THE SURVIVAL RESPIRATION OF MUSCLE[1]. By W. M. FLETCHER, M.A., *Fellow of Trinity College, Cambridge.* (47 Figures in Text.)

(*From the Physiological Laboratory, Cambridge.*)

CONTENTS.

INTRODUCTION.

THE observations which have been made in the past upon the gases taken from or yielded to the atmosphere by excised muscles have been subject to particularly disadvantageous circumstances, resulting in the main from the unsuitability of the methods of analysis to an attack upon physiological problems.

In the first place the material used for the work has in almost all cases been the leg muscles of the frog, which are necessarily small.

[1] This research was undertaken during a tenure of the Coutts Trotter Studentship.

The estimations of the changes produced by the exposed muscles in the composition of the surrounding medium have been made by gasometric (eudiometric), or even by gravimetric methods. The conjunction of these methods with the employment of small masses of muscle has made it impossible to obtain the necessary accurate determinations of changes in the gaseous medium during any but relatively long periods.

Another objection, of very general application, may be based on the fact that during nearly all the observations made upon "muscle-respiration," the muscle investigated has been placed in a closed, usually small volume of air, the changes in which have been determined after the lapse of a relatively long time. It follows that the results so obtained are open to the charge of all the errors which may arise from the long exposure of the muscle to the accumulated products of its own activity.

It must be remembered, lastly, that all the best known experiments upon this subject were conducted at a time when the action of micro-organisms in producing putrefactive changes had not received recognition, and it will be found that the failure to eliminate this very important factor in CO_2 production has rendered whole groups of past experiments valueless.

In the history of the study of "muscle-respiration," the most important advances of knowledge have been those due to the researches of Hermann. Instead of expressing, like Valentin, the gaseous interchanges as the result of a reaction between the atmosphere and the muscle substance, determined in amount rather than in character by the condition of the muscle, Hermann regarded part at least of the interchange as a function of the muscle substance itself and an expression of activities comparable with those of which the muscle is the seat during life. It will be shown below that it is particularly in relation to the study of special activities like those of contraction and *rigor mortis*, as dealt with by Hermann, that the disadvantages belonging to methods of estimation which demand for accuracy long experimental periods, have been most apparent.

From some preliminary experiments upon the rate of the discharge of CO_2 after excision, I found that for the study of changes in that rate, particularly of those produced by special experimental interference, it would be necessary to estimate with accuracy to $\frac{1}{50}$ of a cubic centimetre of CO_2, and to make continuous estimations of the discharge of CO_2 from the same muscle at intervals down to ten minutes. The latter of these conditions amounts to a demand for great speed in estimation.

For the fulfilment of both, only the volumetric method (method of titration) is applicable, for by its use accuracy of estimation need not be obtained at the expense of the ease and rapidity of manipulation.

An apparatus embodying the ordinary method of estimation of CO_2 —the titration of baryta water after absorption of the gas,—by means of standard acid has been arranged by Mr F. F. Blackman, who employed it for researches upon the paths of gaseous exchange between plant leaves and the atmosphere. The apparatus, which was fully described in the *Philosophical Transactions* of the Royal Society, Vol. 186 (1895), has been most ingeniously adapted to meet requirements for great accuracy of estimation associated with considerable speed in manipulation which in general are exactly those of the animal physiologist. I have used in the work described below an apparatus made with some modifications on the same system and have found the Blackman arrangement perfectly suited to the study of the so-called muscle-respiration. It has been possible by its means to follow in detail from the moment of excision onwards the changes occurring spontaneously in the rate of CO_2 discharge from an excised tissue as well as those produced by special experimental interference. I am heavily indebted to Mr Blackman's long experience of the detailed *technique* of CO_2 estimation and to the readiness with which he has always put it at my disposal. Without it, my difficulties in making the complex apparatus efficient and manageable would have been indefinitely prolonged.

The gaseous exchanges of excised muscle are still grouped in some English text-books under the special title of "muscle-respiration," a term which is not easily extensible to the corresponding exchanges of other excised and dying tissues, and which more properly belongs to processes in muscle within the living body. It is suggested here that the word "survival" is suitable for the qualification of the periods after somatic death, or after excision, in which a tissue of any kind is the seat of a new series of chemical activities. The introduction of the word barely needs defence at a time when French physiologists sum up these periods as constituting "*la survie*," and when in Germany the exactly corresponding word "*Überlebende*" is used for tissues passing through these survival periods. It is certainly preferable to the very ambiguous phrase "post-mortem" sometimes taken in the same sense. The expression "survival respiration" will be used throughout this paper for those activities of excised tissues which, when limited to the case of excised muscle, formerly went under the name of "muscle-respiration."

PART I. THE METHODS OF ESTIMATION.

The apparatus referred to depends in principle upon a well-known method of volumetric analysis. It is so arranged that a current of air freed from CO_2 by contact with a strong solution of potash is drawn by a water-aspirator first over the tissue examined and then through a known volume of a weak standard baryta solution. The amount of change in this solution caused by precipitation of the carbonate is measured by subsequent titration with standard hydrochloric acid, the indicator being phenol-phthalein. The special features of the Blackman apparatus are first, those by which greater accuracy in estimation is secured, and second, those to which greater speed in the technical operations is due.

Of the first kind are

(*a*) the adoption of closed absorption chambers with air-tight connexions,

(*b*) the maintenance of a very constant rate of current.

(*a*) The employment of the closed cylindrical chambers for the absorption of CO_2 by the baryta allows not only the exclusion of atmospheric CO_2 but also greater accuracy in titration. A small volume of baryta solution is used for each determination and the whole of this (instead of a measured sample) is subsequently titrated against the standard acid. This removes, it will be noticed, any necessity for washing out the chamber before each estimation, for, after simple emptying, the remaining drops of quite neutral solution introduce no error into the next determination. The burettes containing both the alkali and the acid are connected in an airtight manner with the absorption chamber, and additional amounts from either may be added during titration until a most delicate end-reaction is attained.

(*b*) The maintenance of a very constant rate of air-current, which is necessary if accurate comparisons are to be drawn between the amounts of CO_2 discharged from the tissue during separate short periods of time, is chiefly secured by the special form of Mariotte's bottle used in the Blackman apparatus as the aspirator. During periods in which actual absorption is not going on and the aspirator is not working against the resistance afforded by the small column of baryta solution, any increase in the rate of flow (which would introduce error into the result of the next period of estimation) is obviated by switching the air current to a "resistance-bottle"—a simple wash-bottle in which the height of a column of fluid has been once for all adjusted

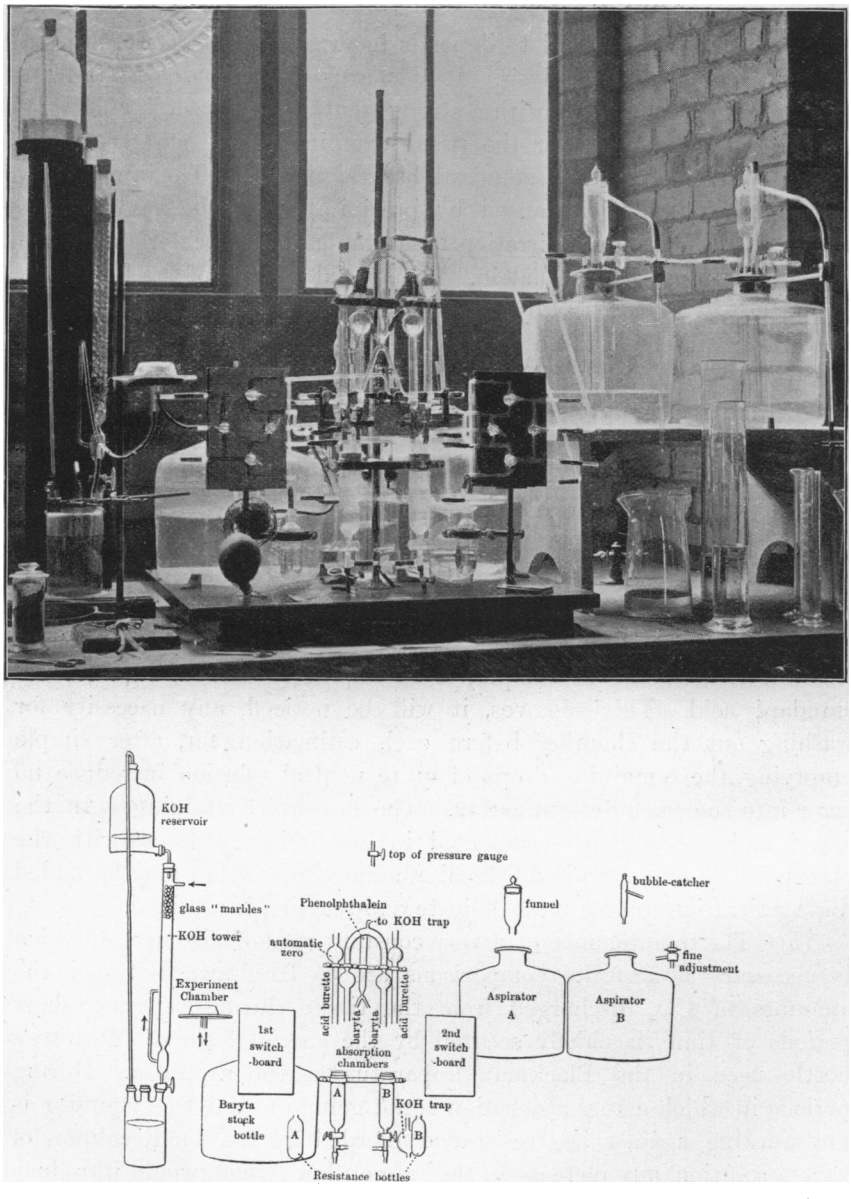
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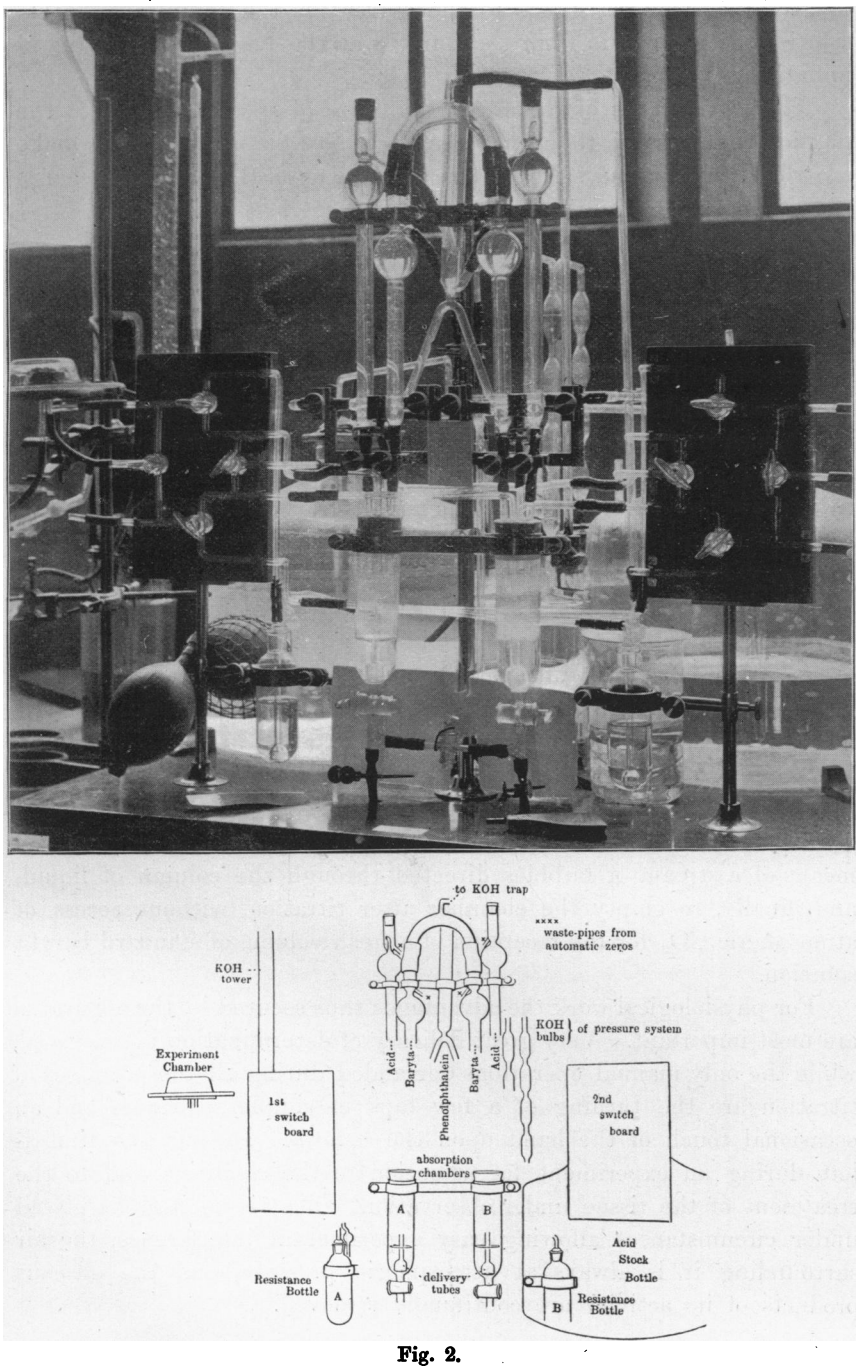
Fig. 1.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KOH bulbs
of pressure system
Experiment Chamber
1st switch board
Acid
Baryta
Phenolphthalein
Baryta
Acid
2nd switch board
absorption chambers
A
B
Resistance Bottle
A
delivery tubes
Acid Stock Bottle
Resistance Bottle
B

Fig. 2.

to give the same resistance to the current as the column of baryta solution usually run in from the burette at the beginning of a determination.

The contrivances of the *second* kind are of special interest to the physiologist, for with their aid it becomes possible, if desired, to make a perfectly continuous series of observations upon the rate of discharge of CO_2 from a tissue during successive short periods of time.

They are,

(*a*) the reduplication of parts of the apparatus,

(*b*) the arrangements for simplifying manual operations.

(*a*) By the reduplication of the necessary parts it is made possible to perform a titration in one chamber while absorption is going on in the second, and thus to make an unbroken series of observations of which each may, if desired, be limited to the few minutes necessary for a titration and the emptying of a chamber. And further it is possible to conduct two parallel and independent series of observations on two separate tissues, the series being only broken by the short intervals necessary for titration.

(*b*) The arrangements securing rapidity of manipulation by the simplification of *technique* (gained in its turn by complexity of apparatus) are easily described in outline. By means of a hand-bellows air may be driven through strong potash into a pressure-bottle, from which it may be supplied for the purposes of doing work in three directions. The compressed air serves to drive the standard solutions from the stock bottles into the burettes as required, to effect the necessary cleanly stirring of the solutions in the chambers during titration by means of a stream of bubbles directed through the column of liquid, and, finally, to empty the chamber after titration, without access of atmospheric CO_2, for the reception of a fresh volume of standard baryta solution.

For physiological work the advantages thus secured by the apparatus are most important. Very great delicacy of determination is preserved, while the only manual operations demanded during all the processes of titration are the turning of a few taps in simple sequences and an occasional touch of the hand upon the bellows. In this way time is left during an experiment for attention to the condition and to the treatment of the tissue under observation. The tissue itself is placed under circumstances allowing easy experimental interference, the air surrounding it is always at atmospheric pressure, and the gaseous products of its activity are continually removed.

In the Blackman apparatus which I have used (see Figs. 1 and 2), certain modifications were introduced to promote still further the rapidity with which accurate titrations could be performed. Instead of using ordinary burettes, I gave a trial at Mr Blackman's suggestion to burettes with an "automatic zero" made by Herrn Greiner and Friedrichs, Stutzerbach. These burettes not only save the labour of standing up to take an upper reading—a labour which becomes excessive when long series of rapid, or double titrations are performed, but also abolish the necessity for the subtraction of the upper from the subsequent lower reading in the calculations after titration. An additional practical convenience resulted from the ease with which suitable quantities of the standard solutions could be run away to waste through the zero apertures of the burettes at the beginning of a new experiment; by this means the first portions of the solutions which might have altered in strength during long contact with the interior of the burettes and their narrow connecting tubes, were not necessarily used in making the first estimations of a series.

I have found no inaccuracy to result from the use of these automatic zero burettes. The convexity of the liquid surface bulging from the small lateral orifice (the zero) at the narrowed upper end of the burette is easily adjusted to the same acuteness in every case; and I have found that a maximal difference arranged between the convexities at the zero points of the alkali and acid burettes when filled, produces an only just noticeable effect upon the burette readings after titration. The burettes are graduated to $\frac{1}{10}$ of a cubic centimetre, and are read, by estimation, to $\frac{1}{100}$ of a cubic centimetre.

The upper surfaces of the baryta solution contained in the two innermost burettes are protected from the atmosphere by the same simple trapping arrangement as in the most recent form of the Blackman apparatus. The upper ends of the burettes are connected with a tube passing to the upper end of a glass bell floating with submerged mouth upon a solution of strong potash. As the solution runs up or down the burettes, the bell floats higher or lower in the potash, and the menisci in the burettes are always at atmospheric pressure.

Instead of the stock bottles of 2 litres capacity containing the standard solutions and connected with the burettes and pressure system as described by Blackman, I have used bottles of 10 litres capacity by which refilling, with its attendant determinations of standard strengths, is rendered less frequent.

In the original Blackman apparatus, while the air current arriving from the experiment chamber was directed by an arrangement of three-way cocks on a "switchboard" to either of the absorption chambers or to either of the resistance bottles, the current on leaving any of these was drawn towards one of the pair of aspirators through a diamond-shaped system of tubes controlled by clips working on indiarubber joints. In my apparatus I have abolished all indiarubber joints controlled by clips and have arranged this second exit system upon a second switch-board, the three-way cocks on which appropriately correspond with those upon the first. I have found that manipulation has been simplified by this arrangement and the risks of mistake considerably reduced. All the tubular connexions of the first system,—that between the experiment chamber and the absorption chambers,—has been made of capillary tubing (1 mm. bore) as in the original apparatus. As the periods in which determinations are to be made become shorter, it becomes more important that changes occurring in the experiment chamber should have prompt representation in the absorption process.

I have used exactly the same form of potash tower for the preliminary removal of atmospheric CO_2 as that described by Blackman. I have found it perfectly convenient and efficient. In testing the completeness with which the CO_2 is absorbed as the air current bubbles from the dilated end of the delivery tube through the baryta solution, I have found that at the rate of flow used in my experiments (from 100—120 c.c. per hour), and when the percentage volume of CO_2 in the air was not more than 3%, the absorption of the CO_2 by the baryta solution was very complete, the amount of CO_2 escaping being always less than $\frac{1}{200}$ of that absorbed.

The aspirators, made like those figured in the original description, but with a slight modification of the siphon-tube by which the accumulation of air bubbles so disturbing to the rate of flow is prevented, have worked with the greatest constancy. It has been a very rare occurrence for their rate of flow to leave the normal (usually 120 c.c. per hour) by more than 2 per cent. The water as it leaves the aspirators is collected in graduated vessels, and the volume of water collected during a given period is taken as equal to that of the air which has been drawn over the tissue and through the absorption chamber during the same time.

The form of chamber in which is contained the tissue to be examined has been adapted in each case to the requirements of the experiment. The special forms which have been used will be described in the accounts

given below of the experiments, but certain requirements essential for every experiment chamber may be mentioned here. The tubes connected with it for entrance and exit of the air current must be arranged so as to diminish any risk of the loss of an estimation through temporary blocking of the current by the collection of moisture, while the exit tube in particular, for the reason given above, should be of fine bore. The whole volume of the chamber should be as small as possible; firstly in order that the period may be as short as possible which must elapse between the final closing of the chamber and the first estimation which shall fairly represent the CO_2 production, and secondly in order to secure prompt representation in the absorbing baryta solution of any change in the production rate. At the same time, for the former reason, the chamber should be so arranged that it may be easily and rapidly closed in an air-tight manner.

It has always been possible to preserve an investigated tissue from drying either by introducing a moist chamber to the course of the air current between the potash tower and the experiment chamber, or by special moistening arrangements within the experiment chamber itself.

It was found that practice readily gave the familiarity with the sequences of manipulation necessary for a successful series of determinations. During the early use of the apparatus the results of many experiments were made useless through the incorrect turning of a tap, an accidental change of rate in the air current or a temporary leakage. These occurrences very soon became comparatively rare.

Material. Nearly all the observations have been made upon the muscles of frogs, partly because of their convenience and partly because they have been the subject of most of the experiments by past observers. A good deal of caution is necessary however if this animal be employed. I have found that sometimes in the case of freshly caught frogs and often in the case of frogs which have been confined for some days, micro-organisms may be found upon the surface of the muscle immediately after skinning, and bacterial putrefaction may begin very soon after death. In some cases it has appeared that the muscles of the leg have been the object of bacterial putrefaction even before death. The CO_2 phenomena of this process however have been found to be so characteristic that its presence may be always detected. It has been noticed that the muscles of a frog kept without food for a day or two provide a curve of discharge of CO_2 different in some particulars from that in the case of a frog fully fed. And, lastly, some results obtained during the breeding season in the spring seemed to show that the

muscles at this time are much more variable in condition than usual.

For the muscle preparation, the legs of the frog have been usually employed. When the "leg" is spoken of, it must be taken to mean the complete thigh and leg, without skin, separated from the body by division of the pelvic girdle at the symphysis and across the iliac process, with minimal cutting injury to the thigh muscles, and divided from the foot by section of the upper ends of the astragalus and calcaneum.

A tubular experiment chamber has, whenever possible, been used, the entrance and exit tube being at opposite ends. In such a chamber the legs of one or more frogs may be placed in longitudinal series, or a chain of two or more *gastrocnemii* linked in series by small platinum hooks. The chamber, whose volume is made as small as possible, is usually in a vertical position so that the muscle preparation hanging freely within it, is exposed on all sides to the slowly moving air current traversing its surface.

A form of chamber very useful for the inclusion of two legs is shown at Fig. 3; it allows an additional arrangement for keeping the muscle surface moist, by means of a small volume of normal salt solution at the lower end. Other special forms of chamber have been used and will be described separately. Under all circumstances the air current, after leaving the potash tower (in which it is found to lose moisture), and before reaching the experiment chamber, is passed through an air-tight moist chamber in a part of which a solution of baryta may be placed as a check upon the completeness of the absorption of CO_2 performed by the potash. With the use of this chamber the muscle may be found as moist at the end of a long experiment as at the beginning.

