contractions of a pair of gastrocnemii.* **Max. break-shock every five minutes. The drum was shifted between each contraction. Load 10 grammes. Temp. 20° C.**

A. **Exposed to oxygen.** ***B.*** **Exposed to nitrogen.**

I have not been able by the substitution of oxygen for nitrogen, after fatigue has been well marked in a muscle exposed to the latter, to obtain such a recovery of the fatigued muscle as to re-establish symmetry in the behaviour of the pair. The late introduction of oxygen appears able always to give long maintenance of the stage of declining irritability reached at a given point, but not a return to a markedly earlier stage. A typical result of this kind is shown in Fig. 10. Here after $8\frac{1}{4}$ minutes of the intermittent stimulation (of which 5 minutes are suppressed in the figure), when the muscle *B* in

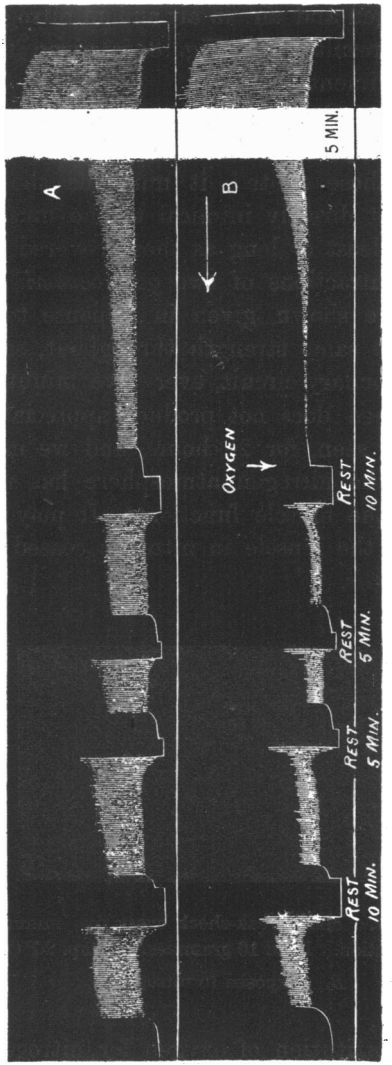

Fig. 10.

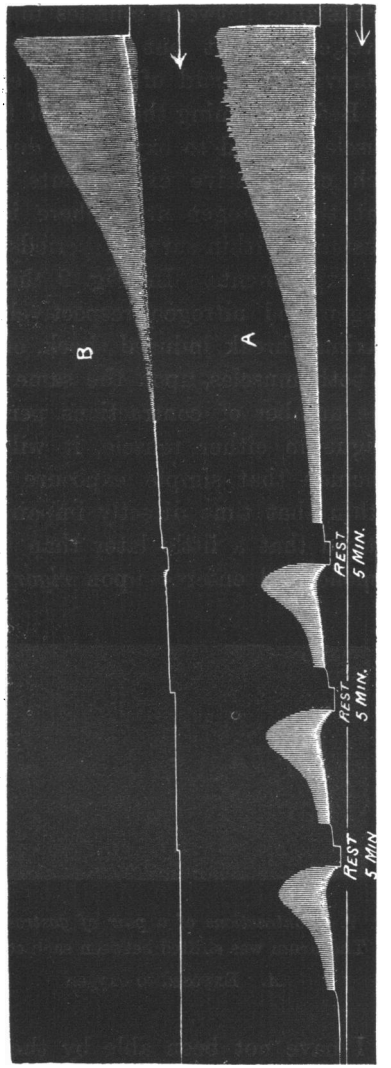

Fig. 13.

Fig. 10. *Fatigue of a pair of gastrocnemii and attempted recovery of one.* Max. break-shocks one per second. Load 15 grammes. Temp. 19° C. Oxygen was substituted for nitrogen in the case of *B* at the point indicated. The lacuna represents five minutes of the first continuous period of stimulation.