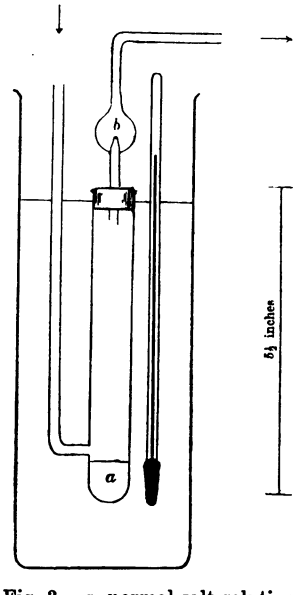

Fig. 3. *a*, normal salt-solution. *b*, condenser.

As soon as the preparation has been arranged in the experiment chamber, the air current is allowed to flow, passing on its way to the aspirator through one of the resistance bottles. For a shorter or longer

time, longer where the volume of the experiment chamber is large, the amount of CO_2 carried away from the chamber does not fairly represent the rate of output from the muscle. In order that actual observation may begin soon after excision, the volume of the experiment chamber has always been made as small as practicable. The rate of flow arranged from the aspirator has usually been 120 c.c. per hour; for this rate, and where the volume of the chamber has been x c.c. I have generally assumed that after the current has flowed for $\frac{x}{2}$ minutes the amounts of CO_2 contained in it represent the production by the muscle.

After more than $\frac{x}{2}$ minutes have passed since the experiment chamber was made air-tight, the current is switched to the absorption chamber for the first period of estimation. From the amount of CO_2 which the subsequent titration shows to have been taken up by the baryta during the period of absorption, the average rate of discharge of CO_2 per unit of time from the muscle during that period is known. The result of any series of determinations of this kind is conveniently shown by a diagram in which ordinates proportional to rates of discharge of CO_2 in cubic centimetres, are raised from abscissæ proportional to periods of time. For any one period of observation only one average rate is determined, so that for each period of observation a rectangular area may be raised proportional to the total volume of CO_2 determined by the titration for that period. The changes in the rate of CO_2 discharge during any series of observations are best exhibited in this integral form, since the preservation of the areas standing for volumes of CO_2 show at a glance the length of each period of observation.

The course of the changes in rate of CO_2 production by a muscle is more easily recognised when the results of the estimations are represented graphically, as in the diagrams at the end of this paper, than from a succession of numerical statements. The protocol of an experiment of each class to be described will be given in full (see Appendix B), but it has been thought sufficient to present diagrams alone to represent additional experiments involving the same details of method.

Part II. The normal course of survival respiration.

§ 1. *Description of the normal curve of discharge.*

Previous observations. The experiments begun by Liebig[1] (1850) and continued by Matteuci[2] (1856) showed that muscle substance after its removal from the body exhales carbon dioxide and absorbs oxygen. Matteuci found that under certain circumstances the discharge of CO_2 was independent of this absorption and must be due to processes of oxidation entered upon before excision.

The work of Valentin[3] (1857) immediately following upon this, probably suffered greatly through the uncertainties introduced by bacterial putrefaction. His method has been fully described by Gamgee[4]. The main conclusion arrived at was that the yield of CO_2 from an excised muscle was to be attributed chiefly to a reaction set up between the atmosphere and the tissue, the character of which was determined by the state of the tissue itself. The yield was not to be attributed to processes at all corresponding with those during life, since, while those processes should decrease after excision or after fatigue, the discharge of CO_2 was found to steadily increase from the moment of excision onwards, and a muscle previously fatigued by long tetanus yielded in the same time more CO_2 than one freshly excised.

Hermann[5] (1867) investigated the gaseous interchanges of excised muscles placed in a closed volume of air over mercury. The changes in the composition of the confined atmosphere were subsequently estimated by eudiometric gas analysis. He did not directly determine the course followed by the survival respiration, but investigated its general nature by numerous experiments. These may be referred to in two classes.

(i) By artificial treatment of the muscle, Hermann found that a special survival discharge of CO_2 might accompany a special survival activity. A muscle made rigid by heat for instance yielded more CO_2 during a long period (such as 15 hours) than a fresh muscle of the same weight. Making the exactly opposite assumption to that of Valentin, who thought an oxidative attack upon an excised tissue from outside increased as the tissue became moribund, Hermann took

[1] *Arch. für Anat. Phys. u. wiss. Med.* 1850.
[2] *Ann. de chimie et de physique*, LXVII. p. 129.
[3] *Arch. f. physiol. Heilkunde*, 1857, p. 283.
[4] *Physiological Chemistry*, vol. I. London, 1880.
[5] *Untersuch. ü. d. Stoffwechsel der Muskeln.* Hirschwald, Berlin, 1867.

the excess of CO_2 to be a phenomenon of rigidity and not the result of a direct oxidation. It appears almost certain, as a matter of fact, from the experiments to be described below, that the large yield of CO_2 from heat-clotted muscle during a long period of observation begun after clotting has been completed, is to be assigned almost entirely to incipient bacterial putrefaction. But the discovery of this large yield led Hermann to the correct belief that heat-rigor is accompanied by an additional discharge of CO_2, and supported his conception of survival respiration as a process largely functional and not wholly due to an attack from outside.

This view was supported by the modifications he found produced in the survival respiration by artificial tetanus (see Part III.), and also by his very important analyses of the gaseous constituents of muscle, removed by the air-pump and by other artificial methods. It is well-known that he showed a formation of CO_2 to be an accompaniment both of artificial rigor and of the act of contraction and that these two processes to give the additional CO_2 drew upon an identical store of material. This functional yield of CO_2 was independent of the presence of oxygen after excision.

The *rigor mortis* of these experiments was that produced artificially, usually by heat, and Hermann does not describe the effect, if any, produced on the character of the CO_2 output after excision during the onset of natural rigor (see § 4).

(ii) In the case of excised muscle, uninterfered with, Hermann found that the absorptions of oxygen by living and dead muscle were practically equal in amount during equal times. It was argued that this was due to an oxidation process of a "putrefactive" kind, wholly unconnected with any characteristic activity of muscle substance and dependent for the extent of its development on the surface area of muscle exposed to the atmosphere. The unassisted discharge of CO_2 after excision was largely explained as a result of this "attack" upon the tissue, being itself accordingly dependent on the exposure area.

In spite, however, of the equality of living and dead muscle in respect of their estimated rates of oxygen absorption, Hermann believed that fresh muscle was also able to absorb oxygen in a functional manner, corresponding probably with that of the intake of oxygen during normal life. By such a slight functional absorption the muscle was said to 'preserve its irritability.' This point was based upon his investigations of the rate of loss of irritability by differently shaped muscles in atmospheres with or without oxygen. The facts indicated

in a general way that the former large absorption of oxygen, described as due to an attack by the oxygen on the tissue, regulated in degree by the extent of surface exposed, was a process tending to hasten the loss of irritability; but that in a muscle the smallness of whose surface relatively to its bulk did not allow this attack to be acute, the smaller functional absorption of oxygen helped on the contrary to preserve the irritability. It may be noted, in passing, that it is not clear why the large absorption of oxygen if it has for its end the loss of irritability, should nevertheless be equal in amount, as originally stated, in both living and dead muscle.

The small "vital" absorption of oxygen was considered to be too small to come within the limits of analysis, and to be only capable of demonstration by the indirect method just described. Its corresponding "vital" discharge of CO_2 accordingly was given no place in the course of the survival respiration as determined by analysis.

Danilewsky[1] (1874) showed that if the CO_2 output from a tetanised muscle be compared with the smaller output from a similar muscle not contracting but kept artificially in vibration through the air, the difference between them diminishes as the temperature increases from 2° C. to 25° C. This was held to show that the chemical attack upon the tissue was responsible for much of the output of CO_2 and was increased if, by agitation, the layers of air next to the muscle were continually renewed. At the lower temperatures, when chemical action should be feebler, this agitation produced less marked effects. The published results of the estimations of actual oxygen absorption are very erratic, but at least show that the yield of CO_2 during contraction is independent of that absorption.

Tissot[2] (1894) has recently repeated many of Hermann's experiments. The frog's muscles investigated were placed in small tubes over mercury, the subsequent analysis of gases being performed according to Bunsen's method. He denied that simple mechanical agitation of the muscle in air produces any increase in the amount of CO_2 discharged or of oxygen absorbed by it. He found an absorption of oxygen in the case of freshly excised frog's muscle which he put down to a true respiration continuing that of normal life ("physiological absorption"). This absorption increased with a rise of temperature till an optimum of 30° C. was reached, after which a sudden decline brought it to zero as the temperature advanced to 40° C. and beyond. Without

[1] *Centralblatt f. d. med. Wiss.* 1874.

[2] *Arch. de Physiol. norm. et path.* 1894, 1895.

any reference being made to the effects of temperature on the solubility of CO_2 in muscle substance, the facts were taken to show that the oxygen absorption rose and fell with the physiological activity of the muscle. No absorption occurred in the case of muscles clotted by heat or fatigued by long tetanus, except such as could be accounted for by simple solution within the muscle ("physical absorption").

Tissot showed, as Hermann had done, that the normal discharge of CO_2 after excision was increased in a muscle killed by heat, or in one fatigued through stimulation, but he described this, by an unfortunate application of terms, as a "physical" output not related to the "physiological" activity of the muscle. This "physical" output he found to be not related to the rate of absorption of oxygen.

Although Tissot denied that dead or fatigued muscle absorbed oxygen, it may be gathered from the figures given for some of his experiments that the rate of CO_2 output by a dead muscle is slightly less in an atmosphere free from oxygen than in air. This point however will be referred to again (§ 5).

The extreme importance of guarding against bacterial putrefaction as a possible error in studying the survival yield of CO_2 was shown by Tissot in a series of estimations of the total CO_2 discharged *per diem* by an excised mammalian muscle, carried out by means of a gravimetric method. He found that under strict aseptic precautions the daily amounts of CO_2 declined to a vanishing point, but that if bacteria were allowed access to the muscle the yield increased from the first day onwards. In these experiments no determination was made of the changes occurring in the rate of yield during periods less than a day.

From this account of previous observations it will be seen that the actual course followed by the gaseous exchanges during the survival periods within the first day has not yet been described. What part of the total survival discharge of CO_2 may be due to the onset of natural rigor has not been clearly shown, and at all events it has been given no distribution in time. Apart from this, it is indicated by Tissot's results that the CO_2 discharge dependent on contemporary oxygen absorption declines as the muscle becomes less fresh. But it has not been shown whether this process ends before or after the beginning of rigor.

The initial stages of the bacterial putrefaction have not been studied, nor the extent to which this process may introduce error into experimental results during periods earlier than those at which its more obvious chemical accompaniments have become evident.

The normal curve. The changes in the rate of CO_2 output from the leg muscles of a frog, freely exposed to the air but protected from superficial drying, are expressed in the series of estimations tabulated in the first of the Protocols at the end of the paper, and represented graphically in Fig. 4.

The curve indicated in this graphic expression exhibits from the first a fall in the rate of discharge,—a fall which is continued, except for certain irregularities, during many hours. The decline in rate is most rapid immediately after excision, but becomes progressively more gentle during the first five hours, towards the end of which it follows a nearly straight course only slightly inclined towards the base line.

From this point it enters upon a stage of considerable irregularity. A rise in the rate of discharge at about the 6th hour after excision leads to an irregular 'hump' upon the curve, of which, however, the highest crest represents a lower rate of CO_2 production than that found in the first periods after excision. Passing onwards, the curve descends from this 'hump' to a level of production, in this case lower than any yet exhibited, which is maintained at a very constant height above the base line for nearly 20 hours. This constant rate of CO_2 production, represented in the diagram by a long flat 'plateau,' at last sinks towards zero.

But in the example shown at Fig. 4, the curve has not fallen far towards zero before a great change comes over the character of the survival discharge. The curve takes a sudden bend upwards and indicates at this point the occurrence of a strongly marked rise in the output of CO_2 from the muscle. The elevation of the curve leads in a few hours to levels of CO_2 production far above the previous maximum reached during the earliest periods after excision.

The course of the survival respiration of different muscle preparations varies in some particulars from the form just described. The extreme limits of these variations in three main directions are shown in Figs. 5, 6, and 7. In all of them the curve takes a smooth course downwards for the first 5—6 hours, the descent rapid at the beginning, but afterwards more gentle. In all of them, too, the course followed by the discharge curve changes its direction markedly at or soon after the 6th hour. In Fig. 5, the curve at this period simply enters upon a long 'plateau' extending over many hours. In Figs. 6 and 7 the curve enters upon the 'plateau' after a preliminary rise corresponding with the irregular hump shown in Fig. 4.

In Fig. 6 the rate of discharge does not sink below this constant

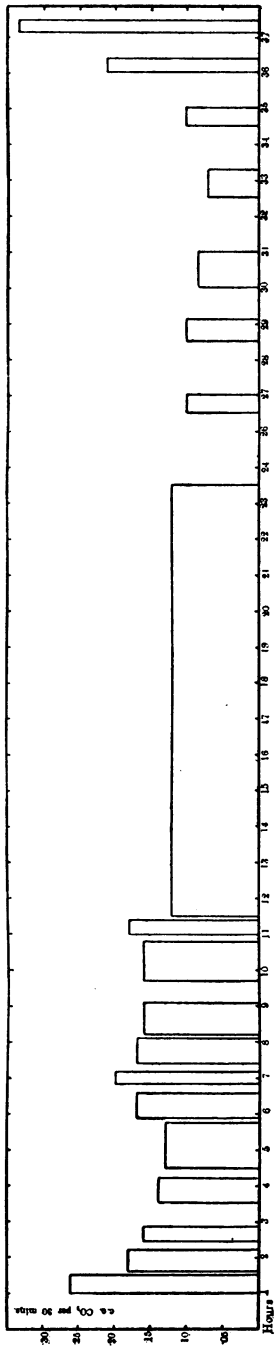

Fig. 4. Survival discharge from legs of large frog.

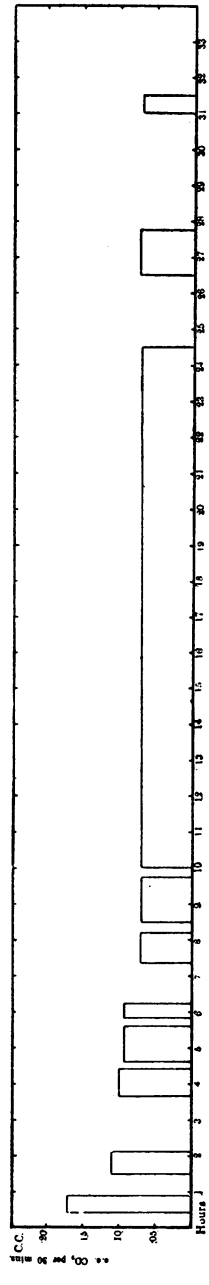

Fig. 5. Survival discharge from legs of frog.

level, for the 'plateau' is suddenly interrupted by the decisive upward direction of the curve leading to relatively enormous rates of CO_2 production. In Fig. 7, on the other hand, the curve of discharge maintains a very constant level after the first irregularity of the 6th hour, and it is only at the end of 50 hours that the final decisive uplift in the curve makes its appearance.

In this last experiment two *gastrocnemii* were employed, but for each of the others both legs of the frog. The muscle preparation for Fig. 4 was obtained from a particularly large frog; the absolute measurements of rates of CO_2 production indicated in Figs. 5 and 6 are more nearly representative of the average production from the legs of the frog. In all the observations which I have made upon the natural course of discharge of CO_2, the same sequence of events has been found. In general, the variations from the type are of only two kinds. These are, first, variations in the prominence of the 6th hour hump which inaugurates the plateau of steady production; and, second, variations in the time of appearance of the ultimate lofty ascent of the curve. With these reservations it may be taken that the general form of curve which has been described is constant in all essential points for any of the muscles of the frog's leg.

The results obtained from the leg muscles are not materially affected by the presence of blood in their vessels. At the beginning of many of the early experiments I washed out the vessels by injection of normal salt solution from the aorta. It was found that this did not affect the results obtained except in so far as it led to a waste of time between somatic death and the first period of determination. Any excess of blood is readily avoided by hanging the frog from its toes for a few minutes after decapitation. In (§ 2) the results of observations upon the rate of CO_2 discharge from freshly shed frog's blood will be stated, and these will be found to confirm what has been said with regard to the unimportance of washing out the vessels.

Upon a preliminary examination, only one feature of the curves which have been described has an immediately obvious meaning. The sudden and final change of direction of the curve, showing a rapidly increasing rate of CO_2 discharge, is invariably found to be accompanied by a distinctly putrefactive smell. Some of the phenomena of the high rates of discharge accompanying the foul odour will be described below, but for the present their consideration may be dismissed in the study of the normal survival respiration; since, if the access of micro-organisms to the muscle be prevented by the usual aseptic precautions,

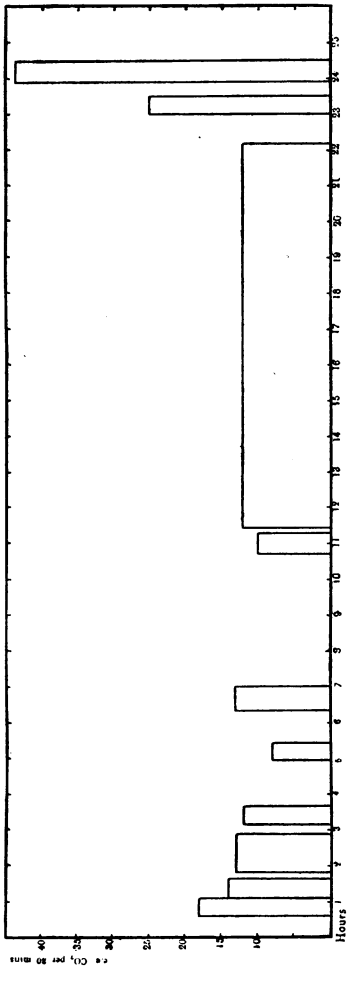

Fig. 6. Survival discharge from legs of frog.

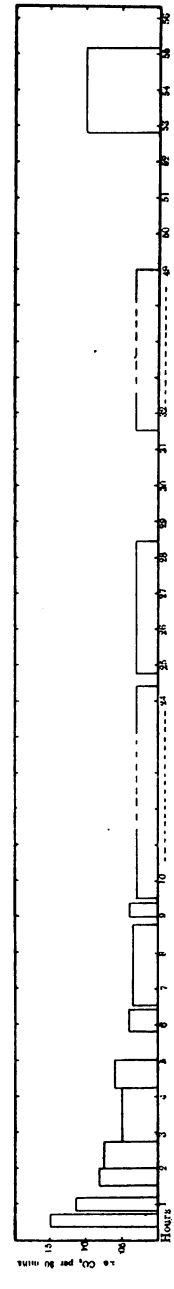

Fig. 7. Survival discharge from two *gastrocnemii*.

the rapid rise in output of CO_2 normally concluding the course of the respiration, fails to make its appearance; and, in its absence, the natural curve of discharge more and more closely approximates to zero. The rise is not a phenomenon of the survival discharge from the muscle *per se*.

It remains to find an interpretation of the normal curve by reference to factors responsible for the discharge and for the changes in its rate.

§ 2. *Phenomena of diffusion from muscle substance.*

Determinations have been made of the changes in the rate at which CO_2 escapes from the surface of muscle substance uniformly impregnated with it, to an atmosphere which contains none. The impregnated muscle was placed in the experiment chamber from which the CO_2 was swept away in the air current to the absorption chamber as it became liberated. The results of this work are important for two reasons. In the first place they give an indication of the extent to which simple effects of diffusion are responsible for the course of the survival discharge from a muscle in which CO_2, formed during activities prior to excision, may have been contained. And, secondly, they give a measure of the truthfulness of a given curve of discharge in its representation of the changes in rate of CO_2 production by a muscle during given periods, and allow an estimate to be made of the promptitude with which any given change in that rate, experimentally produced, may be expected to find its appropriate representation in the form of the discharge curve determined by the results of successive estimations.

Similar observations have been made of the changes in the rate of diffusion of CO_2 from egg-albumen and water, which had been uniformly charged with it; and also of the changes in rate of output of CO_2 from freshly drawn frog's blood.

For the experiments on muscle, three methods were adopted for uniformly charging the tissue with CO_2:

(*a*) The leg muscles (of frog) were exposed freely until the rate of discharge had sunk to near zero; they were then placed in an air-tight vessel, from a capsule in which CO_2 was liberated by the action of acid upon a carbonate. After 8 hours the muscle was considered to be uniformly impregnated.

(*b*) The leg muscles were confined in a small air-tight tube in which a drop of chloroform had been placed. From other experiments (see § 6) I have found that a muscle exposed in this way to chloroform

vapour yields CO_2 actively at first, but rapidly reaches a state in which that yield permanently ceases. Putrefaction in such a chamber is indefinitely postponed, and to make uniform impregnation certain the muscle may, if desired, be left enclosed for days.

(*c*) The leg muscles at a time after excision when the rate of discharge is known to be fairly constant for some hours, were enclosed in a small tube. Self impregnation occurs in this case without loss of power to yield additional free CO_2.

Exactly similar results have been obtained from muscles charged according to the first two methods. In each case the rate of discharge of CO_2 upon exposure in the experiment chamber is highest at first, very rapidly declines from the initial rate during the early periods, but afterwards more slowly. The fall of rate becomes so slow when zero is approached that for many hours traces of CO_2 may be detected in the air current leaving the muscle. In the diagram at Fig. 8,

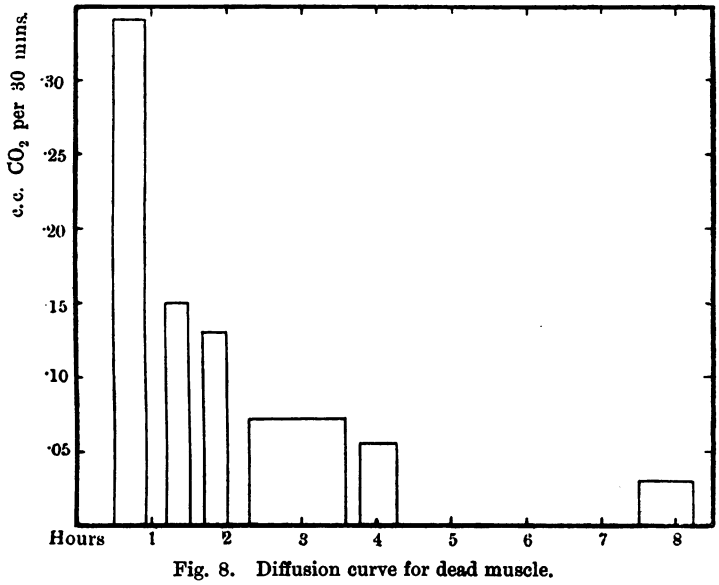

Fig. 8. Diffusion curve for dead muscle.

representing a typical diffusion escape from muscle unable to produce additional CO_2 on its own account, the initial rate of discharge is seen to be higher than that of the normal survival respiration of the legs of a frog. To compare the diffusion effect from the muscle substance when the initial rate is less, the leg muscles charged with CO_2 according to method (*b*) were exposed for an hour and then confined

again in small air-tight tube. After saturation of a lower degree had become uniform throughout the tissue, the rate of discharge upon exposure was determined. The diagram of this discharge is shown at Fig. 9; it is seen to be exactly comparable with that starting from a higher initial rate.

In the case of muscles charged with CO_2 by the method (*c*), a similar rapidly falling curve of discharge is found during the first periods after exposure in the experiment chamber. Here, however, the muscles are still producing free CO_2 *de novo* at a certain rate, and the rate of the pure diffusion discharge, instead of nearing zero, approaches at first very rapidly, but later more slowly, the rate of

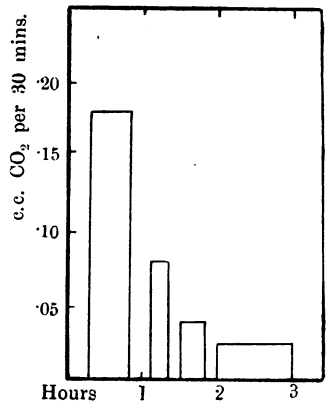

Fig. 9. The same, with lower initial rate.

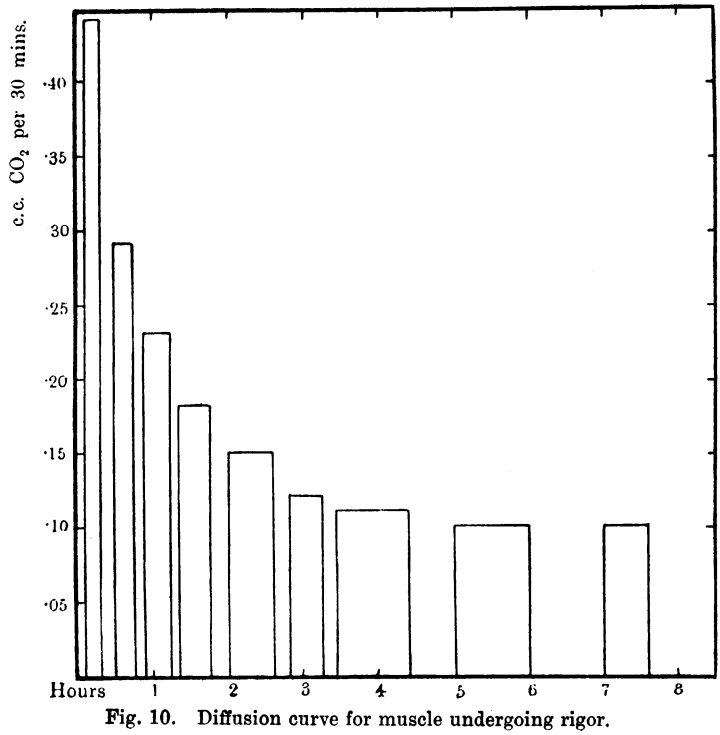

Fig. 10. Diffusion curve for muscle undergoing rigor.

discharge characteristic of the muscle at that period,—a rate which is maintained, in the example chosen, for a considerable time (Fig. 10).

Curves exactly corresponding with those of the former case, where all formation of CO_2 *de novo* within the muscle had ceased, have been obtained for the diffusion discharge of CO_2,

(*a*) from the white of an egg which had been placed for 48 hours, with intact shell, in a closed tube containing CO_2.

(*b*) from soda-water of a uniform low saturation, prepared by the recorking of diluted commercial soda-water.

A diagram for the changes in the rate of discharge from water is given at Fig. 11, and on the same diagram is plotted the curve for the discharge from the egg albumen.

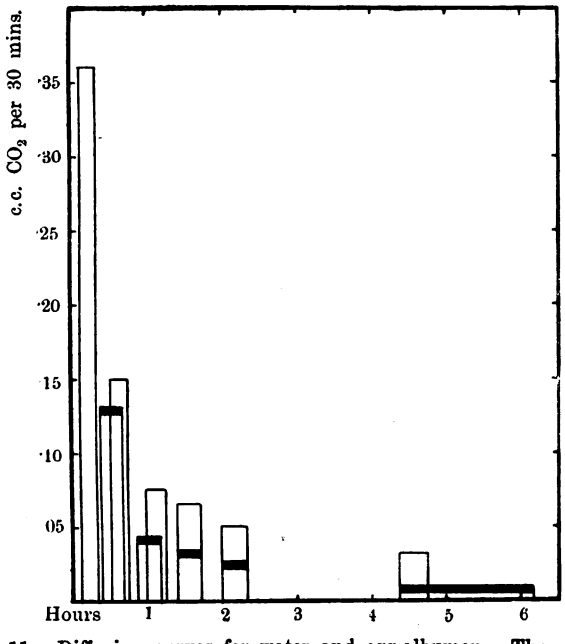

Fig. 11. Diffusion curves for water and egg-albumen. The upper curve is that for water.

These curves, illustrating the changes in rate of escape by diffusion from muscle and other substances are all approximately logarithmic in character, and approach very closely to those which might be constructed on the assumption that the rate of escape at any moment is proportionate to the degree of saturation of the muscle with CO_2 at

that time. It appears that no greater resistance is offered to the escape from muscle substance than to that from structureless egg-albumen or from water.

These results may now be used to aid in the interpretation of the course of the normal survival discharge of CO_2. In view of the facts just detailed, an escape by diffusion to the air-current of CO_2 which has been set free within the muscle before excision could only noticeably affect the general course of survival discharge during the earliest periods after excision. It is during these early periods however that the shape of the normal curve (Figs. 4—7) does appear suggestive of a diffusion effect. The course followed at first by the normal survival discharge is obviously comparable, on inspection, with the course of discharge from the muscle prepared by the method (*c*), in which the rate of output sinks, first rapidly and then slowly, until it reaches the level which represents the activity of the muscle at that time in the production of CO_2. If this resemblance between the natural and artificial cases be a real one, it is necessary to believe that a freshly excised muscle, at a given moment soon after excision, is charged more highly with free CO_2 than is permitted by the relation between its rate of *de novo* production at that time, and the rate at which diffusion allows the CO_2 to be swept away by the current of air. Diffusion would, accordingly, rapidly proceed, as in the artificial case, until the right balance had been struck between the rate of fresh production and the rate of diffusion.

There is other evidence to support this suggested comparison. If a frog in which the brain has been destroyed and the skin removed from the hind legs, be kept for an hour or two with intact circulation in a moist chamber, the curve of the survival discharge, obtained after excision of the leg-muscles, is found to be a normal one except that the first "diffusion effect" is absent. The curve for such a preparation as this (see Fig. 12), where diffusion has been allowed to go on before stoppage of the circulation and the excision of the muscles, begins at a point lower than the corresponding curve for a muscle preparation in which skinning and excision have been simultaneous, but after 2 to 3 hours the two curves in this comparison meet, and continue together.

Another kind of experiment, giving a remarkable result, supplies evidence which I suggest is to the same effect. In this series the changes in the rate of CO_2 output from the time of skinning were followed in the case of leg-muscles with intact circulation. An example is quoted at p. 78 and Fig. 41. The result shows that a very nearly

normal curve was obtained, in spite of the fact that during the first two periods of estimation the blood-flow through the muscle was uninterrupted. After the stoppage of circulation—which in one sense corresponds with the moment of excision for the normal curves already

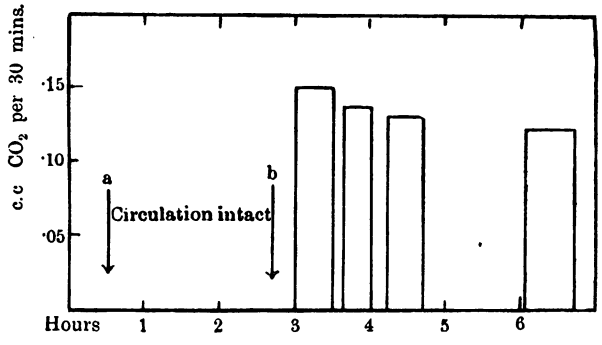

Fig. 12. Survival discharge from legs of a very large frog.
At *a*—legs skinned,
at *b*—legs excised.

given,—the curve indicates not a new decline in output, but a relatively constant rate of production like that of the muscle just mentioned in which the diffusion effect was at an end.

These experiments seem to show that it is immediately after the skinning and exposure of the muscle that the maximum rate of survival discharge is exhibited. And that the rapidly declining rate of CO_2 output following on exposure is not directly representative of activities of the muscle which, themselves declining, give a quickly lessening yield of CO_2, is shown by the fact that the initial course of the discharge from a muscle exposed to the air current may be the same under quite different physiological conditions. In § 10 it will be discussed how far it is possible to account for the presence of free CO_2 in a muscle at the time of excision, in quantities out of proportion to the survival rate of fresh production.

It has been seen that in the latest stages of diffusion from muscle or other substances uncomplicated by any continued production of CO_2, the decline in rate of discharge is very slow indeed. In the case of the muscle killed with chloroform vapour, a discharge of CO_2 at the rate of ·02 c.c. per half-hour continued for many hours. From 15 c.c. of egg-albumen I have found a discharge in the end stage of diffusion of ·03 c.c. CO_2 per half-hour for 20 hours. In the natural survival curve it was seen

that even when putrefaction was very late in making its appearance the later rates of discharge never fell actually to zero (see Figs. 5 and 7). This result is to be expected from the phenomena of diffusion just described, and in general it may be said that very low rates of CO_2 discharge from a tissue following upon higher rates by no means indicate any production of CO_2 *de novo* on the part of the tissue. During the yield of traces of CO_2 in the latest part of the normal survival curve, it may be taken that the actual formation of CO_2 has reached zero.

It was said in § 1 that the normal curve of discharge does not appear to be affected by the presence of blood within the vessels of the muscle, and that on that account the washing out operations before experiment had been replaced by a removal of excess through careful bleeding. I have followed the changing rate of CO_2 yield from freshly shed frog's blood, at atmospheric pressure, exposed in the experiment chamber. The result of a typical experiment is expressed in the diagram at Fig. 13. The decline in rate of discharge is seen to

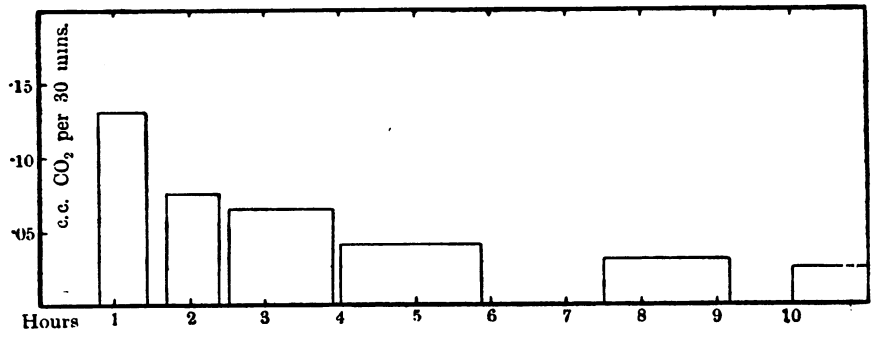

Fig. 13. Escape of CO_2 from frog's blood.

be more gradual than that found in the cases already described. No irregularities of evolution occur, however, which could introduce special complications into the study of the normal survival respiration.

§ 3. *Influence of surface area.*

In experiments on the influence of surface extent upon the normal survival respiration of muscle, I have not used muscles of different shapes, as Hermann[1] did, in order to obtain different ratios of bulk to surface, but have varied the surface relations by artificial means. If

[1] *op. cit.*

different muscles be used, it is possible that variations in respiration due to specific differences in metabolism may be wrongly connected with particular differences in shape.

In one series of experiments, the natural survival discharge was followed, without interference, for the earliest periods, until the 'diffusion effect' was nearly at an end. The chamber, constructed to open and close rapidly, and whose volume was as small as practicable, was opened at this point and the muscle substance cut across many times with a sharp knife so that a greatly increased surface area was displayed. After this the rate of CO_2 discharge was again followed.

The effect of this exposure of fresh surfaces upon the survival curve is shown in the diagram at Fig. 14. After the cutting had taken place the curve rose temporarily, but again descended along what is apparently the normal course. That a rise in the curve should follow the cutting injury is probably to be explained either as due to the production of

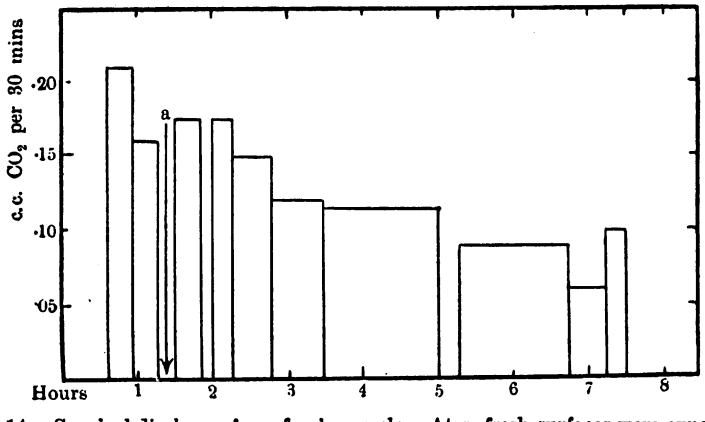

Fig. 14. Survival discharge from frog's muscle. At *a*, fresh surfaces were exposed.

additional free surfaces through which outward diffusion may proceed, or as the result of a partial localised hastening of rigor. As a matter of fact it has been found difficult to avoid the rapid onset of rigor among all the cut pieces of muscle after the operation. The experiment described was one of only two among the series in which general clotting was avoided, and in this case it is probable that part at least of the temporary rise resulted from rigor occurring in spite of precaution.

On account of this tendency of muscle cut up an hour or two after excision to pass immediately into *rigor mortis*, the cutting in a second

series of experiments was performed immediately after removal from the body. The pieces of fresh muscle, carefully cut with a sharp knife, were arranged in a clean sterilised chamber upon a little platform of platinum wire, so that a maximum of free surface, on all sides of the pieces, was obtained.

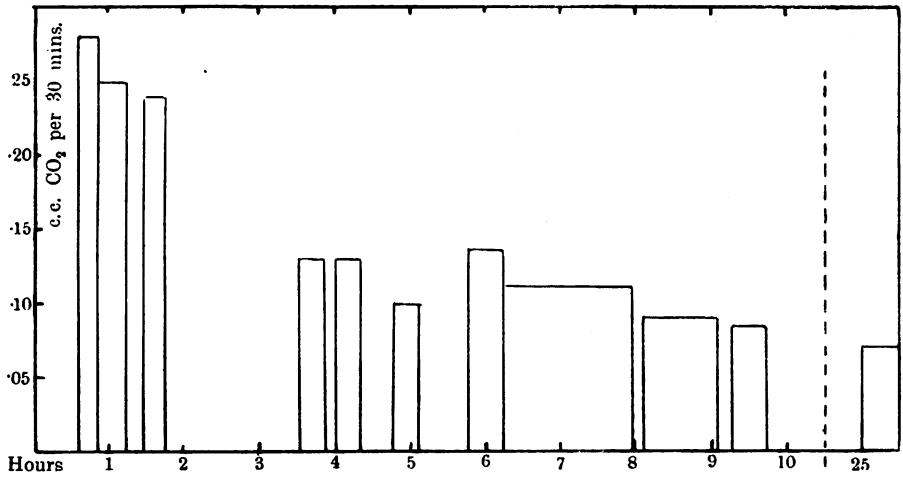

Fig. 15. Survival discharge from slices of frog's muscle.

It will be seen that the curve (Fig. 15) is practically the same as that for the normal survival discharge from intact muscles. The beginning of the irregularities of rigor is obvious in the 5th hour and has probably been hastened by the slicing. On the second day the curve declines from the long 'plateau' and sinks nearly to zero. It is only in the earliest periods that the curve appears to be at all abnormal. It will be noticed that though the frog in this experiment was particularly large the initial rates of discharge are higher than normal in proportion to the subsequent rates.

The result of this experiment is more conclusive than that of the former series in deciding the relation of the surface of exposure to the rate of production of CO_2. The fact that the numerous small slices of muscle provide a curve of survival respiration, similar in nearly all essentials to that for a muscle perfectly intact, points very decidedly to the conclusion that the greater part at least of the survival discharge of CO_2 is to be accounted for in terms of the activities of the muscle substance itself. That the minced muscle ceases at an appropriate time to yield more than 'traces' of CO_2 is a fact entirely opposed to

the supposition that 'dead' muscle is the object of destructive processes of oxidation. In further opposition to it is another kind of evidence.

If the total survival discharge of CO_2 from a muscle depend in the main upon the activity of surface oxidation (the discharge due to natural rigor being excepted) it should be much greater per unit weight of substance for small masses of muscle than for large. Hermann[1] gives the result of one experiment upon this point. He found that from two portions of fresh muscle whose weights were in the ratio of 1 : 4, the discharges of CO_2 (at 20—22° C.) to a closed volume of air during 15½ hours were in the ratio of 1 : 1·3. He does not describe the method of preparing the two portions of muscle. My experience has been that great care is required to avoid putrefaction during 15 hours at this high temperature; especially in the case of a muscle with large artificial surface. Some experiments I have made on the relation of muscle weight to the rate of CO_2 discharge have given another result. Comparative simultaneous estimations were made of the total CO_2 discharged during the second and third hours after excision,

(*a*) by frogs' legs from which the *gastrocnemii* had been removed, and

(*b*) by the isolated *gastrocnemii* taken together. It is easy to remove the *gastrocnemii* so carefully as to avoid any injury to them or to the adjacent muscles. In a comparison of this kind it is essential to avoid any cutting injury which, by hastening rigor, might prejudice the result. The 2nd and 3rd hours after excision were chosen as being fairly independent both of the early 'diffusion effect' and of the irregularities near the 5th hour.

Of six experiments the average ratio of the total yield during these two hours by the two *gastrocnemii* to that by the legs without them, was as 1 : 4·7. The results of ten weighings shewed that the average ratio of the weight of the *gastrocnemii* to that of the other muscles of the leg together was as 1 : 5·3. These figures render it impossible to explain in terms of surface relations more than a very small proportion of the total CO_2 output during these periods.

§ 4. *Natural rigor.*

The relation of the onset of natural or 'death' rigor to the survival discharge of CO_2 from excised muscles has not been determined in the

[1] *op. cit.*

past. Hermann[1] demonstrated by means of air-pump analysis the large outburst of CO_2 which accompanies the almost instantaneous clotting induced by a temperature of 38°—40° C. And in a comparison of the CO_2 discharges from 'living' and 'heat-clotted' muscles respectively to closed volumes of air during 16¼ hours, he found that the former exhaled 16·3 c.c. of CO_2 per cent. of muscle volume, the latter 25·7 c.c. per cent. Such a result as this has no clear meaning. For in the 'heat-clotted' muscle giving the larger yield, the process of rigor was presumably rapidly completed by heat before the beginning of observation, while in the 'living' muscle, the processes of rigor had probably begun, on the other hand, before the end of the experiment.

In view of Hermann's well-known researches on the relation of the process of contraction to that of rigor it has been naturally supposed that in an excised muscle the onset of rigor, occurring parallel with the disappearance of the power of contraction, is marked by an evolution of CO_2 whose total amount should be equal to that obtainable during sudden heat-rigor caused in a similar freshly excised muscle. And Ranke[2] (1865) has shown that the total amount of acid developed in an excised muscle is approximately equal whether its development accompanies the slow process of natural rigor, or the rapid one set up by heat.

With regard to the changes in shape of a muscle which mark the occurrence of natural rigor, Walker[3] (1871), with Hermann, shewed that in the *hyoglossus* of the frog the first spontaneous shortening begins from 4 to 5¼ hours after excision, and in the *gastrocnemius* about 2 hours later.

I have followed the natural death-shortening of the three chief muscle masses in the leg; the results are shown graphically at Fig. 16. The curves show that for the *gastrocnemius*, *triceps femoris*, and the hamstrings, the shortening appears between the 4th and 9th hours. For about 12 hours it remains feeble, but in all the three examples it shows later on a sudden and marked increase. The shortening has reached its maximum in all cases soon after the 30th hour.

These time relations of the natural death-shortening must now be considered in relation to the course of the survival discharge of CO_2 as already determined. It is at once obvious that there is no disturbance in the rate of survival discharge corresponding in point of time with the period of most rapid death-

[1] *op. cit.* [2] *Tetanus*, Leipzig, 1865.
[3] *Pflüger's Archiv.* IV. 1871.

shortening, which may be placed between the 15th and 30th hours after excision. The only elevation in the normal curve after excision

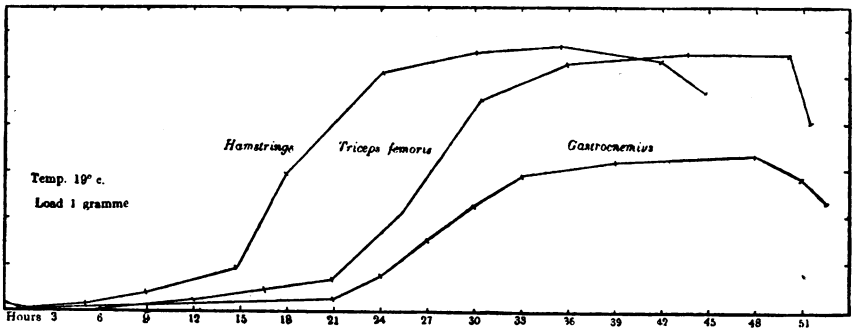

Fig. 16. Muscle contractions during onset of *rigor mortis*.

is that which has been described as a long 'plateau,' extending unchanged for 20—40 hours and introduced in most cases by an irregularly raised 'hump' near the 6th hour after excision.

That this long-continued constant level of CO_2 production should be the accompaniment of the loss of irritability and the advance of rigor in a muscle, agrees with what is known of the latter processes. The loss of irritability, more than the subsequent death-shortening, occurs in different muscles at different times; and, within the same muscle, begins earlier in the outer than in the inner parts of it. At a certain time for instance after excision the *triceps femoris* may be found quite unirritable and slightly stiff, while the *gastrocnemius* responds actively to slight stimuli. Later on, while the surface of the *gastrocnemius* is irresponsive to the strongest stimuli, an inner core of the muscle remains irritable.