A. Exposed to oxygen. *B*. Exposed to nitrogen.

Fig. 13. *Fatigue in a pair of sartorii.* Max. break-shocks one per second. Load 6 grammes. Temp. 19° C.

A. Exposed to oxygen. *B*. Exposed to nitrogen.

nitrogen was responding only just perceptibly, the nitrogen was swept out of the chamber by the oxygen current. The marked maintenance, with a slight improvement of its response subsequently, after various periods of rest, may be seen in the record.

The single muscle twitch. Comparative records were taken by the use of the same apparatus and methods to show the effect of an oxygen atmosphere upon the single muscle contraction, in delaying the onset of fatigue. The result of such an experiment is shown in Fig. 11. Both muscles, *A* and *B*, in oxygen and nitrogen respectively, were arranged as usual to receive similar and synchronous induced break-shocks, and the ordinary tracings to show progressive fatigue were taken upon a rapidly moving drum. The usual fatigue effects are seen in the record in both cases, but they are strikingly exaggerated and hastened in the case of the muscle *B* deprived of oxygen during contraction.

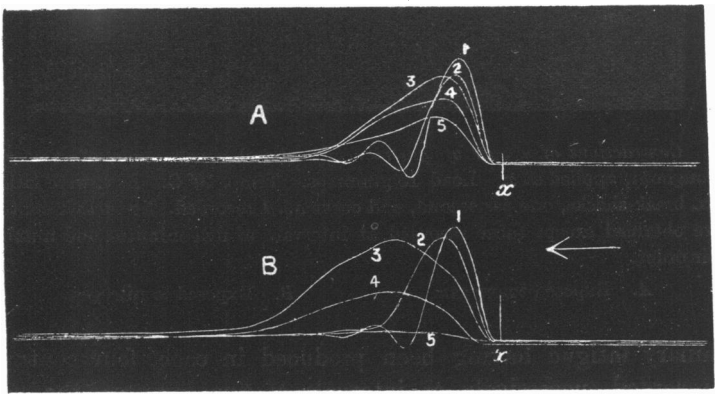

Fig. 11. *Contractions of a pair of gastrocnemii.* Break-shocks only of same strength throughout. Load 10 grammes. Temp. 18·5° C. Stimulus applied at *x*. Contraction no. 1 was the first given. Between each two successive recorded contractions as numbered, 120 unrecorded contractions were given, a little slower than one per second.

A. Exposed to oxygen. *B*. Exposed to nitrogen.

When air is used in place of oxygen, in this comparison, the same effect is obtained, and the record of fatigue occurring in air may be roughly described as intermediate between the two sets of curves figured. The figures which are given in text-books of the effects of fatigue and other agents upon the muscle contraction are, in general, taken from muscles exposed to air, and the phenomena described owe more or less of their intensity to the presence of oxygen.

When the effects of fatigue have once shown themselves in a muscle deprived of access to oxygen, the intoxication continues to proceed after all stimulation has been withheld. In a muscle supplied with oxygen, on the other hand, the intoxication does not advance during rest, but is found to diminish. The relation has been indicated in the records already given. It is shown very clearly in Fig. 12. In this case

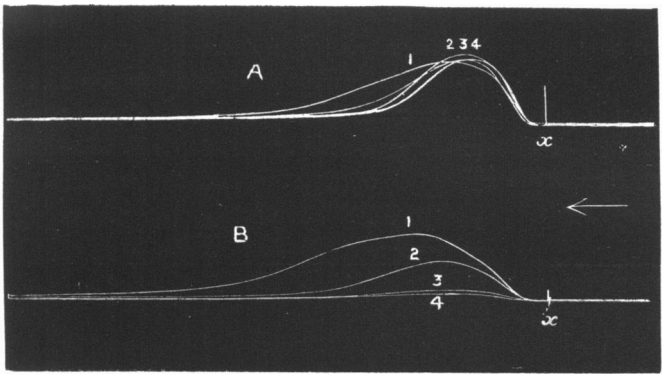

Fig. 12. *Contractions of a pair of gastrocnemii.* Break-shocks only, of same strength throughout, applied at *x*. Load 10 grammes. Temp. 19° C. Both were fatigued by max. break-shocks, one per second, and curve no. 1 recorded. No further contractions were obtained except those recorded at intervals of five minutes and numbered in their order.