From an inspection of the normal curve it is obvious that a naturally clotting muscle discharges CO_2 in any given period actually more slowly than the same muscle when freshly excised. This contradiction to the common statements of the relation of *rigor mortis* to the CO_2 output is more apparent than real. For the freshly excised muscle with which, as 'living,' the clotting muscle must be compared, is itself in an extremely anomalous condition. The rate of CO_2 discharge from such freshly excised muscle is probably due to the co-operation of more than one factor, and is almost certainly not simply representative of its physiological condition *qud* living muscle. On the other hand, the long-continued discharge during the rigor 'plateau' is probably entirely dependent upon the particular processes which originate and

accompany the death-shortening. It will be shown in support of this that any circumstances which prevent or delay rigor correspondingly affect the appearance of the plateau, and agents known to hasten rigor, on the other hand, increase the height of the plateau and proportionately shorten its length by hastening its end. (See §§ 5, 6, and 8; and also Parts III. and V.)

The end of the long rigor 'plateau' marks the conclusion of the activity shown by an excised muscle in the production of CO_2. It is the natural ending of the course of survival respiration. For when bacterial putrefaction is successfully avoided, the curve sinks very closely to zero, and the very small traces of CO_2 which usually continue to appear (at the rate of ·01—·04 c.c. per hour) have been already described as probably due to the extreme slowness of diffusion where the intensity of saturation is at a minimum (§ 2).

It will be noticed on a comparison of the curves of death-shortening (Fig. 16) with the normal curve of CO_2 discharge, that the continual discharge accompanying natural rigor is mainly the result of the processes which precede the most pronounced shortening and does not show any relation to the shortening itself. The highest part of the rigor plateau is the 'hump' which marks its beginning, and this is usually reached before the earliest death-shortening is perceptible even in the movement of a magnifying lever. And the plateau itself has in many cases already descended towards zero before the very marked final death-shortening is near its maximum. The special formation of CO_2 during natural rigor appears therefore to be largely independent of the final act in the process,—the marked shortening, with the obvious changes in the consistency and appearance of the muscle.

§ 5. *Influence of atmospheric oxygen.*

By most observers in the past, the atmospheric oxygen has been regarded as determining to a very important degree the course of the survival discharge of CO_2. Hermann's[1] general conclusions on this point have already been noted (p. 23). One or two remarks may be made in reference to particular experiments.

He found that living and clotted muscle absorbed very nearly equal amounts of oxygen, during periods ranging from $15\frac{1}{4}$—$22\frac{1}{6}$ hours. It will be seen that the distinction between clotted and unclotted muscles becomes lost when the period of examination is so extended. The

[1] *op. cit.*

relationship of the destructive oxidation to the extent of surface area was determined by an experiment already discussed (p. 24). Tetanised muscle, lastly, was found to absorb more oxygen than resting muscle during periods less than 4 hours, but the difference was abolished when the resting muscle was kept in passive agitation, without contraction, during the experiment.

That agitation of this kind has any effect in raising the absorption of oxygen has been directly contradicted by Tissot[1]. He found that while fresh muscle does absorb oxygen, the absorption is diminished if the muscle be fatigued, and entirely abolished, save for negligible traces, when the muscle has been forced into heat rigor. The absorption was said to be one of the manifestations of life, and to "follow faithfully the variations in the muscle's irritability." (See p. 25 above.)

I have made some preliminary determinations of the survival discharge of muscles exposed to an atmosphere of nitrogen. The nitrogen was prepared by passing air over copper filings heated to redness in a combustion-tube. This tube was introduced between the potash tower and the experiment chamber, so that after the beginning of an experiment the air which had traversed the potash tower in the usual way reached the muscle in the chamber deprived of its oxygen.

In all experiments simultaneous observations were made upon two muscle-preparations exactly similar, one being supplied only with nitrogen, the other, for control, with the usual moist air current. The similarity of the two preparations was secured by using 'crossed pairs' of legs. The right legs of two frogs constituted one pair, the two left legs the other. The air which had passed down the potash tower was drawn by one of the aspirators through the moist chamber, over the control muscle preparation, and, during periods of observation, through one of the two absorption chambers. By the second aspirator, air from the potash tower was taken through the long combustion-tube containing red-hot copper filings, in which it was deprived of oxygen, through a moist chamber to the muscle preparation, whence it passed either to the second absorption chamber or during periods in which no observation was being made, to the second 'resistance' wash-bottle. Between the combustion furnace and the moist chamber (which contained water previously boiled), in this circuit was introduced a U-tube filled with fresh soda-lime for the absorption of any oxides of nitrogen produced in the combustion tube (see Fig. 17). The moist-chambers

[1] *loc. cit.*

on both circuits were submerged in a common vessel of water maintained at 15°—17° C. The two experiment chambers also were

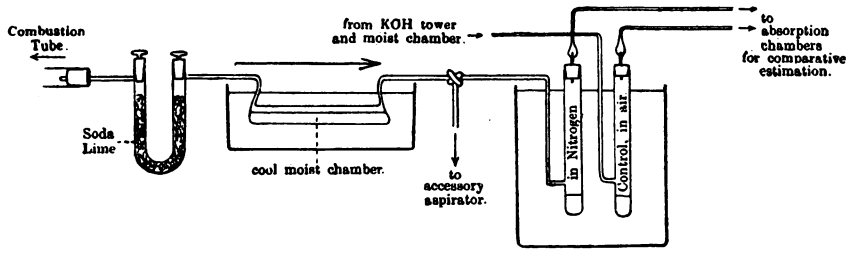

Fig. 17. Apparatus for supply of nitrogen (furnace omitted).

side by side immersed in water kept within the same temperature limits. The two aspirators were carefully regulated to work at the same rate. For at least three hours before the beginning of a series of determinations an air current was drawn by means of an accessory aspirator through the whole system from the heated combustion tube up to and including the empty experiment chamber, in order to sweep out the oxygen already contained in it.

When the course of the survival discharge of CO_2 from the two muscle preparations was followed, it was found that during the earliest periods of observation the muscle exposed to an atmosphere free from

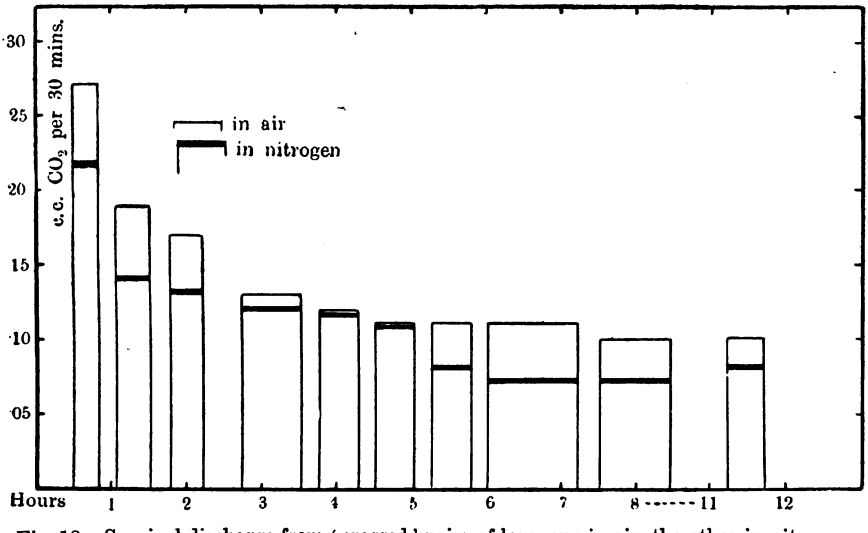

Fig. 18. Survival discharge from 'crossed' pairs of legs, one in air, the other in nitrogen.

oxygen discharged CO_2 at a lower rate than the control preparation. As the survival discharge proceeded, however, this difference did not increase, but diminished progressively until a point was reached when both muscles showed the same rate of CO_2 discharge. This point, it is interesting to notice, was reached at about the 5th hour. In Fig. 18, in which the course followed by the discharge from each preparation is shown on the same diagram, this convergence of the two curves is

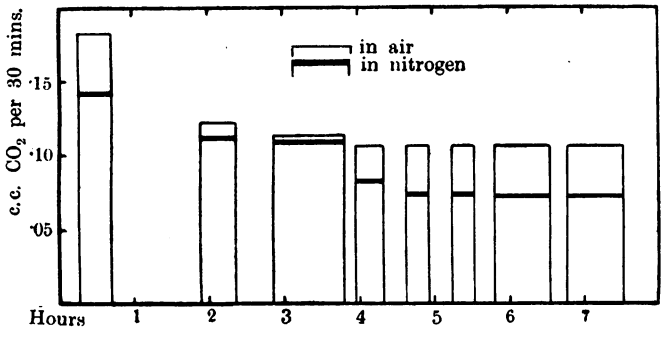

Fig. 19. The same, at higher temperature.

complete at the end of the 5th hour. In Fig. 19 where the temperature was higher (20° C.) the curves meet in the 4th hour. Up to the time when both preparations are discharging CO_2 at the same rate, the total discharge after excision is slightly greater (in the approximate ratio of 14 to 12) in the case of the muscle exposed to atmospheric oxygen.

From this point however the difference between the rates of discharge from the two muscles is again established and continues for at least 5 hours. The difference is established almost immediately after the first point of convergence, and though at first very steadily maintained, shows signs of diminishing after 6—7 hours.

The control muscle enters upon the sustained level of production which has been shown to be characteristic of the normal muscle undergoing rigor progressively in its different parts. The other muscle, which has been in the atmosphere of nitrogen, from excision onwards, exhibits a lower level almost immediately. The difference at this point is usually as well marked as the greatest difference shown between the curves in the first period after excision. For several hours at least it is maintained.

Very considerable practical difficulties have up to the present stood in the way of my following the course of discharge beyond this period

in the case of a muscle to which the access of oxygen had not been allowed from a time immediately after excision, for more than twelve hours. This has so far been my longest series of observations. For such a series as this the furnace must be kept at a red heat altogether for 15 hours, while constant attention is necessary during the whole time, not only for the repeated estimations, but to guard against leakage and against irregularities of the aspirator flow.

It may be said that indications from imperfect series point to the fact that the divergent curves meet again before the long period of *rigor mortis* is ended. It is to be noted that the lower curve immediately rises to the upper level if a temporary access of oxygen be allowed to the preparation it represents (see Fig. 20).

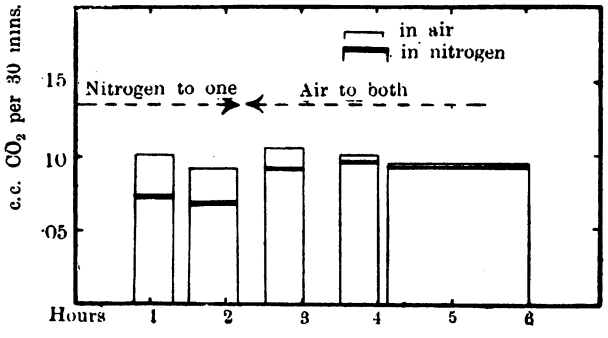

Fig. 20. Discharge from crossed pairs of legs undergoing *rigor mortis*.

If one of the crossed pairs of legs has been deprived of oxygen not until a time considerably after excision, very contrary results are obtained. The curves of the discharges in the two cases naturally run together during the early periods after excision. If at the end of the 6th hour pure nitrogen only be supplied to one of the preparations, no divergence can be detected in the curves indicating the subsequent rates of CO_2 output (Fig. 21). The supply of nitrogen to one of the preparations at a given time is effected by the use of an accessory aspirator, shown in Fig. 17. Up to the moment at which the nitrogen is supplied to the experiment chamber, an air current has been for 2—3 hours drawn by the accessory aspirator through the combustion-tube system and its moist chambers so that, the last traces of oxygen having been swept out from them, the current of nitrogen may be switched through suitable connections to the main system of experiment and absorption chambers.

Even after one of the muscle-preparations has been exposed to an atmosphere containing no oxygen for 6—7 hours, so long as the

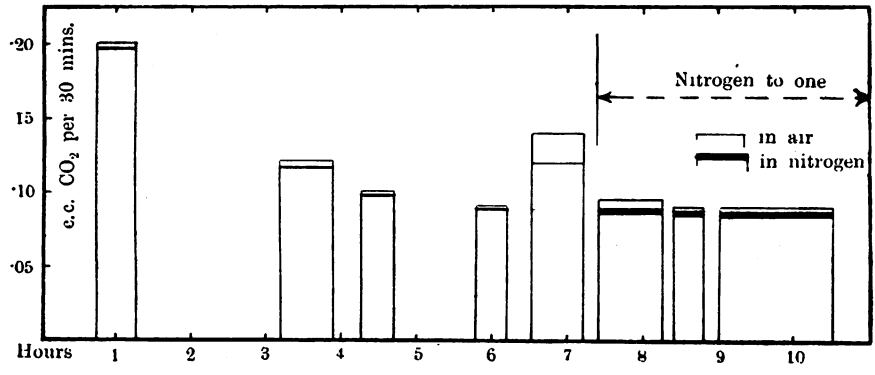

Fig. 21. Discharge from crossed pairs of legs.

deprivation of oxygen was not suffered until 5—6 hours after excision, no decline is found in its rate of discharge. It is apparently true that in order that the diminution in height of the rigor plateau due to the absence of oxygen shall be exhibited, the oxygen must have been absent and remained absent from a time very soon after excision.

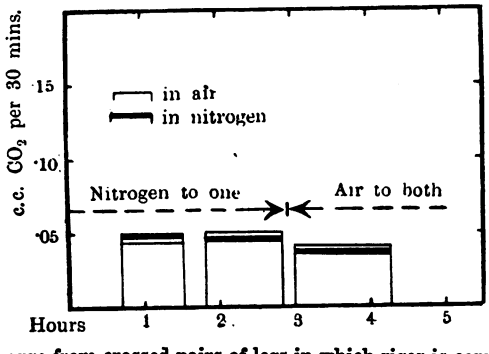

Fig. 22. Discharge from crossed pairs of legs in which rigor is complete or nearly.

If crossed pairs of muscle preparations, taken from the body after natural rigor is nearly completed, be arranged as described, one exposed to air, the other to nitrogen, no difference can be detected between their small rates of CO_2 discharge (see Fig. 22). And, similarly, the absence of oxygen has no effect in diminishing the CO_2 output from one of two preparations excised immediately after death and artificially clotted by means of chloroform.

In summing up the results of these experiments, we may speak provisionally of an excised muscle as being at different periods in three different conditions, that previous to rigor, that during the onset of rigor, and lastly that after rigor has been completed.

In the first condition the rate of CO_2 discharge is determined, but only to a small degree, by the presence of oxygen. As the muscle however passes through this condition on its way to the second, this dependence becomes gradually less marked, until at the end of this stage the rate of CO_2 discharge is the same for a muscle in air as for one in an atmosphere of nitrogen.

In the second condition, extending over the long period during which first one and then another part of the muscle passes into rigor, a similar and well-marked depression is found in the rate of discharge from a muscle to which free oxygen has been denied from the very beginning of the survival periods. This dependence of part of the discharge upon the presence of free oxygen has so far only been shown for the first few hours of this stage in the muscle's survival history, and has indicated in some imperfect observations a tendency to decrease as this stage advances. Experimental difficulties however have so far prevented more complete observations on this point. But it has been found that this diminution in CO_2 output does not make its appearance during the early part of the 'rigor stage' in the case of a muscle in an atmosphere of nitrogen if it has been allowed access to free oxygen during its passage through the first condition. In other words, part of the CO_2 output at this time is dependent, not upon the presence of free oxygen in the same period, but upon its presence in periods prior to it.

In the third condition, last of all, when the CO_2 output has sunk low after the completion of rigor, the presence or absence of free oxygen has no effect whatever upon the rate of CO_2 production, whether this last stage has been reached in the normal sequence of survival events, or has been hastened by the rapid completion of rigor caused by chloroform vapour.

These results, although incomplete, seem perfectly incompatible with the existence of an oxidative attack made upon the tissue which becomes more and more successful as the tissue itself declines in activity; and they are not in agreement with the conclusions of Hermann by which direct oxidation is to account for the bulk of the survival CO_2 discharge, and equally so in living and in clotted muscle.

The depression in the first part of the natural curve produced by the absence of oxygen, and which gradually disappears, supports the

observations of Tissot[1], who found that the absorption of oxygen by an excised muscle, speaking roughly, diminished *pari passu* with the irritability. The facts indicate that in these earliest survival periods there is a slight and progressively disappearing respiration resembling and presumably continuing that during the normal resting life of the muscle. This respiration decreases in amount as the changes occur which accompany loss of irritability and inaugurate rigor.

The depression due to the absence of oxygen during the early part of the rigor plateau presents great difficulties. It is hardly suitable to discuss it at all until it has been shown whether and when in the gradual development of the rigor the depression is removed. But my own observations, which show that it is only upon the presence of oxygen before the beginning of rigor, that this part of the discharge of CO_2 during rigor depends, are in harmony with those of Tissot, who finds no actual absorption of oxygen by clotting muscle.

For the present the suggestion may be tentatively made that the absence of oxygen during the survival stages before rigor leads to a delay, or a distortion of the process of rigor appearing subsequently. The facts as they have been found already are fully explained if this be true. If rigor be merely delayed the depression in the curve for the muscle exposed to nitrogen should gradually disappear, as indeed it has already shown indications of doing. If it be prevented in whole or part, the depression should continue until rigor in the control muscle exposed to air has been completed.

Such an explanation if tenable would remove the difficulty raised by the observation of Tissot mentioned on page 25. The muscle which had ceased to produce CO_2, during confinement since excision in an atmosphere of hydrogen, absorbed oxygen and discharged CO_2 on its exposure to air. At first sight opposed to Tissot's denial of the absorption of oxygen to clotted muscle, the fact may be explained on the assumption that a part of the rigor process had been delayed or prevented by the previous entire seclusion from free oxygen.

I have not gone into the relation of the appearance of putrefaction in muscle to the presence of free oxygen. In two well marked cases the rapid rise in CO_2 output due to putrefactive bacteria appeared simultaneously in a muscle exposed to nitrogen, and in its control pair in air.

[1] *op. cit.*

§ 6. *Effects of special poisons.*

The effects of chloroform vapour. Chloroform vapour has been found to produce very striking modifications in the normal course of CO_2 discharge from excised muscle. These modifications are different for the different stages in the discharge passed through after excision, and for this reason the use of chloroform has been very important in supplying an additional test of the reality of the distinctions which have already been drawn between them.

For the application of the vapour a simple elongated chamber containing chloroform was introduced between the moist and the experiment chambers. A by-pass was provided so that the air current could flow as usual from the moist chamber direct to the muscle, but could be switched at will and for definite periods through the chloroform tube to receive its charge of the vapour.

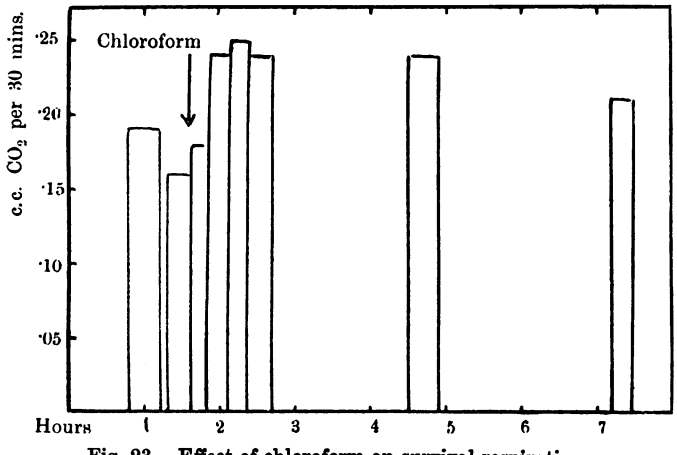

Fig. 23. Effect of chloroform on survival respiration.

During the periods before the beginning of rigor, the effects of the vapour upon the normal curve of discharge may be two-fold. If the vapour be switched to the muscle for five minutes or more, and especially if the chloroform be warmed at the same time, a marked rise is produced in the rate of discharge (see Fig. 23). This is the effect most commonly obtained. If the chloroform be applied however for a very short time, the exactly opposite effect may be produced of depressing the rate of discharge. This result is more difficult to obtain, and

has only been noticed in cases where the chloroform has been unheated, and its application only for 1—2 minutes. It is well shown at Fig. 24,

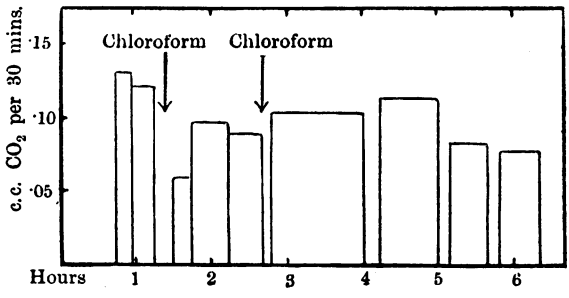

Fig. 24. Early 'anæsthetic' and later exciting effect of chloroform on survival respiration.

in which also the opposite effect due to a subsequent larger dose is given. The effect upon the curve of very slight doses of this kind sometimes appears to be intermediate in character, a temporary depression being followed by a temporary rise (see Fig. 25).

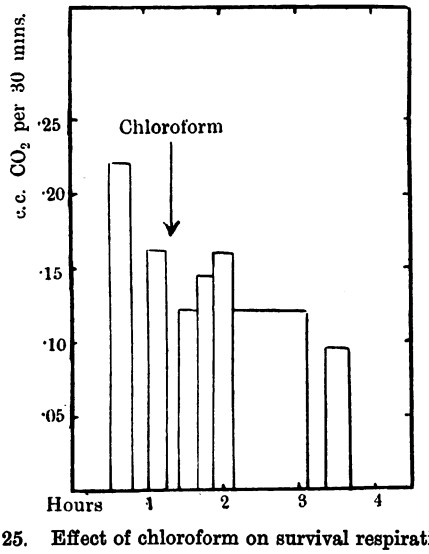

Fig. 25. Effect of chloroform on survival respiration.

After these earliest periods the effect of chloroform vapour is for many hours of only one kind,—an elevation in the curve of discharge. During the whole of the long stage in which the muscle is undergoing

rigor, the vapour invariably produces a rise in the curve. As the rigor becomes more complete throughout the preparation the maximal effect of the vapour diminishes. The latest time at which the effect can still take place exactly marks the end of the rigor 'plateau.'

During the stage after the completion of rigor, and before the onset of putrefaction, no dose of chloroform, however intense, has been found to effect the low rate of discharge characteristic of this period.

When, finally, the time has come for the steep elevation of the curve due to bacterial putrefaction, the chloroform vapour has again an influence upon the rate of CO_2 discharge. It produces an immediate and marked decline in the output, rapid at first but slower afterwards even if the application of the vapour be long continued. This effect of chloroform will be more fully discussed in § 9.