A. Exposed to oxygen. *B.* Exposed to nitrogen.

preliminary fatigue having been produced in each, four contractions were recorded successively at intervals of five minutes, the muscles remaining otherwise at rest. In one muscle, *A*, in oxygen, there is a maintenance of the condition of preliminary fatigue and some restoration to an earlier one. In the other, in nitrogen, the record shows progressive intoxication.

Sartorius. In Part I. it was shown that the resting *sartorius*, in spite of its shape, reacted to oxygen in the same way as the more bulky *gastrocnemius*. So also when it is maintained in activity, the *sartorius* gives results exactly corresponding with those just described and in an even more advanced degree. The facts suggest indeed that the delay of fatigue produced by exposure to oxygen is more striking in the case of this muscle because of the thinness which allows ready access of the gas to all its parts—a ready access which Hermann believed on the other hand rendered it defenceless against the oxygen attack he

described. In Fig. 13 (p. 492), a tracing taken from a pair of *sartorii* is given. The conditions of experiment were those already stated for the *gastrocnemius*.

In the tracing given in Fig. 13, the *sartorius B*, in nitrogen, came to a standstill in just over 3 minutes—having been stimulated like the other by maximal break induced shocks given once a second. After the first rest interval of 5 minutes it gave a barely perceptible movement. Subsequently, after further rests it showed no movement at all, and entered upon the preliminary lengthening of *rigor mortis*, as the tracing shows. This lengthening, in the particular case given in Fig. 12, passed after a few hours into well-marked death-shortening. The muscle *A* showed on the other hand a much more slowly advancing fatigue effect. After the rest period it showed a marked improvement in its response to the same stimuli as before and gave nearly equivalent series of contractions after each rest period subsequently. It continued to give responses to the same stimuli after its pair *B* had begun to show death-shortening.

In the record in Fig. 13, it may again be noticed that the 'staircase' phenomenon at the beginning of the series of contractions is more pronounced in the case of the muscle deprived of oxygen. Such a relative exaggeration of the 'staircase' effect has been generally observed throughout the comparative experiments in the case of the muscle less adequately supplied with oxygen. I do not propose to deal with this point in detail in the present paper.

In all the experiments precautions were taken to avoid contamination with CO_2 of the gases used. The nitrogen in particular, made by the action of hypobromite upon urea, was freed from CO_2 by passage through a wash-bottle of strong potash. The nitrogen current beyond the wash-bottle, like the oxygen current, was tested from time to time by means of the estimation apparatus and was found to be free from traces of CO_2. Von Lhota[1] has recently published an account of the action of CO_2 upon muscle contraction and his comparative records taken from muscles contracting respectively in air and CO_2 show a great similarity in many respects to some of those given above. He does not refer to the circumstance that the effects assigned to the action of CO_2 must include effects due to the absence of oxygen. In one section he shows that the action of CO_2 in abolishing irritability is hastened by the activity of the muscle, but the absence of oxygen in a

[1] *Arch. f. Anat. u. Phys.*, Oct. 1902.

CO_2 atmosphere is a factor in this result to which he makes no reference. It is clear that the specific action of CO_2 upon muscular contraction can only receive pure demonstration when the control muscle for comparison is exposed to an indifferent, and therefore oxygen-free, gas.

The foregoing observations show that in an excised muscle, irrespective of its shape, the development of fatigue following upon active contractions is controlled by the contemporary supply of oxygen, the onset of fatigue being slower, the more abundant the oxygen supply; and that the intensity of fatigue phenomena, at a given moment, in a recently excised muscle from which all oxygen is withheld, is a function not of the time elapsed in the absence of oxygen, but of the previous activity of the muscle.