That chloroform should produce an increase in the production of CO_2 by an excised muscle is to be expected from its well-known property of hastening *rigor mortis*. When the vapour reaches the muscle in a sufficient dose to produce the rise in the CO_2 discharge, the surface of the muscle is observed to undergo local twitchings representative of rapid but localised rigor. The results already detailed show that chloroform-rigor like the rigor naturally occurring is accompanied by a special discharge of CO_2; and the elevation which is produced by a dose of chloroform in the rigor 'plateau' must be regarded as due to a hastening of the gradual natural rigor which causes a proportionate shortening of the time over which the plateau would otherwise have extended. If the dose of chloroform be intense enough to produce complete rigor throughout the muscle, the rate of CO_2 discharge declines rapidly from the height it has reached to the low level characteristic of the last stage (before putrefaction) of the normal curve.

That chloroform produces no effect at all upon the normal curve at this period after the completion of rigor mortis supports what has been said already with regard to this stage.

The cases in which a minute dose of the vapour has led to a temporary decline in the rate of discharge during the earliest survival periods provide an additional illustration of the differences between the character of the CO_2 discharge at this time and that during the onset of rigor. It is natural to suppose that a depressing effect ('anæsthetic effect') of this kind would be of rarer occurrence in face of the opposite tendency of the vapour to produce *rigor mortis* and its accompanying CO_2 production.

The effects of ether vapour. The effects of ether vapour applied like

that of chloroform have given similar results. Larger doses (*i.e.* longer application of vapour) are necessary for equal effects in hastening rigor and elevating the curve of discharge.

I have not obtained an 'anæsthetic' effect in the early periods by means of ether.

The effects of carbon disulphide vapour. This vapour has a very marked effect in hastening the process of *rigor mortis* as that is represented by the rate of CO_2 production. To the eye, however, its effect upon the muscle in stiffening is much less obvious than that of chloroform. The carbon disulphide produces an immediate rise in the rate of CO_2 discharge from the first periods after excision (see Fig. 26). After the completion of natural or artificial rigor, however, it has no effect upon the discharge.

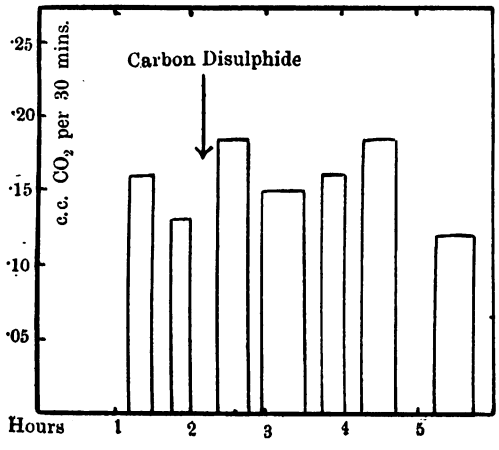

Fig. 26. Effect of CS_2 on survival respiration.

Carbon disulphide was said by Kühne, with Jani, to act only as a stimulant of nerve.

The effect of alcohol vapour. I have not been able to obtain any positive results with the use of alcohol vapour applied according to the method described for chloroform. The passage of the vapour over the muscle substance has in no case been found to produce at ordinary temperatures any effect upon the rate of CO_2 discharge. The longest exposure to the vapour was for $3\frac{1}{2}$ hours. Alcohol is one of the substances which Kühne grouped as stimulants of nerve but not of muscle.

§ 7. *Effects of acids.*

The experiments under this head are at present very incomplete, but some of the results I have obtained with solutions of lactic acid may be definitely stated.

Catharine Schipiloff[1] (12) found that weak solutions of lactic acid (·1—·25 p.c.) upon injection through the blood vessels of a frog caused immediate rigidity of the muscles. This rigidity was not only removable on injection of stronger solutions (·3—·5 p.c.), but was said not to be caused at all if the stronger acids were applied at the first. Schipiloff suggested that the development of lactic acid during survival periods was the cause of natural *rigor mortis*, the ultimate disappearance of which was due to the solution of the myosin clot in the stronger accumulated acid.

I have examined the effect of lactic acid solutions upon the survival CO_2 discharge and upon the occurrence of *rigor mortis*. The lactic acid was dissolved in normal salt solution in amounts from ·05—5·0 per cent. This series of strengths is a more extended one than Schipiloff refers to. Every solution of lactic acid was found to produce *rigor mortis* in an excised muscle immersed in it. The onset

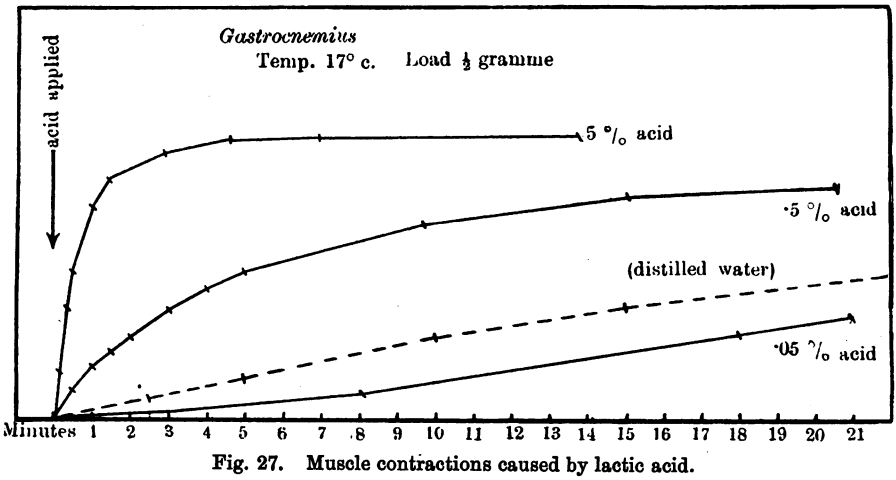

Fig. 27. Muscle contractions caused by lactic acid.

of rigor was more rapid as the solutions increased in strength and was practically instantaneous when such a very strong solution of acid as that of 5 p.c. was applied. At Fig. 27 are given curves illustrating

[1] *Centralblatt f. d. med. Wiss.* 182.

the shortening of the frog's *gastrocnemius* upon the application of lactic acid solutions of 5·0, ·50 and ·05 per cent. respectively. It will be seen that the strongest of these solutions is ten times more concentrated than the solution quoted by Schipiloff as altogether preventing rigidity.

The effects of these lactic acid solutions upon the survival discharge of CO_2 are in agreement with their influence upon death-shortening. All the solutions used, of strengths varying between the limits stated, have markedly increased the rate of CO_2 output. The experiment given at Fig. 28 shows that a ·05 per cent. solution applied to the muscle increased within ten minutes the rate of output from ·42 c.c. to ·68 c.c. per hour. The 5 per cent. solution of acid had an effect upon the CO_2 discharge only comparable with that produced by heat. The rate of discharge rose in five minutes from ·32 c.c. to 1·50 c.c. per hour, sinking gradually to zero during the continued application of the acid.

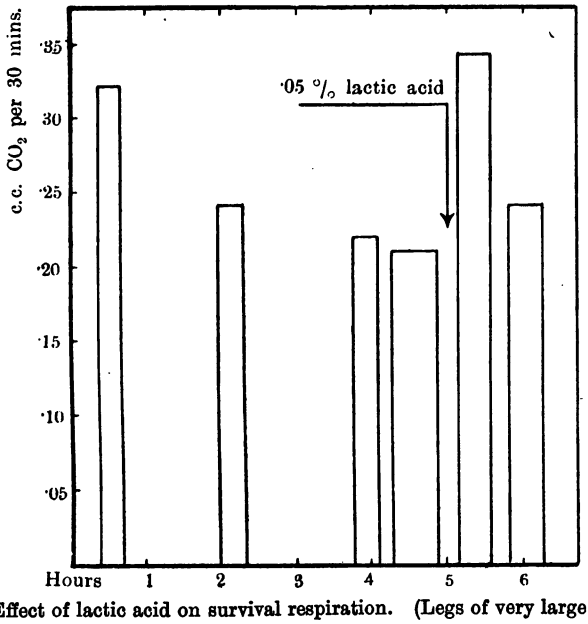

Fig. 28. Effect of lactic acid on survival respiration. (Legs of very large frog used.)

The application of the acid was always effected within the experiment chamber itself; 10 c.c. of the solution placed at the lower end of the tubular chamber, was brought to the muscle at will by the tilting of the chamber (see Fig. 42).

The results of some experiments I have recently begun have

suggested that it may become possible to draw a distinction between the mode of action upon muscle of very weak lactic acid solutions and of the stronger ones. The sudden rigor produced in a muscle subjected to immersion in fluids containing more than ·1 per cent. of acid seems to be of a different nature from the slower shortening caused when less acid is present. The shortening produced by a ·9 per cent. NaCl solution containing ·05 per cent. of lactic acid is partially removable by subsequent immersion in the same NaCl solution containing no acid. Such a recovery is shown at Fig. 29. I have so far been quite unable

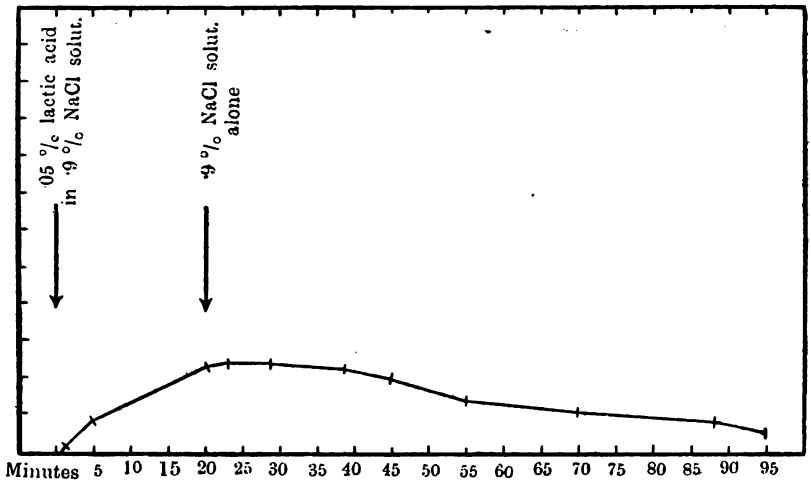

Fig. 29. Recovery from contraction due to weak lactic acid solution.

to find a similar recovery from the shortening produced by the stronger acid solutions (·1—5 per cent.). This possibility of recovery from the lactic acid rigor which has been produced by the weakest acid solutions indicates that the shortening in these cases may not be due to a chemical reaction which introduces or accompanies *rigor mortis*, but to a physical effect connected with osmosis based upon the presence of the lactic acid in solution about the muscle. It is difficult otherwise to account for the relaxation undoubtedly caused by the subsequent washing with the neutral salt solution of exactly the same concentration as that previously holding the acid. Such a physical effect, if it occur, must be present too when the stronger acids are employed, but here it is presumably overshadowed by the very pronounced chemical phenomena with the rapid shortening and rigidity.

This consideration, at present only suggested, is of interest in

relation to the effects of the acid upon the natural CO_2 discharge already described. The weakest as well as the strongest acids have been found to increase the rate of CO_2 output. But if the shortening due to the weakest be a physical phenomenon, and not comparable with true rigor, the additional CO_2 produced by the weak lactic acid solution must have a different source from the greater part of that due to the strong solutions. It is conceivable that the very weak acid solutions, too weak to cause immediately the chemical disturbance accompanying *rigor mortis*, but able to affect the physical condition of the muscle in such a way as to produce slight shortening, might affect, as it does, the amount of CO_2 output by liberating CO_2 held within the muscle in loose combination. The action of the stronger acids would be the same, but would cause in addition the pronounced shortening and the additional CO_2 yield of true *rigor mortis*.

§ 8. *Influence of temperature variations.*

Under this head will be very briefly described the effects produced in the normal rates of discharge:

(*a*) by variations of temperature within the limits 13° C.—25° C., and

(*b*) by cooling to points between 0° C. and 5° C.

(*a*) The effects of variations of temperature between 13° C. and 25° C.—the upper limit being well out of reach of the heat-clotting temperature—have not presented any special points of interest. I have not yet worked out any accurate quantitative relations between the temperature (between these limits) and the course of the survival respiration. But some general qualitative relations may be roughly stated.

The initial rate of the first diffusion effect—of the discharge of CO_2 during the first period after excision—is increased with the temperature within the stated limits, an increase probably related in a simple way to the decrease in solubility of CO_2 as the temperature advances. The beginning of the period of progressive *rigor mortis* is hastened as the temperature is raised. The earliest indications of it may be found in the 4th hour of excision for temperatures of 19°—22° C., or as late as the 7th hour for temperatures of 13°—15° C.

The most important factor in regulating the time of appearance of bacterial putrefaction is the temperature. Putrefaction is hastened as the temperature is higher. Other factors, such as the cleanliness

of the materials used and the nature of the muscle surface, are prominent enough to make it difficult to express this relation more accurately.

(*b*) Kühne[1] (1864) has shown that the frog's muscle plasma is prevented from clotting by temperatures below 0° C., and that at temperatures just above this the clotting proceeds extremely slowly. I have followed the rate of survival discharge in muscles kept at temperatures between 0° C. and 5° C., chiefly with the object of finding how far the rigor produced by chloroform was comparable with natural rigor in relation to cooling. At Fig. 30 is shown the course followed

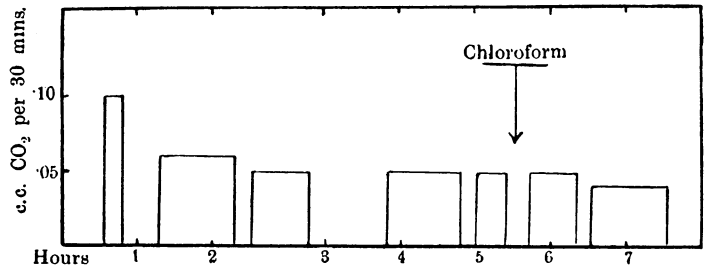

Fig. 30. Survival discharge from legs of frog at 0°—5° C.

by the discharge from a muscle cooled to this extent. The low initial rate of discharge is soon succeeded by a very low and steadily maintained rate continuing for many hours. After 24 hours (during part of which time the temperature rose as high as 8° C.) no rise from the low rate of discharge had made its appearance.

At 5° C. chloroform vapour has no effect at all upon the rate of discharge. In the experiment shown at Fig. 30 the vapour from heated chloroform applied for an hour did not appreciably affect the yield of CO_2.

§ 9. *Phenomena of bacterial putrefaction.*

The sudden upward tendency of the normal curve of CO_2 discharge accompanying the development of a putrefactive smell makes its appearance at a very variable time after the first exposure of the muscle-preparation. This rise in the curve leads rapidly to rates of CO_2 discharge much higher than those attained during any of the earlier survival periods and is the natural conclusion of the whole account of the survival production of CO_2.

[1] *Untersuch. ü. das Protoplasma*, Leipzig, 1864.

That the daily increase in the output of CO_2 from an exposed mammalian muscle is a function not of the muscle itself but of micro-organisms infecting its surface has been shown by Tissot[1] (1894), who found that under aseptic conditions the output from the muscle soon declined to zero. In the case of frog's muscle I have found it possible to ward off the putrefactive rise in CO_2 output for at least six days after excision. It has been already said that the discharge of CO_2 eventually sinks to the 'traces' equivalent to zero, in the absence of micro-organisms. The study of the CO_2 phenomena of bacterial putrefaction is accordingly irrelevant to the survival respiration of muscle, but a short account of their relations to the normal curve is necessary to illustrate the objections which have been raised to many of the older observations upon 'muscle-respiration,' in which precautions were not taken against this source of error.

The steep rise in the rate of CO_2 discharge due to putrefaction has been shown at Figs. 4, 6 and 7. The putrefactive rise varies markedly in the time of its appearance, the most important factors aiding its early development being warmth and uncleanliness. The average time of occurrence of putrefaction, in experiments where clean instruments and sterilized chambers have been used, is from the 36th hour onwards from excision, for temperatures 14°—17° C. One of the latest times of occurrence is that shown at Fig. 7, where the putrefactive rise began at the 50th hour, and was not very highly developed even at the 70th. Under ordinary circumstances however the average time of occurrence is about the 24th hour after excision, at 20°—22° C. The rate of increase of CO_2 output during the early stages of putrefaction may be very great. In the example given at Fig. 6, for instance, the rate of output is rather more than trebled within 4 hours.

The influence of cleanliness in work was shown in three immediately consecutive experiments carried on in the same chamber which was only rinsed with water at the end of each, instead of being washed out with mercuric chloride solution as usual. In the first, the putrefactive rise began at the 40th, in the 2nd at the 19th, and in the 3rd at the 15th hour after excision. A similar muscle-preparation was employed in each case, the temperature for all being 17° C. The very first beginning of a rise in output of CO_2 is nearly simultaneous with the earliest occurrence of a putrefactive smell. It is often however only just possible to detect a putrefactive smell from muscles in which a well-marked rise of the CO_2 output has already begun.

[1] *op. cit.*

The effect of chloroform vapour upon the putrefactive production of CO_2 is well-marked. A dose of the vapour given for an hour, according to the method already described, produces invariably a rapid decline in the rate of output. It is usually observed however that though an hour's dose of chloroform will rapidly bring the production of CO_2 to a lower level, a subsequent larger dose (from 3 to 5 hours for instance) carries the decline no further. To bring the level of CO_2 production approximately to zero it is necessary to apply the vapour for an enormously longer time. The following typical example illustrates

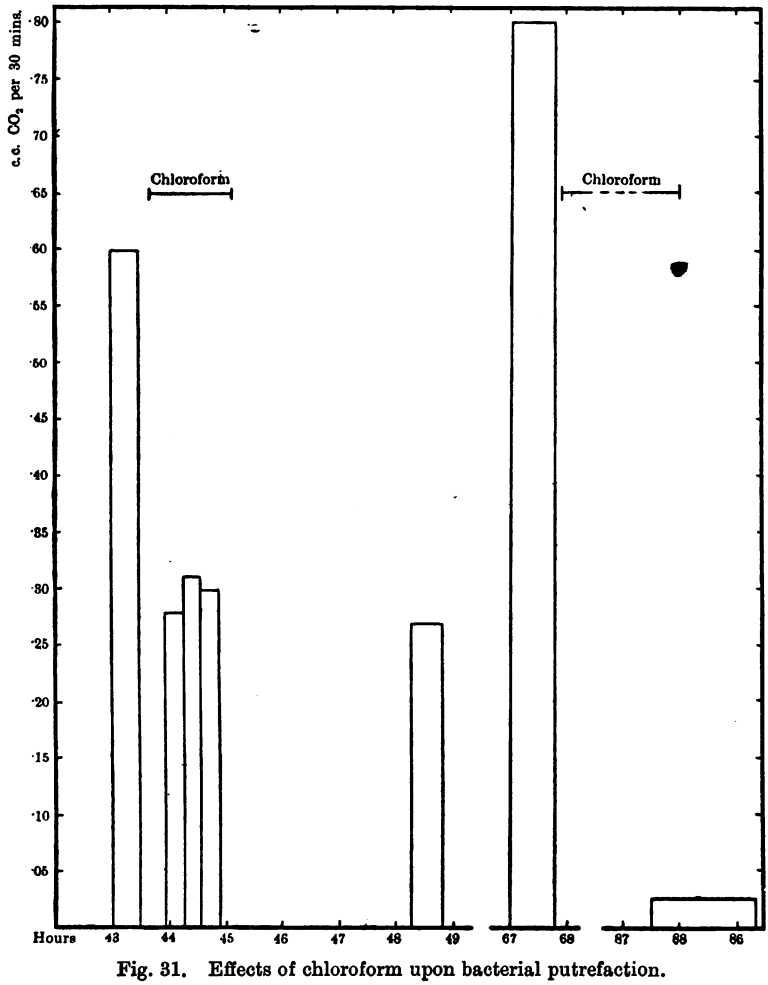

Fig. 31. Effects of chloroform upon bacterial putrefaction.

this. To a putrefying muscle discharging CO_2 at the rate of 1·2 c.c. per hour, chloroform vapour was applied for 1½ hours. The rate sank to ·54 c.c. per hour at the end of 3 hours, but declined no further. Subsequently the rate of discharge again rose and reached 1·4 c.c. CO_2 per hour. During six hours of chloroform the rate became ·34 c.c. per hour at the end of 3—4 hours, but did not fall lower. The temperature was 16°—18° C. After 24 hours from the beginning of the experiment the putrefactive discharge rose to the rate of 1·6 c.c. per hour, and was brought by means of chloroform applied for 4 hours to ·35 c.c. per hour. After an application lasting 20 hours the rate of discharge fell to ·05 c.c. per hour, *i.e.* approximately to zero (see Fig. 31).

The facts agree with the accounts of the products of putrefaction which have been based upon other considerations (see Gotschlich[1] (1896)). These bodies are to be considered in two classes. On one hand are the immediate products of the cell-metabolism of the bacteria, while on the other are the products of the chemical disturbance set up in the culture medium through their agency. The large respiratory gas-exchange of certain bacteria has been demonstrated by Hesse[2] and others. It is reasonable to suppose that the part of the putrefactive yield of CO_2 which is immediately checked by chloroform is a measure of the direct respiratory activity of the micro-organisms upon the surface of the muscle. The remaining part, which is not checked until the application of the chloroform has been continued for many hours, cannnot have a similar source. It is probably to be assigned to chemical changes already set up in the muscle-substance, presumably through enzymes secreted by the organisms,—changes which the chloroform can only control by killing the bacterial life, and so preventing their continued origination.

§ 10. *Summary of Part II.*

The normal course of the survival respiration of excised frog's muscle has been expressed by a curve representing the changes in the rate of discharge of CO_2, from excision onwards. Without precautions the normal course may be interrupted by the special output of CO_2 due to putrefactive bacteria, but it has been shown that this output is a purely accidental and independent phenomenon which, when recognised, may be left out of consideration.

[1] Flügge's *Micro-organismen*, Leipzig, 1896.
[2] *Zeitschr. f. Hygiene u. Infektionskankheiten*, xv.

The normal curve of discharge (see Fig. 32) may be divided into three stages. The first stage extends over about 5 hours and is irregularly declining. The second stage, described as the 'plateau,' is many hours longer and shows a very slowly declining rate of discharge. It passes by gradual degrees into the third stage in which only traces of CO_2 are yielded by the muscle. Reason has been shown (§ 2) for believing that during this last stage the production of CO_2 *de novo* by the muscle has reached zero. The first and second stages of the curve are merged at a point near the 6th hour which is marked by a slightly raised 'hump.' The hump may be well or badly marked and has been absent in one out of every three or four curves obtained.

The results stated in § 2 have shown that a given evolution of CO_2 at any time within the muscle is to be expected to have punctual representation in the curve of general discharge. And they indicate that the curve as a record of the course of events in the muscle is approximately faithful in its time relations.

From the consideration of the schematic normal curve at Fig. 32, which marks out an area expressing the total output of CO_2 by volume, we may first remove the only part of it which has been shown to be dependent upon the immediate presence of free oxygen in the atmosphere. This forms a small fraction ($\frac{1}{5}$—$\frac{1}{6}$) of the output during the earliest period after excision, but the fraction decreases as the survival discharge proceeds until, after a point near the 6th hour, no part of the CO_2 output seems due to contemporary oxidation (§ 5). The shaded triangular area at the lower part of the diagrammatic scheme represents this diminishing discharge of CO_2.

For the present it seems most natural to suppose that such a declining oxidation is similar to and actually continues part of the respiratory process of normal life. And the presence of such a slight but 'vital' respiration after excision supplies an explanation of the slight 'anæsthetic' effect produced by small doses of chloroform vapour during these early periods (§ 6).

After subtracting the CO_2 due to this declining and imperfect 'life,' there still remains the bulk of the survival discharge (the unshaded area in the scheme) for consideration. The part of this which seems to have the more obvious meaning is that subsequent to the 6th hour already described as the plateau. This has already been taken as an indication of the constant production of CO_2 due to the gradual onset of natural *rigor mortis* in all parts, successively, of the muscle (§ 4). The formation of free CO_2 during this time is accompanied by the continued

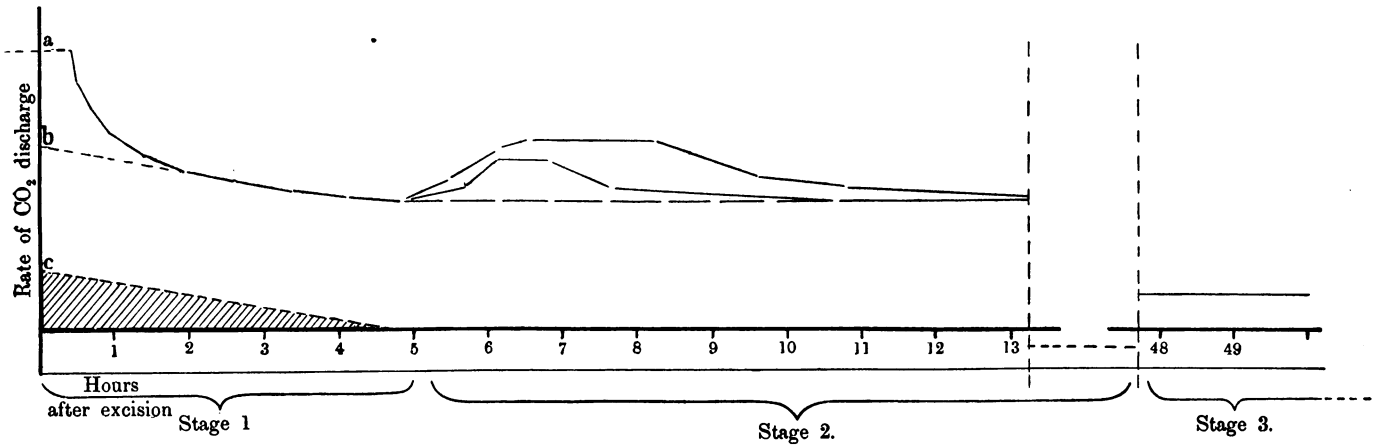

Fig. 32. Scheme of the survival discharge of CO_2.

formation of acid and the progressive shortening of the muscle, and may be hastened, like these, by the application of chloroform vapour, of other irritants, of lactic acid, and of heat; it is retarded by cooling and also, apparently, by the absence of free oxygen from the muscle during the previous survival periods (§ 5). In the normal curve, uninterrupted by putrefaction, the end of the rigor plateau, which is the end of the spontaneous activity of an excised muscle in the production of CO_2, is definable by the absence of any chloroform effect (§ 6).

Turning lastly to the unshaded area of the scheme during the early survival periods from excision to the 6th hour, greater difficulties of interpretation arise. The activity of discharge declines from the beginning, first rapidly, and then more slowly, until it reaches a rate of production equal to that at the first part of the rigor plateau, exclusive of the hump. It is not easy to explain this part of the discharge as arising from any one source alone within the muscle.

To interpret the whole of it as due to splitting processes like those responsible for the rigor plateau, and which decline from an initial activity caused by the shock of excision to a steadier spontaneous level, is made difficult by the shape of the curve. Whenever by interference the rate of CO_2 discharge is increased temporarily during the plateau, the subsequent decline is first slow and then rapid, giving a convex outline very different from the concave descent under discussion. The facts given in § 3 are also opposed to such a view.

Another explanation would be found in the view that this part of the discharge is the result of processes of destructive metabolism like those during normal life associated with the maintenance of irritability in a resting muscle. After excision, it might be taken that these processes, declining with the disappearing irritability, gave rise to the falling curve under discussion. It is however impossible under this interpretation to explain the close similarity between both the early and later course of discharge from a muscle in which the circulation has continued after the beginning of exposure and observation, and those in the case of a muscle excised in the ordinary way. It has been found that it is on the time of skinning and exposure and not on the time of stoppage of circulation that the occurrence of the rapid decline from relatively high rates of CO_2 discharge depends (§ 3). All we know moreover of the decline of the functions of muscle during survival periods, show it to occur as a diminishing arithmetical series, and the curve representing it to be a straight line.

It has been suggested (§ 2) that an account of the CO_2 discharge

during these early periods might be given in terms of diffusion of an excess of CO_2 from the muscle substance. The shape of the normal curve is such as to suggest that the muscle at the moment of excision contains more free CO_2 in its substance than represents its activity at that time in production. The curve of discharge, as in the artificial case for comparison, sinks rapidly and then more slowly until it has reached the level maintained by the actual rate of fresh production. In the absence of the initial surplus of CO_2, the curve would begin (see p. 34) at a level not very much higher than that just before the 6th hour or that of the rigor plateau. Such an explanation is applicable to the case in which the circulation was continued after the beginning of observation (p. 78). Here, too, the rapid initial descent is not directly representative of declining production but of the establishment of equilibrium between the CO_2 contents of the muscle substance and of the air surrounding it.

On this suggested explanation, the earliest stage of the whole survival discharge from excised muscle is to be accounted for in terms of three separate factors. The bulk of the discharge of CO_2 is due to destructive processes going on within the muscle independently of the surrounding medium. In addition to this are smaller quantities of CO_2 of two distinct origins. Part of the earliest discharge is CO_2 existing already as such within the muscle and which escapes with the rest by diffusion outwards. The process of diffusion is rapid enough to allow the escape to attain first rapidly and then more slowly the rate of output representing the real rate of production by the muscle. The remaining part of the discharge, finally, is due to a process in which oxygen is absorbed from the air and CO_2 produced, and which is to be taken as an imperfect continuation of part of the normal process of respiration during somatic life. The rate of this yield of CO_2 possibly only contributed by the layers of muscle which have direct access to the atmosphere, declines steadily from excision until near the 6th hour, when it has reached zero.

After this time, usually at the 6th hour from the first exposure, the rate of discharge is at last truly representative of the destructive processes going on in the muscle independently of its surroundings which have for one end the appearance of free CO_2. To processes of this kind have been already assigned the CO_2 production during the long rigor plateau. They are to be considered for the present as extending also over the earlier periods, but masked in these by the initial diffusion effect, and supplemented as well by the slight and declining oxidative

process continuing normal respiration. In the scheme at Fig. 32 these factors in the discharge have been expressed diagrammatically.

This interpretation of the course of survival respiration in muscle can be only provisional in view of the many additional experiments which have still to be made. It gives at present however an adequate expression to the results already obtained. Some difficulties raised by its adoption may be briefly considered in conclusion.

It has been said that the most satisfactory explanation of the form of the early part of the normal curve involves the presence in the muscle, just after excision, of free CO_2 in excess of that being formed at the time. And although it is extremely probable that the rate of CO_2 production within a living muscle whose circulation is intact is greater than that during any survival period, the presence of any excess at excision which is quickly removable by diffusion outwards, seems to imply at first sight that the blood-stream is actually a less efficient means for the removal of the CO_2 than the process of diffusion to the air current after excision.

At present there is no reliable ground on which to base a comparison between the two processes, nor is there direct evidence of the completeness or rapidity with which removal of CO_2 is effected by the blood. The presence of an excess of CO_2 in the muscles just after excision from a freshly killed frog is not difficult to understand. The rate of production of CO_2 within the living body, when nutriment is being supplied to the muscle as well as oxygen by the blood, is probably very much higher than at a time after excision when nutriment is not supplied and the oxygen reaching the tissue does so by diffusion from outside. And the fall from one level of production to the other, though sudden, is probably not exactly synchronous with the moment at which the circulation ceases, since after such cessation the building up processes by which the high level of CO_2 production is attainable, may continue for a time. Such a brief continuation would give the excess needed by hypothesis. The completion of the escape by diffusion of this excess would allow the normal curve soon after the earliest period to represent the truly survival discharge on the lower level. Such an explanation does not include the case quoted at p. 34 and p. 78 (Fig. 41), if under those conditions the circulation through the leg muscles be really normal. The experimental method however is sufficiently imperfect to allow a doubt on this point. The legs had been skinned, and the waist was necessarily slightly constricted; these conditions might be enough to lead to an excess of production over removal of CO_2 in the living muscles.

Leaving this question on one side, there can be no doubt that the bulk of the survival discharge of CO_2 after excision is due to chemical processes occurring spontaneously within the muscle, in which complex molecules are replaced by simpler ones, with the conspicuous results of the appearance of the acid and of free CO_2. This process, masked as we have seen during the earliest survival periods, becomes more and more evident as its resulting CO_2 discharge ceases to be augmented from other sources. There has appeared so far no reason at all for dissociating this destructive decomposition, as it occurs before the 6th hour, from that giving rise, later on, to the plateau with or without its introductory hump. It is a decomposition during the whole course of which no line can at present be drawn dividing one variety of CO_2 origin from another. The presence in most cases of the 6th hour's 'hump' is however an irregularity in the otherwise uniform advance of destructive decomposition for which no counterpart has yet been described either in the physical condition or acid reaction of the muscle.

The variability of occurrence of this hump, taken together with the effects of weak solutions of lactic acid upon muscle (§ 7) have suggested a meaning for it which is advanced with hesitation, but which at least supplies a direction for future experiments. It was shown that extremely weak lactic acid solutions, whose effect in causing shortening of the muscle was apparently rather physical than chemical, since it was reversible by a simple alteration of conditions (see p. 56), nevertheless produced an increase in the output of CO_2 from the muscle. It was suggested that this increased output was due to the liberation of CO_2 previously held in loose combination within the muscle and that the increase was not of the same nature as that caused by the strong acid solutions which undoubtedly have a direct chemical effect upon the muscle substance and lead to a large fresh production of CO_2.

The destructive decomposition advancing in an excised muscle is accompanied by the production of acid and of CO_2. It is easy to imagine that at a certain point the acidification becomes intense enough to liberate loosely combined CO_2 within the muscle, whose output would cause a 'hump' on the curve. That such a 'hump' should be variable in its occurrence could then (and it is not easy to see how otherwise) be accounted for, since such loosely combined CO_2 might be of variable occurrence, and depend on the previous activity or nutrition of the muscle.

The production of acid still advances, however, and as it accumulates more and more in the muscle-substance it is reasonable to suppose,

from its effect when artificially applied, that it acts in a kind of vicious circle by aiding the processes of decomposition which gave rise to it. Our knowledge of the different stages in these processes is extremely slight. One of the stages, and probably itself a late one, is the production of the myosin clot, but the relation of this to the other occurrences introducing or accompanying it is still unknown.

The comparison of the normal curve of CO_2 discharge with the curves expressing the death-shortening of the frog's leg muscles (Fig. 16), shows that the chemical processes leading to the production of CO_2 correspond in point of time with the early stage of very slow shortening, and are not only nearly at an end when the rapid shortening begins, but exhibit no signs of increased activity at that period. It is suggested here that the slow shortening associated with the steady production of CO_2 may be analogous to that produced apparently as a physical effect by weak solutions of lactic acid, and may be due to the slowly accumulating acid whose formation is an accompaniment of the CO_2 production at this time. At a later time, the noticeable precipitation of the myosin clot occurs. This is associated with a rapid shortening of the whole muscle, but a shortening which, probably like the change from myosinogen to myosin, has no representation in the form of the normal curve for the CO_2 discharge.

Part III. The course of survival respiration during periods of contraction.

In the previous observations upon the rate of the CO_2 output from active as compared with resting excised muscles, results have been obtained to show that a special outburst of CO_2 is the inevitable accompaniment of contraction in an excised muscle.

In the early accounts by Matteuci[1] and Valentin[2] (1857) of muscle respiration, it was stated that the amount of CO_2 discharged into the closed experiment chamber was increased if, during a given time, the muscle had been stimulated by the interrupted current. This statement was repeated by Hermann[3] after his later (1867) experiments upon this point; he found that more CO_2 was discharged by a muscle which was tetanised than by a similar muscle at rest during the same time, but that this difference between their rates of discharge was diminished if the resting muscle were frequently agitated.

[1] *op. cit.* [2] *op. cit.* [3] *op. cit.*

A part therefore of the excess CO_2 in the case of the tetanised muscle was due to the continued renewal of the layers of air next to the muscle:—

EXP. 27. 2 muscles, each in tube. Period of estimation 3 hours 10 minutes.

Muscle at rest yielded 1·43 per cent. of CO_2 by volume.
Sim. muscle tetanised yielded 9·33 „ „ „

EXP. 31. 2 muscles, each in tube. Period of estimation 2 hours 35 minutes.

Muscle at rest but agitated yielded 9·31 per cent. of CO_2 by volume.
Sim. muscle tetanised „ 11·31 „ „ „

The results of determinations of the kind quoted, are not very satisfactory. For since tetanus is known to hasten *rigor mortis*, and the latter process has been shown to be accompanied by an increase in CO_2 output, the experimental result may be equally well explained as due to the inclusion of some of the CO_2 due to rigor in the one case within the long period of observation upon the two muscles.

The latest work on this question has been that of Tissot[1] (1895). The leg of a frog, deprived of its skin and with its foot removed, was passed up to the known volume of air contained in a test tube inverted over mercury. After the experiment the changes in constitution effected in the air were determined eudiometrically.

Tissot denied that mechanical agitation aids the evolution of CO_2 from the surface of a muscle, and, with regard to the effect of contraction upon the discharge of CO_2, found that the muscles of a stimulated leg yielded more CO_2 than those of a leg kept at rest during the same time. He gives the figures:—

Leg at rest yielded ·34 c.c. CO_2 in 70 minutes.
Leg stimulated to contract yielded ·54 c.c. CO_2 „ „

The possibility of the hastening of rigor in the muscle which has been severely tetanised is a serious weakness in the conclusions which Tissot has drawn, and it is a possibility which apparently he has not considered. Whether or not placing the muscles in a confined and small volume of air, so that they were exposed to the accumulating products of their activity, could also have resulted in the hastening of rigor, is another point which is not discussed as a possible source of error.

[1] *op. cit.*

I have followed the changes accompanying contraction in an excised muscle by the volumetric method already described, and have found the method especially convenient for the purpose. The periods of estimation may be so short as to be contained within the earliest survival periods before irritability has declined far, and the course of the CO_2 discharge may be readily followed after the period of activity, without interruption. At the same time the fact that the current of air causes a continual renewal of the layers of gas nearest the muscle, renders it unnecessary to consider possible complications due to the movements of the contracting muscle.

The muscles of the frog's leg have in all cases been used. The experiment chamber has usually been tubular, with entrance and exit tubes at opposite ends, the whole volume being made as small as possible. In the earliest experiments the pelvis of a frog was prepared with both legs attached; the feet were amputated and the skin removed. The preparation was enclosed in a flat discoidal chamber, two platinum electrodes passing to the exterior having been hooked one behind each *gastrocnemius* tendon. The result of a typical experiment of this kind was as follows:—

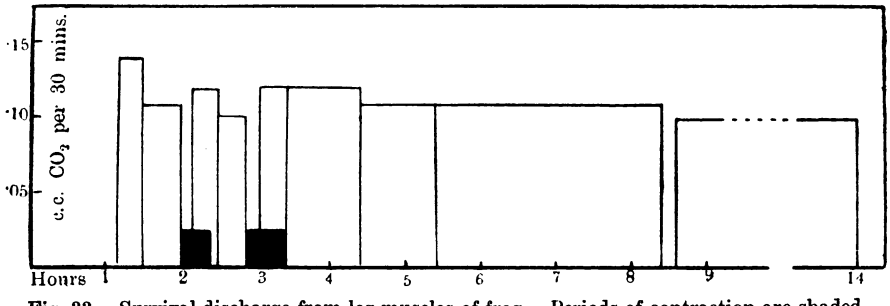

Fig. 33. Survival discharge from leg muscles of frog. Periods of contraction are shaded.

EXPERIMENT I. (See Fig. 33.)

The two legs arranged as described and current started 11.30 a.m.

11.45 a.m.	—12.5	yielded	·14	c.c. CO_2	per ½ hour.
12.5 p.m.	—12.35	„	·11	„	„
12.41 „	— 1.1	„	·12	„	„
1.1 „	— 1.21	„	·10	„	„
1.25 „	— 1.45	„	·12	„	„
1.45 „	— 2.45	„	·12	„	„
2.45 „	— 3.45	„	·11	„	„
3.45 „	— 6.35	„	·11	„	„
6.45 „	—12 a.m.	„	·10	„	„
[12 noon	—11 p.m.	„	·016	„	„]

From 12.35—12.55 muscles tetanised during alternate minutes (coil at 17 cm.).

From 1.21—1.45 muscles tetanised during alternate minutes (coil at 6 cm.).

It is seen that in this experiment severe intermittent tetanus extending over 20 minutes produced an extremely slight addition to the total CO_2 output (about ·02 c.c. CO_2). A second period of tetanus of the same kind, however, caused a rise in the rate of output no higher than the first, but which led to a long maintained level of production corresponding closely with the plateau in the natural curve already associated with *rigor mortis*. Here then is evidence that while no distinct outburst of CO_2 is associated with contraction, the contraction may cause a very marked hastening of the advent of *rigor mortis*. If the total discharge from this tetanised muscle collected for one estimation during the first 3 hours had been compared with that from a similar resting muscle, an excess amounting probably to nearly 1·5 c.c. of CO_2 would have been put down in the former case to the occurrence of contraction as such.

In all the experiments remaining to be described however it was found that if care be taken in the application of the stimulus, even the effect of hastening rigor may be avoided and the period of tetanus gone through without apparent modification in the normal curve of discharge.

In the following example the preparation was arranged as in Experiment I., but the lumbar plexus of each side was laid across the electrodes.

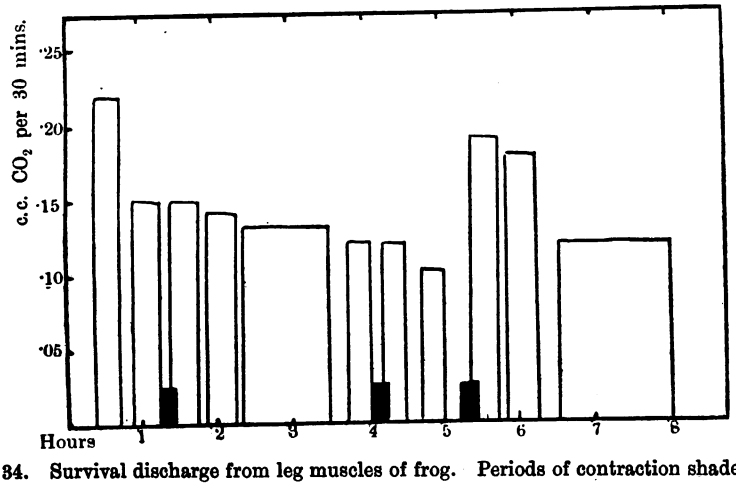

Fig. 34. Survival discharge from leg muscles of frog. Periods of contraction shaded.

EXPERIMENT II. (See Fig. 34.)

Current started 11.0 a.m.

11.22—11.42 a.m. yielded ·22 c.c. CO_2 per ½ hour
11.50—12.10 p.m. „ ·15 „ „

[12.11—12.16 muscle stimulated through nerves to violent tetanus every 15″. Coil at 20 cm.]

12.20—12.42 p.m. yielded ·15 c.c. CO_2 per ½ hour
12.50— 1.10 „ „ ·14 „ „
1.20— 2.30 „ „ ·13 „ „
2.41— 3.1 „ „ ·12 „ „

[3.3—3.8 stimulation as before; similar response with coil at 12 cm.]

3.10— 3.30 p.m. yielded ·12 c.c. CO_2 per ½ hour
3.40— 4.2 „ „ ·10 „ „

[4.12—4.20 stimulated as severely as possible every 10″, coil at 0 cm. Irritability soon abolished.]

4.20— 4.40 p.m. yielded ·19 c.c. CO_2 per ½ hour
4.47— 5.7 „ „ ·18 „ „

In this experiment two periods of contraction, active but not excessive enough to produce rapid loss of irritability, were marked by no rise in the curve. The third period of excessive contraction at a late survival stage led to a sustained rise, but, while the stimulus applied was much more intense than before, the resulting contractions were even at the first not greater than the previous ones, and were absent during the last two minutes of the stimulation.

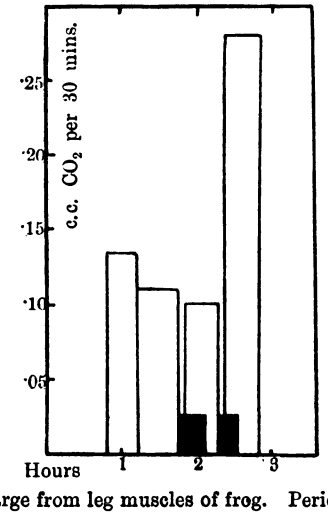

Fig. 35. Survival discharge from leg muscles of frog. Periods of contraction shaded.

In a similar experiment, two legs of a frog were used, connected in series by platinum wire in a tubular chamber. The induced current was sent through the whole muscle chain.

EXPERIMENT III. (Fig. 35.)

Current started 5.35 p.m.

5.51—6.11 p.m. yielded ·13 c.c. CO_2 per ½ hour
6.11—6.41 „ „ ·11 „ „
[6.41—7.0 p.m. strong tetanic contractions every 15″. Coil at 7 cm.]
6.45—7.5 p.m. yielded ·10 c.c. CO_2 per ½ hour
[7.5—7.20. Coil at 0 cm. Tetanic spasms produced in rapid succession so that muscles perfectly unirritable after the first five minutes, after which they remained passive.]
7.5 —7.20 p.m. yielded ·28 c.c. CO_2 per ½ hour

In this example the first period of stimulation is marked by more numerous and effective contractions than the second. But while during the first no change in rate of CO_2 discharge is noticed, during the second fatiguing stimulation, the rate is nearly trebled. The method adopted here is, in short, the best for securing the maximum effect upon the rate of output dependent on fatiguing tetanus. Strong direct stimulation leading to very rapid loss of irritability and response produces the most marked increase in yield of CO_2. This Experiment III. provides a case for comparison with the results obtained by Hermann and by Tissot, given above.