Attributing the phenomena of fatigue to the presence within the muscle of toxic substances, we have to explain the mode in which an abundant supply of oxygen can prevent the formation, or effect the removal or neutralisation, of these poisons. Loeb[1] has discussed the effect of a lack of oxygen upon the heart-beat in two species of fish-embryos. He found that the heart of an embryo *Ctenolabrus* deprived of oxygen soon came, after a slowing of its rhythm, to a very abrupt standstill. In the allied *Fundulus* embryo, on the other hand, the lack of oxygen gave a similar slowing of the rate of beat, but the rhythm after falling first rapidly and then more gradually, was maintained at a low level for some hours. The sudden standstill of the *Ctenolabrus* heart he was inclined to attribute to an intoxication acting possibly through a structural change. The decline in rate of the *Fundulus* heart-beat he put down to a decline of energy freed by oxidation, while its long maintenance was evidence against the formation of poisonous bodies like those which gave the abrupt stoppage of beat in the former species. It seems that so marked a difference in the effects of withdrawal of oxygen in two closely allied species must more probably depend upon some secondary circumstances than upon an essential difference between the chemical processes of contraction in the cardiac muscle of the two species. Budgett[2] subsequently in Loeb's laboratory showed that the visible structural changes and the resultant disorders of function produced in certain Protozoa by many drugs were closely imitated by the changes following a simple withdrawal of oxygen. He concluded

[1] *Pflüger's Archiv*, LXII. p. 249. 1896.

[2] *Am. Journ. of Phys.* I. p. 210. 1898.

that either the poisons had their action by interfering with oxidation, or that interference with oxidation (by removal of oxygen) had its action by producing poisons.

Reason has been given in Part II. above for believing that the chemical processes of muscular activity, like the survival processes of resting muscle, do not reach their natural end in the production of CO_2 without an adequate oxygen supply. With or without oxygen available at the moment, the excised muscle gives rise slowly if at rest, rapidly during activity, to bodies believed to be precursors of CO_2, and known to be poisonous, whose action is marked by the onset of fatigue and hastening of *rigor mortis*. It is reasonable to suppose that the beneficial action of oxygen, in delaying both fatigue and *rigor mortis*, is a sign of the greater completeness with which in its presence the metabolic products of muscle are expressed in the form of liberated CO_2.

IV. Summary.

1. The progressive loss of irritability in a surviving muscle is markedly delayed by an abundant supply of oxygen.

2. The advent of natural *rigor mortis* is delayed, apparently indefinitely, by the same influence, even when special means are taken to hasten it.

3. These preservative actions of oxygen appear to be independent of the shape of the muscle and its surface relations, and no confirmation has been found for the view of Hermann that the action of oxygen on muscle-substance is twofold, one effective at the surface, the other, opposite in character, effective below the surface.

4. The phenomena of fatigue resulting from active contractions in an excised muscle are, irrespective of its shape, diminished in intensity and in rapidity of onset, by an adequate contemporary supply of oxygen, and marked recovery from the effects of fatiguing contraction may be seen in a muscle deprived of nutriment but exposed freely to oxygen.

5. The survival discharge of CO_2 from an excised muscle is increased during periods of contraction in the presence of abundant oxygen, the increase being proportionate, or roughly so, to the number and degree of the contractions. In agreement with observations already published, this additional yield of CO_2 accompanying contraction is absent or incomplete in the case of a muscle made to contract in air or in nitrogen.

6. It is suggested that the delay of *rigor mortis* and of fatigue effected by the presence of available oxygen marks the completeness with which, in the presence of oxygen, the dissociative processes alike of resting and active muscle, advancing slowly in the former, rapidly in the latter, result in the formation of free CO_2, and that the hastening of *rigor mortis* and fatigue in a muscle from which oxygen is withheld are due to an increased accumulation, under circumstances of deficient oxidation, of the metabolic products within the muscle which are the potential precursors of CO_2.