It may be said that while a period of contraction produces no rise in the curve of discharge for an excised muscle unless the stimulation be excessive, or deferred until irritability is nearly lost, the rise even under these conditions is not temporary or in correspondence with the period of contraction, but of long duration, usually reaching its maximum well after the stimulation has ceased. These points are illustrated by a set of three experiments to be described. Here a chain of 6 *gastrocnemii* was used in each case, bearing a load of 10 grammes. The muscles, connected in series by platinum hooks joining each lower end of the femur to the tendon of the next muscle, were arranged in an experiment chamber similar to but longer than that shown at Fig. 36. The induced current was sent during stimulation through the whole series. To save space, the figures for volumes of CO_2 are not quoted in full, but are expressed like those above in

diagrammatic form (Figs. 37, 38, and 39). The periods of stimulation are marked in the diagrams.

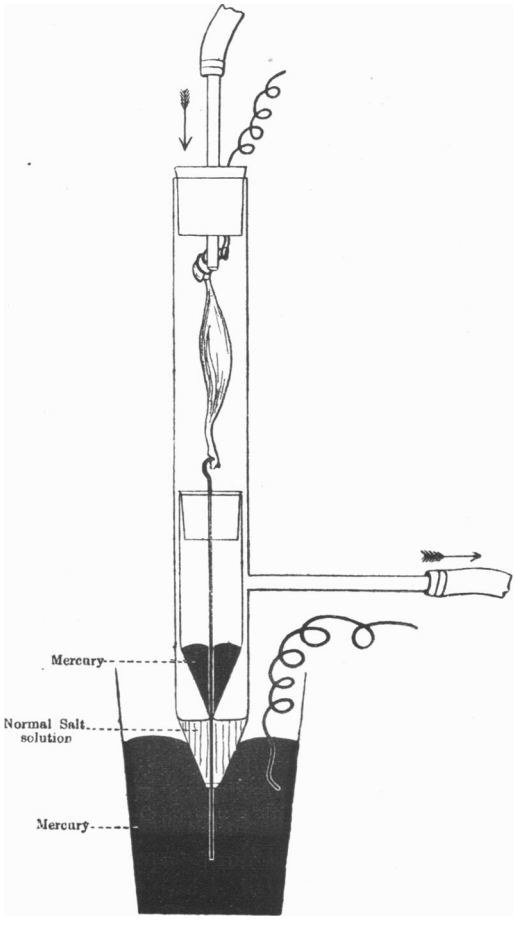

Fig. 36. Experiment chamber.

EXPERIMENT IV. (Fig. 37.) Temp. 16° C.

1*st period of stimulation* lasted 10 minutes. Coil at 7 cm.

Key held open every 15″ till maximum tetanic spasm reached.

Total contraction $\frac{3}{4}$ inch. No appreciable loss of irritability during this time.

No effect on curve.

2nd period of stimulation lasted 5 mins. Coil at 3 cm.
Similar stimulation every 15″. Total contraction $\frac{3}{4}$—$\frac{1}{2}$ inch.

No effect on curve.

The rigor hump appears in the subsequent curve of discharge a little earlier than normal.

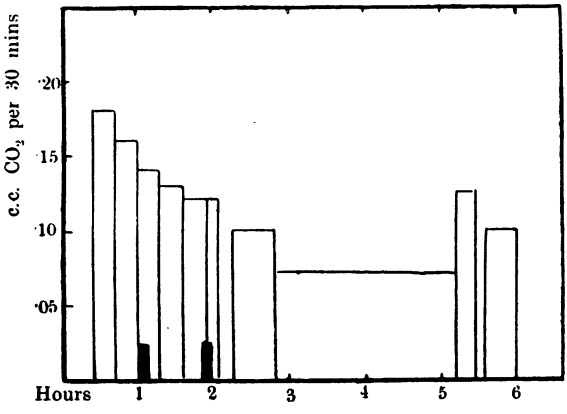

Fig. 37. Survival discharge from six *gastrocnemii*. Contraction periods shaded.

EXPERIMENT V. (Fig. 38.) Temp. 14·5° C.

Period of stimulation lasted 10 minutes. Coil at 4 cm.

Stimulation as before, every 5″. Strong contraction at first, but in second 5 mins. fatigue very pronounced. Contraction total fell from initial $\frac{3}{4}$ inch to $\frac{1}{8}$ inch. At end of period there was a permanent shortening of $\frac{1}{5}$ inch.

Rise in curve, reaching later maximum, and lasting 3 hours.

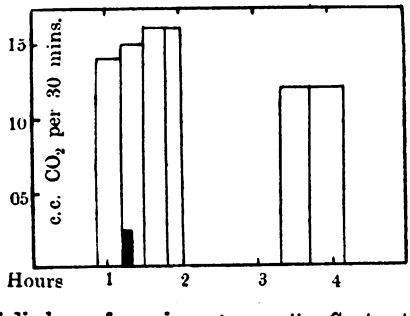

Fig. 38. Survival discharge from six *gastrocnemii*. Contraction periods shaded.

EXPERIMENT VI. (Fig. 39.) Temp. 13°C.

1st period of stimulation lasted 10 mins. Coil at 8 cm.

Stimulation as before, every 10", no loss of irritability apparent. Contraction total ½ inch.

No effect on curve.

2nd period of stimulation, 4 hours after excision. Lasted 5 mins. Coil at 0 cm.

Stimulation every 5". Contraction at first violent—total 1 inch,—but rapidly lessening until total ¼ inch. Permanent shortening of ¼ inch.

Advancing rise in curve.

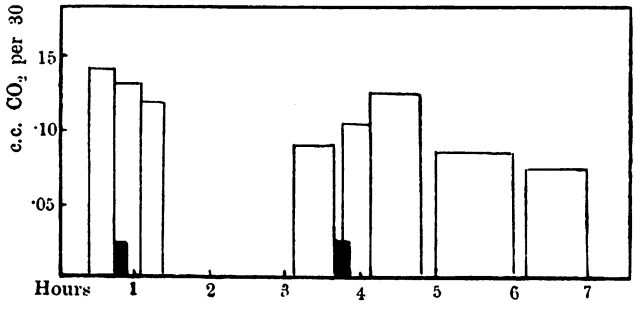

Fig. 39. Survival discharge from six *gastrocnemii*. Contraction periods shaded.

In a muscle-chain composed of 6 *gastrocnemii* it is very easy to observe the permanent shortening due to severe direct stimulation. This fatigue effect was always found in those cases in which stimulation produced any change in the normal curve of discharge.

In the last method of experiment to be described arrangements were made to secure the greatest possible irritability in the muscle preparation. A flat circular chamber was prepared with glass sides and wooden edges (see Fig. 40). At one point the wooden edge, complete elsewhere, was interrupted by a gap of ⅛ of an inch. The wood employed had been previously kept at a high temperature for half-an-hour, while immersed in liquid paraffin wax, so that all its pores had, on cooling, become filled up with solidified wax. One glass side was permanently fixed to the woodwork forming the sides of the chamber.

A healthy frog was lightly anæsthetised with ether and a piece of stout sheet india-rubber pierced in the centre was drawn over the

frog's waist, so as to fit well but not too tightly to the skin. The frog was placed with its waist in the space interrupting the wooden wall of the chamber so that the india-rubber could block the main interstices left between the frog's body and the wood. The unfixed glass side was

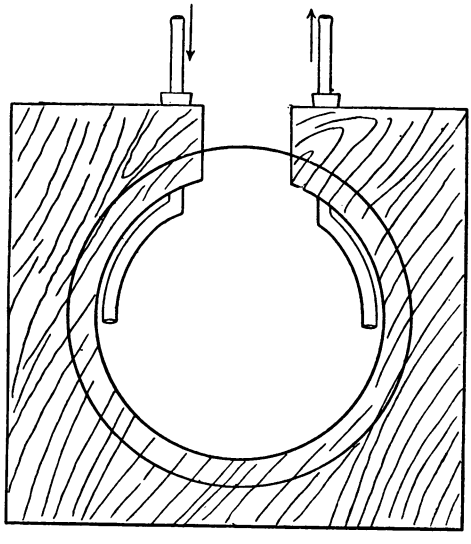

Fig. 40. Experiment chamber.

brought so as to completely enclose the discoidal chamber containing the frog's hind limbs. Before finally fixing the glass, the skin was rapidly removed from the frog's leg in such a way as to avoid any but the most insignificant bleeding, and the feet were fixed by elastic cords to a hook fixed to the wooden wall. The interstices remaining between the frog's body and the wood were packed with cotton-wool over which a solution of collodion in ether was poured. When the collodion had set, it was found that a very air-tight union had been effected at all points between the woodwork, the frog's india-rubber girdle and the frog's waist. Blocking the entrance tube to the chamber, when the aspirator was working, instantly stopped the succession of bubbles through the resistance bottles. This stoppage would not have occurred if any weak spots in the walls of the chamber had allowed the entry of air from outside. Details of the course of this experiment are as follows:—

EXPERIMENT VII. (See Fig. 41.)

Frog etherised 9.50 a.m. Placed in chamber, legs skinned, and chamber finally air-tight 10.3 a.m. Current through pressure bottles 10.5. Current switched to baryta 10.21.

Estimations.

10.21—10.56	a.m.	yielded	·22 c.c.	CO_2 per ½ hour.	Rest, circulation intact.
11.15—11.45	„	„	·16	„ „	Contraction.
11.58—12.28	p.m.	„	·14	„ „	Rest, no circulation.
12.55— 1.25	„	„	·10	„ „	Contraction.
1.41— 3.1	„	„	·11	„ „	Rest.
3.37— 4.37	„	„	·10	„ „	„
5.0 — 7.0	„	„	·10	„ „	„
7.17—10.17	„	„	·13	„ „	„
10.50—11.50	„	„	·13	„ „	„

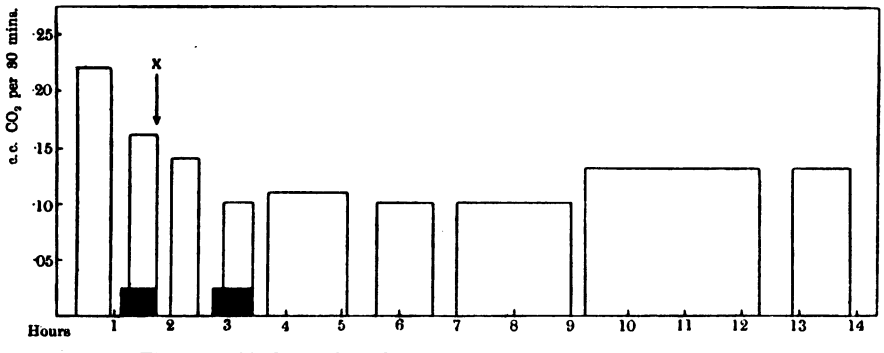

Fig. 41. Discharge from legs of frog. Circulation stopped at *x*.

From 10—10.56 a.m. the frog under the anæsthetic was breathing regularly, and its heart beat strongly. Its legs in the chamber were quite motionless.

At 11 a.m. the spinal cord was exposed for stimulation. Very little bleeding.

From 11.5—11.43 the legs were kept in an almost continuous condition of tetanus by stimulation of the cut lower end of the spinal cord and of the anterior roots of the appropriate spinal nerves. At first the secondary coil was at 16 cm.—later, the coil was moved to 15 cm., 13 cm., and 8 cm. Brief intervals of rest were allowed. Irritability was maintained until 11.43. The leg muscles contracted in opposition to one another and in stretching the india-rubber bands

of the feet performed external work. The breathing and heart-beat were maintained throughout.

At 11.50 the heart was exposed, the sinus cut across and the frog bled as completely as possible, by tilting the chamber so that the legs were higher than the head.

From 11.50—12.28 no movements occurred.

At 12.40 the electrodes were placed on the lumbar plexus of each side. With coil at 8 cm., the legs were tetanised by stimulation for 2 minutes at a time, with rests of one minute between each period of contraction, beginning at 12.45. After 1.7, rests of two minutes were given. Contractions were good till 1.25, when stimulation ceased. Chamber perfectly air-tight at 1.15.

There can be no doubt that the conditions of this experiment were such as to secure greater irritability of the leg muscles than in a preparation stimulated after the necessary delay between excision and the first estimation. The severe stimulation produced however no rise in the curve of discharge. It will be noticed that a well-marked 'hump' made its appearance at about six hours after the stoppage of the circulation.

The curve of discharge found in this experiment is of considerable interest, and has already been discussed (p. 66). The initial course of the curve is such as to suggest that an excess of CO_2 previously existent in the muscle tissue was liberated when skinning took place. For it can hardly be possible that the decline represents a decline in the activity of the muscle, since in that case it should show some relation to the passage from the condition in which circulation is normal to that of the first 'survival' periods. Although such a curve has been obtained in all experiments of this kind, it may still be possible that the circulation under the conditions is not so rapid and complete as during normal life.

The results of these experiments may now be summed up. They show that, under suitable conditions, the occurrence of active contractions in an excised muscle is not accompanied by an increase in the rate at which CO_2 is yielded by the muscle. The conditions are fulfilled when the stimulation causing contraction is not so severe as to produce marked fatigue and permanent shortening, and when it is applied during an early survival period.

Stimulation during a late period may cause a rise in the rate of CO_2 discharge, even though the contractions evolved are much feebler than previous ones not accompanied by a rise.

Finally, it has been shown by following the course of survival respiration after periods of activity, that the rise associated with fatiguing or late stimulation is not a temporary one but has the prolonged character already described as typical of the development of natural *rigor mortis*.

Our knowledge of the excretion by muscular tissues of simple carbon-containing bodies, has been derived from observations of very different kinds. Of these, one class includes investigations of the carbon dioxide gas yielded freely as such by muscles exposed to an innocuous atmosphere, while a second and larger class contains the estimations which have been made of the carbon dioxide obtainable either by artificial devices directly from the muscle substance itself, or indirectly from the muscle by examination of the blood which has passed through it.

The results of experiments of the first class have already been discussed. The second class contains experiments whose results depend on analyses

(*a*) of the gases obtained artificially from muscles under varying conditions,

(*b*) of the blood entering and leaving muscles at different times,

(*c*) of the gaseous exchanges at the lungs during varying conditions of bodily activity.

It is well known that the results of all three groups have indicated that the special carbon excreta are largely increased as the result of muscle contraction. The methods which have been employed are too well known to need more than the briefest mention. They may be noticed under the three groups.

(*a*) Hermann[1] found that the CO_2 obtainable by the air-pump from a muscle was increased by tetanisation. This experiment and the conclusion from it that the extra CO_2 yielded to the air-pump was due to the act of contraction as such, is subject to the same objection as that already applied to the experiments by Hermann on the relation of tetanus to the 'muscle-respiration.' For the tetanus induced at intervals during an hour may also in this series of experiments have given rise to fatigue-rigor. The same observer has shown that a muscle undergoing rigor yields to the pump additional free CO_2, and it seems very probable, in the absence of special

[1] *op. cit.* p. 116.

precautions, that during the hour's tetanus the muscle should have passed into the condition in which, as my own experiments have shown, fatigue effect is associated with an increased production of CO_2.

(*b*) Ludwig, with Sczelkow[1] (1862) found the venous blood coming from a group of muscles in the dog, stimulated to contraction within the living body, yielded more CO_2 when analysed by the aid of Ludwig's blood-pump than that flowing from the same group of muscles at rest. The results, however, were variable, and the rate of blood current difficult to control. The ratio (Q) of the CO_2 yielded to the oxygen absorbed, was usually found to be increased during contraction. This was followed by the experiments of Ludwig and Schmidt[2] (1868), upon the changes produced in blood artificially circulated through excised muscles. It was found that the blood circulating through the muscle received in most cases a greater addition of CO_2 during activity than during rest; but this result was not invariable and in several instances the opposite held true, an actual diminution of CO_2 being determined in the blood during a period of activity or of exhaustion. The quotient, Q, moreover, did not vary in a constant direction when the muscle was stimulated.

The later experiments of Minot[3] (1877), in Ludwig's laboratory, depended on a similar method of artificial circulation, in which the fluid employed was serum (prepared from the same animal). The increase of CO_2 in the serum leaving the muscles was found to have no relation whatever to the conditions of rest or activity. Minot concluded that CO_2 is not one of the decomposition products formed within the muscle substance during contraction, and that the discrepancy between that conclusion and those reached by other observers (Hermann[4], etc.), could only be explained on the assumption that the chemical accompaniments of contraction are different in a living muscle from those in a muscle removed from the body.

More recently Max von Frey and Gruber[5] (1885), have described and used an elaborate method for the artificial circulation of blood through an isolated muscle of the dog, and for the estimation of the changes produced in the blood during circulation. They found that, in general, the occurrence of contractions in the muscle was accompanied by an increase in the CO_2 added to the blood, but that this increase

[1] *Sitzungsber. d. k. Akad. Wien*, XLV. 1862.
[2] *Ludwig's Arbeiten*, Leipzig, 1868.
[3] *Ludwig's Arbeiten*, Leipzig, 1877.
[4] *op. cit.*
[5] *Archiv f. Anat. u. Phys.* 1885.

was very variable, and not very striking (ranging from 46 per cent. to 10 per cent.). The increase of CO_2 was found in many cases to be less than the corresponding absorption of oxygen, so that Q became lowered during contraction. The highest value of Q was 1·12, the lowest ·72.

Chauveau and Kaufmann[1] (1887) found that blood circulating through the *levator* of the horse's upper lip gained more CO_2 during activity than in rest, the output of CO_2 containing more combined oxygen than that absorbed at the same time.

The results then of analyses of the fluids circulating through muscles within the body, or isolated under conditions as normal as possible, have led to very varied conclusions. The increase of CO_2 due to contraction has been usually, but not always, found when blood has been circulated, and did not appear at all when serum was used. The respiratory quotient was said to be increased during contraction by Ludwig and Sczelkow[2] and others, to give no constant variation for activity, by Ludwig and Schmidt[3], and to be diminished during contraction, by von Frey[4].

(*c*) The last class of investigations, on the other hand, in which determinations have been made of the changes produced in the gas exchanges at the lungs during periods of muscular activity, have yielded very constant results. A large number of observers (Regnault and Reiset[5] (21), Pettenkofer and Voit[6] (23), Zuntz[7], Ed. Smith[8], Schnyder[9] (25) etc.) have found the CO_2 discharged from the lungs to be markedly increased during and just after periods of muscular exercise. The respiratory quotient is also raised during muscular activity.

From the foregoing it will be seen that the study of the alleged outburst of CO_2 within a muscle during the act of contraction has established three very different grades of evidence upon the point. There is a unanimity of opinion that the blood reaching the lungs contains an increased amount of CO_2 during muscular exercise when the body is intact. There is evidence, but evidence which is inconstant and conflicting, that the blood leaving a group of muscles contains

[1] *Comptes Rendus de l'Acad. franç.* 1887.
[2] *op. cit.* [3] *op. cit.* [4] *op. cit.*
[5] *Ann. de Chimie et de Physique* 1849.
[6] *Sitzungsber. der k. bayer. Akad. d. Wiss. zu München* 1866.
[7] This *Journal*, 1890. [8] *Phil. Trans.* 1859.
[9] *Zeitschr. f. Biologie* 1896.

more CO_2 when the muscles contract than during rest. And lastly while there is evidence that the air-pump can extract from an excised muscle of a cold-blooded animal more CO_2 after contraction than before it, and also that such an excised muscle can spontaneously yield to the atmosphere additional amounts of CO_2 during contraction, both these statements can be readily explained under the experimental conditions described, as the result of confusion between the allied processes of contraction and *rigor mortis*.

The results of my own experiments have shown that the unaided discharge of CO_2 from freshly excised muscle is not increased during contraction within fatigue limits. If this be the case it must be argued either that CO_2 is produced as such during the act of contraction, but that it is held in some kind of chemical combination by fixing substances within the muscle, or else that the chemical decomposition accompanying contraction does not advance so far as the formation of the simple molecule of CO_2. The former alternative is not easily tenable if we consider that since CO_2 is continually being produced within a muscle during survival periods, the bodies capable of 'fixing' the CO_2 of contraction should be satisfied before contraction can begin. No evidence moreover of the presence of such CO_2 fixatives is found in connection with the action of poisons on the survival respiration.

Taking the second alternative, the act of contraction which has been shown on 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. Such a relation as this has been indicated as the true one in all the experiments I have myself made upon the relation of contraction to the survival respiration, and is perfectly compatible with all the experimental results if not with the theoretical conclusions of the investigations upon the same subject in the past.

This hypothesis is not violated by the results of the other and more indirect methods for determining the relation of contraction to free CO_2 formation. For while we may suppose the chemical processes during contraction to result in bodies more complex than CO_2, free CO_2 might be derived from these products of activity either by the air-pump or through an exchange between them and the blood. The results obtained by means of the air-pump are not yet free from the possible confusion

between contraction and rigor which would beg the question at issue. The class of experiments on the other hand, based upon analyses of the blood circulating through muscle, does not suffer from this objection since fatigue and the hastening of rigor are probably prevented by the supply of nutriment.

If it be true that after contraction the muscle substance is impregnated with an additional quantity of free CO_2 for the removal of which the blood is the passive vehicle, the irregularities of the observations which have been made in the past remain unaccountable. In nearly all the experiments on artificial circulation a greater or less degree of failure to obtain evidence of such simple removal of free CO_2 by the blood has been met with. Ludwig and Sczelkow could only occasionally obtain an increase in the venous blood after contraction, von Frey found the increase very variable, and Minot (using serum, it is interesting to note, instead of blood), denied that such an increase occurred at all.

If, on the other hand, the blood in reaching a recently active muscle is not supplied directly with free CO_2 due to the contractions but is set the definite task of obtaining it from some product of contraction which holds it in combination, it is easy to understand that blood tampered with by artificial conditions of circulation is not always an efficient medium for the removal, or that transfused serum should fail to obtain additional CO_2 from the active muscle altogether. Where the conditions are all normal, on the other hand, as in the intact body, no such discrepancies in observation should occur, and, as a matter of fact, the results of analyses of the CO_2 excreted at the lungs during rest and activity have been very uniform.

Von Frey[1] was so struck by the irregularity of his own artificial circulation results, and by the fact that, according to them, Q diminished during contraction instead of increasing as the observers of the general excreta have agreed, that he has suggested that some of the products of contraction within the muscle, destined to split further and to yield CO_2, pass to the blood unchanged as bodies of acid character. Gruber, with von Frey[2], found a very striking increase of sarcolactic acid in the blood after contraction, in confirmation of the old observation of Spiro[3]. Von Frey shows that the amounts of lactic acid so yielded in his experiments are amply sufficient to account for the CO_2 which escaped his observation, but which would presumably have appeared at the

[1] *op. cit.* [2] *op. cit.*
[3] *Zeitschrift f. phys. Chemie*, 1878.

lungs in the normal animal. He suggests that the lactic acid formed during the course of experiment may not, owing to the artificial conditions, be further split up, and may remain stored up either in the blood or in the muscle-substance itself. And, going further than this, he even points to the possibility that in the intact living animal the muscles may not themselves be the seat at which the lactic acid arising from them is split up and elaborated. This possibility is one indicated also by the observations of Meyer[1], who found that the lowering of CO_2 production caused by phosphorus and arsenic poisoning was accompanied by an accumulation of lactic acid, especially within the liver.

However this may be, it remains clear that the astonishing irregularity of the results of the experiments in which the method of artificial circulation has been used, does not agree with the supposition that the muscle-substance liberates within itself during contraction a quantity of free CO_2, which is free independently of some agency within the blood or even within some other organ of the body. In the past, however, that supposition has always had the support of the peculiarly direct evidence which the phenomena of survival respiration can supply. Minot[2] indeed, who was hostile to the supposition, felt the weight of this evidence so much that he postulated some essential difference in kind between a muscle in the body and one freshly excised. It has been shown, however, that the evidence from the survival respiration of muscle in the past has not been free from one obvious source of confusion, and that my own experiments by a new method have given results entirely opposed to the occurrence of a special production of free CO_2 within the muscle-substance during normal contraction.

PART IV. THE PHENOMENA OF WATER-RIGOR.

A muscle plunged into distilled water rapidly loses its irritability, and after preliminary localised twitchings apparent on its surface, becomes stiffened and turgid. The nature of this so-called 'water-rigor' is still obscure. Du Bois Reymond[3] (1859) found a muscle treated with distilled water to be clotted and acid within a very short time, and this water-rigor has been very usually classed with the condition of rigor produced by heat, or by chloroform. But Biedermann[4]

[1] *Arch. f. exp. Path. u. Pharmakol.* Bd. XVII. [2] *op. cit.*
[3] *Monatsbericht der Berliner Akad.* 1859.
[4] *Sitzungsber. der Wiener Akad.* LXXXI.

(1885) has shown that the electromotive phenomena of water-clotted muscle are not at all comparable with those of true rigor, since the difference of potential between a normal and a water-clotted part of the same muscle was insignificant. He concluded that the condition of water-rigor could not be attributed to any chemical change in the muscle-substance of the same kind as that characteristic of *rigor mortis* or of heat or chloroform rigor. It is said indeed that the irritability of a water-clotted muscle may be recalled upon simple dehydration with strong NaCl solution.

In view of the stimulation by water-clotted muscle of some but not of all the phenomena of true rigor, it becomes interesting to determine the relations of water-rigor to the survival output of CO_2. Hermann[1] showed that the CO_2 yield was slightly greater in water-clotted than in fresh irritable muscle and upon this has been based the common view that water-rigor, like heat-rigor, is characterised by a large discharge of CO_2.

In Hermann's experiments the muscle preparation was left all night in distilled water, and was subsequently placed in the analysis tube for 21 hours. A similar control preparation had been placed during the night in normal salt solution and was observed in the same way and for the same long period. The object was to show that the oxygen absorption was nearly equal in each case. So far as the yield of CO_2 was concerned the experiment affords no basis for definite statement. The control 'living' muscle must have been the seat of natural rigor during the observation, while the water-clotted muscle was under examination only during a period subsequent to the completion of the clotting process. And since the temperature during the experiment was 21°—22° C. it is probable that the initial stage of bacterial putrefaction had begun in each case.

I have used for my experiments a tubular experiment chamber nearly twice as long as was necessary for the inclusion of the muscle preparations (see Fig. 42). The other length was filled at the beginning of the experiment with distilled water. The production of water-rigor could be obtained at any moment by tilting the tube so as to immerse

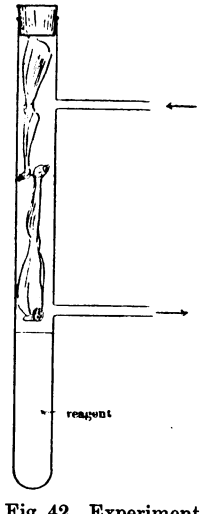

Fig. 42. Experiment chamber.

[1] *op. cit.*

the muscle substance in the water. During the whole time of immersion the air current, if desired, could be maintained and the estimations continued. A similar muscle preparation in another similarly arranged tube was observed at the same time for control. The results showed that no additional output of CO_2 accompanies the action of distilled water upon muscle substance; the normal course of survival discharge was not affected during the destruction of the muscle's irritability by the water, and the onset of the turgescence.

In some other experiments the muscle preparation was removed from the experiment chamber when the course of survival discharge had been followed for 90 minutes and placed in a large bulk of distilled water. After 5 minutes the turgid muscle was replaced in the chamber and the observations resumed. In no case was evidence obtained of an increase in rate of CO_2 discharge. A subsequent dose of chloroform vapour produced in these cases the usual elevation in the discharge curve.

The absence of any special production of CO_2 by a muscle during its stiffening in distilled water supplies another fundamental distinction between this stiffening and the clotting occurring spontaneously in excised muscle, or that due to other poisons. That the water-clotted muscle exhibits the same rate of survival CO_2 discharge as fresh irritable muscle, affords a parallel to the fact that the electromotive phenomena in the two cases are also identical, and gives the strongest confirmation to the opinion based by Biedermann upon that circumstance with regard to the non-chemical nature of the shortening and stiffening of the muscle fibres which distilled water produces.

PART V. THE EFFECTS OF HEAT ON THE CO_2 DISCHARGE.

Hermann[1] (1867) found that on heating an excised frog's muscle to 40° C., a production of CO_2 took place as the muscle rapidly clotted, removable by the air-pump without the addition of acid. The same observer found that muscle plunged rapidly into water at temperatures from 70° C. upwards not only did not lose any gases but actually entered a condition in which future rigor was impossible. The substance which suffers decomposition in rigor was said to be 'fixed,' so that the 'scalded' muscle was regarded as containing the same amounts of gases as freshly excised muscle, but to be incapable of subsequently

[1] *op. cit.*

entering heat-rigor at 40° C. For the process of heat-rigor then there are, on these statements, minimum, optimum and maximum temperatures of 0° C., about 40° C., and about 70° C. respectively; and some writers have compared the process for this reason with ferment action.

The well-known experiments of Pflüger and Stintzing[1] (1878) showed however that mammalian muscle subjected for some hours to a temperature of 100° C. yielded 100 volumes per cent. of CO_2 to a current of air passing over it, and that the amount of yield was not diminished if the muscle had been previously acidified (and afterwards washed) for a long time at 0° C. The yield was accordingly due to real destructive decomposition and was not due to the liberation of preformed CO_2. The CO_2 arose from the same substance as that concerned in the chemical processes of contraction and rigor, since previous activity or rigidity might reduce the maximum 100 vols. CO_2 per cent. of muscle used to 20 or 30 vols. per cent. A previous subjection of the muscle for instance to a temperature of 40° for 22 hours lowered this yield to 30 per cent.

Nasse[2] (1879) attempted to explain the contradiction, and the failure of Hermann to find the yield of CO_2 at 100° C., by suggesting a double origin for the CO_2. One of the processes leading to the production of CO_2 he imagined to have its optimum near 40° C. and to be inhibited permanently at 70° C., while the second was active proportionately with advances of temperature from 0° C. to 100° C. There appears to be at present no evidence on which to base such a hypothesis.

Tissot[3] (1894) has more recently estimated the CO_2 output from muscles heated to different degrees in order to support his view that the CO_2 yielded has no relation to the physiological condition of the excised muscle (see p. 24). The CO_2 discharged was found to be greater in a muscle as the temperature was higher, within the observed limits, although the 'vitality' of the muscle should decline with the rise of temperature at least above 40° C. So far as the experiments bear on the present point their results are quantitatively valueless. The figures given are very variable, and in one series of observations the higher temperatures are found to give less CO_2 than the lower. This discrepancy is explained by the author himself as due to the fact that in some cases the heating took place during the collection of gases, while in others it was completed beforehand.

[1] *Pflüger's Archiv*, 1878. [2] *Hermann's Handbuch.* [3] *op. cit.*

The incompleteness of our knowledge of the effects of heat on the discharge of CO_2 has resulted in conflicting statements in modern text-books. In one account[1], combining the results already given, the discharge is said to be large at 40° C., to be absent on rapid submission to 70° C., at which temperature the material providing the CO_2 is 'fixed' so that a later application of 40° C. is without effect; and lastly to be at a maximum at 100° C. In another[2], a muscle plunged into boiling water is said to discharge no CO_2 whatever.

I have made some experiments on the effect of these higher temperatures upon the survival respiration of excised frog's muscle, which have been undertaken so far with the object of testing the value of the method of estimation I have described, in this direction. The muscle in the experiment chamber is heated by means of water in which the whole chamber is plunged. Since the experiment chamber is only connected with the potash tower and moistening arrangements on one hand, and the absorption chambers on the other, by means of flexible tubing, the heating can be managed very simply and effectually. The experiment chamber has always been tubular, to contain two legs in series, and has been made of the thinnest glass practicable, so that any temperature change in the surrounding water is rapidly communicated to the muscle (cf. Fig. 3, above). The entrance tube for the air current runs down if desired as a coil, through the water, to open at the lower end of the chamber. In this way the air-current is also warmed to the given degree before it reaches the muscle. For the higher temperatures a simple condensing arrangement is necessary at the beginning of the exit tube leaving the upper end of the experiment chamber.

One of the greatest advantages of the volumetric method of estimation already described is in the fact that any alteration in the temperature of the muscle examined does not affect the accuracy of the determination made of the evolved gas, as it would without special and elaborate precautions where the eudiometric method is employed. And it may be noticed as an additional advantage that owing to the reduplication of the absorption chambers comparative and simultaneous observations may be made on 'crossed' pairs of muscle preparations (see p. 43 above) at the same or different temperatures. By the same reduplication, moreover, the extremely important advantage is secured of being able to follow the changes in CO_2 output succeeding as well as those accompanying the application of a given temperature.

[1] Waller, *Human Physiology*, London, 1893, p. 138.

[2] Stewart, *Manual of Physiology*, London, 1895, p. 516.

The results I have already obtained are chiefly concerned with the CO_2 output caused by the heat rigor at 38°—40° C., and with the alleged 'fixing' effect of higher temperatures. To save space, full protocols of each experiment have not been given. The diagrams are more expressive and contain exactly the same information.

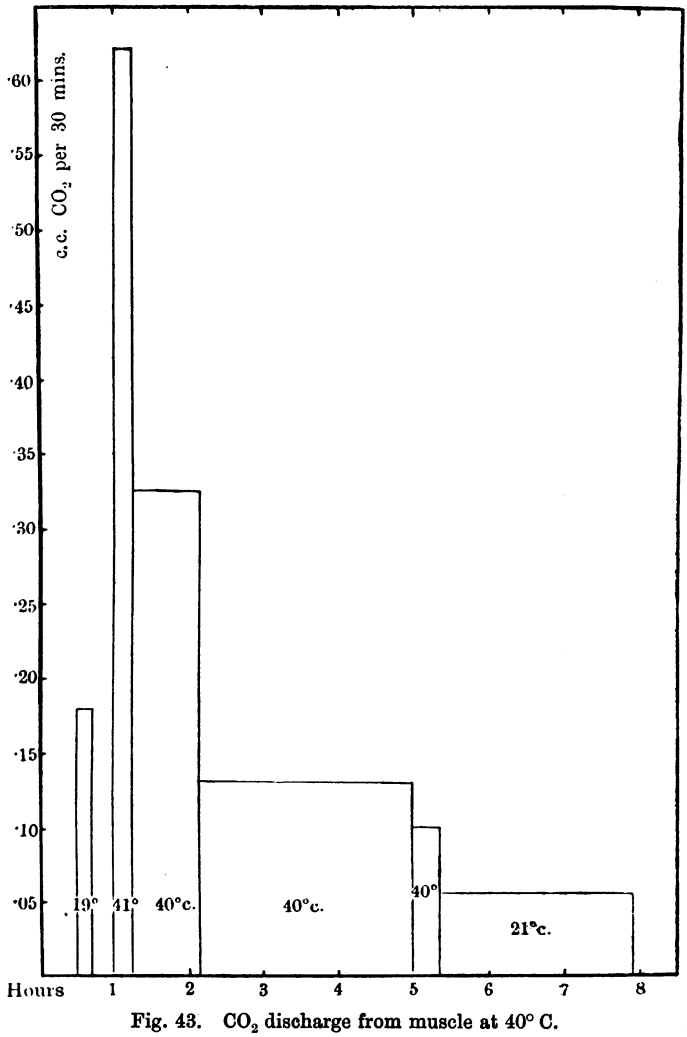

Fig. 43. CO_2 discharge from muscle at 40° C.

If the muscle soon after excision be raised to 40° C. an immediate rise in CO_2 output is seen (Fig. 43). The rate of output is, on the

average, rather more than trebled. From this rate, however, while the muscle is still maintained at 40° C., a rapid decline occurs until in the course of 3—4 hours it has fallen to a very low level. This low level may be taken as virtually zero. On cooling after this to 15°—20° C. the rate of output is *nil*, and the muscle, in the absence of putrefaction, has lost all power of producing CO_2 either spontaneously or under the influence of such irritants as chloroform (see p. 52).

The decomposition yielding CO_2 in heat-rigor is then both instantaneously produced and rapidly completed at 40° C. An average of four experiments in each of which the whole amount of CO_2 yielded at

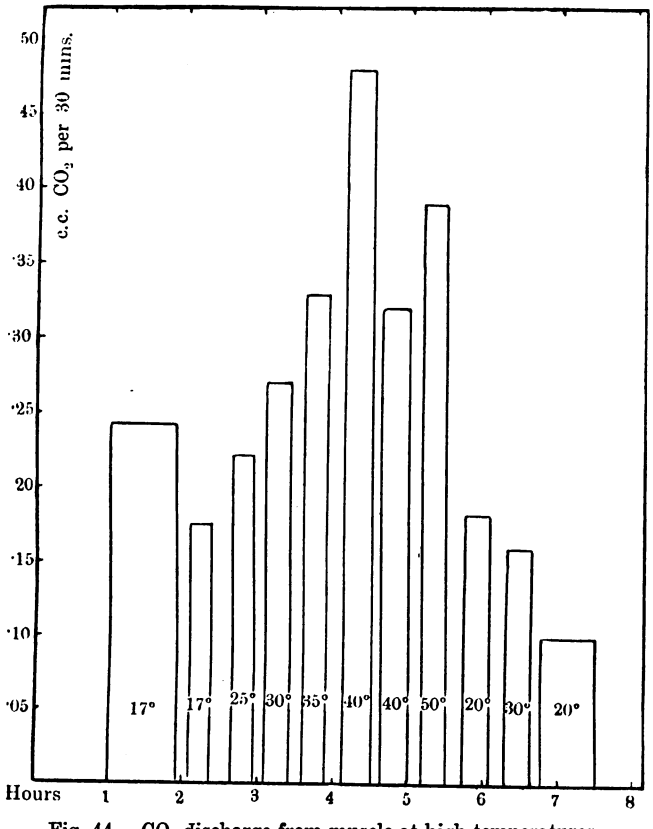

Fig. 44. CO_2 discharge from muscle at high temperatures.

40° C. for three hours was collected by one absorption, showed the yield to be approximately 30 per cent. by volume of the muscle used.

When the muscle is more gradually heated to 40° C. the rate of

discharge rises very strikingly in proportion with the rise of temperature until the passage from 35°—40° C. occurs (see Fig. 44). Here a slightly disproportionate rise in rate is found. The result however of such a gradual approach to the degree of heat rigor is that the rate of discharge when 40° C. is reached is always less than that found on the sudden rise to 40° C. In the same experiment it is seen that a rise from 40° C. to 50° C. after the first maximum was reached, only delayed the decline in rate but did not produce a rate above that due to 40° C. in the first instance.

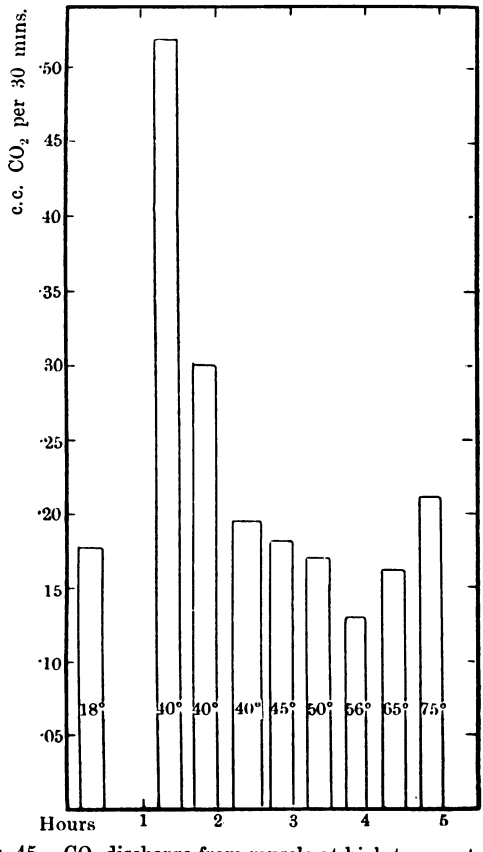

Fig. 45. CO_2 discharge from muscle at high temperatures.

The effect of such subsequent heating after the 40° effect is better shown at Fig. 45. Here, in spite of the advance to 45°, 50° and 56° C., the rate of output declined persistently. This result has been quite

uniformly obtained in these experiments when the application of 40° C. has been long enough to complete the rigor changes (unlike the example at Fig. 44). When 65° C. is reached, however, the rate rises, and again for the rise to 75° C. and to 90° C.

The detailed course and the meaning of this rise after complete rigor, for these later increments of temperature, have still to be worked out. It may be possible to express them simply in terms of the diminished solubility of CO_2 at higher temperatures, while they have also perhaps to be associated with definite chemical changes during the coagulation at certain degrees of temperature of different constituent proteids of the muscle substance.

For the present however it is clear that the definite chemical change associated with rigidity and yielding CO_2 for temperatures between 35° and 40° C. is the most conspicuous event beyond all comparison occurring in the muscle as the temperature rises from 20°—100° C.

When the muscle is heated in the first instance to one of the higher temperatures an immediate yield of CO_2 occurs, associated with immediate rigor. The effect is precisely of the same kind as that due to the temperatures between 38° and 42° but is more rapid and intense. In Fig. 46, the muscle was raised to 70°; it shows a rate of output comparable with that of Fig. 43, but larger than the control preparation heated at the same time only to 40° C. Three hours after (the muscle having cooled) the temperature was raised to 40° C., from which resulted a rise in output quite insignificant as compared with the heat-rigor effect, and probably only dependent on the decreased solubility due to the rise of temperature, of the CO_2 already liberated, which the diagram shows had not completely escaped.

When the experiment chamber is plunged into boiling water, the same result, intensified, is again obtained (Fig. 47). In this case the application of boiling water for a second time just after cooling produced a rate of discharge only equal to about a third of that due to the first application (which only lasted 20 minutes).

Summing up briefly the results of which I can so far speak positively, the effect of heat upon survival respiration depends for its most marked phenomena on the heat rigor which is produced at 38°—40° C. or by any temperature above that. This heat rigor is accompanied by a large outburst of CO_2 amounting (speaking roughly for the present) to about 30 per cent. by volume of the muscle substance at 40° C. and at atmospheric pressure. An exactly similar outburst is caused on the application of any temperature above 40°, and its magnitude, though rising with

the temperature producing it, does not do so proportionately. That it rises with the temperature is certainly to be partly explained in terms

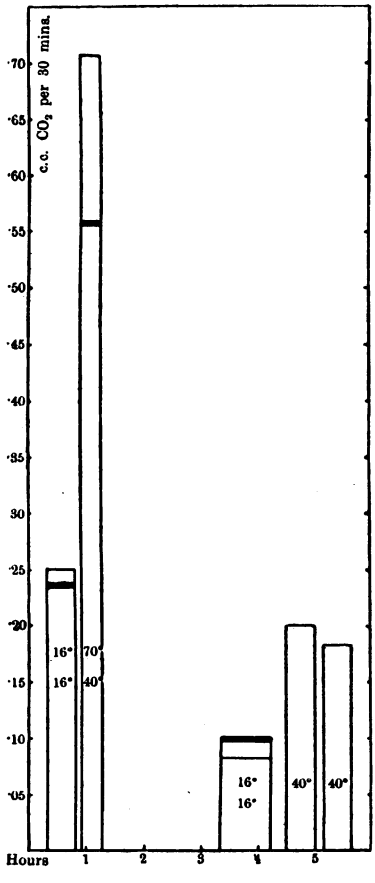

Fig. 46. CO_2 discharge from muscle at high temperatures. The escapes from two muscle preparations differently heated are shown.

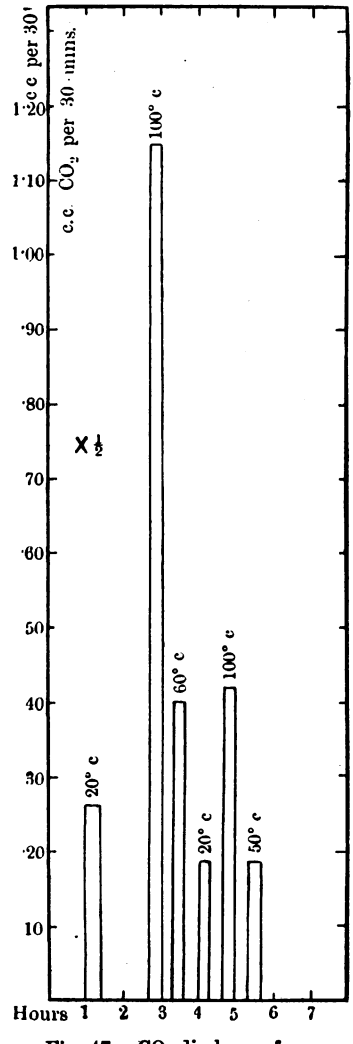

Fig. 47. CO_2 discharge from muscle at high temperatures.

of the solubility of CO_2 and is probably also due to the fact that the rigor caused by higher temperatures is more sudden and the initial rise of rate of CO_2 output more rapidly at its maximum than when lower

temperatures, above 40° C., are applied. It is entirely untrue that a muscle plunged into boiling water does not yield CO_2. The application of boiling water is, on the contrary, the readiest method for rapidly obtaining a maximum CO_2 discharge from an excised muscle. It is true at the same time that to boil a muscle for a short time and then to cool it is, in virtue of the rapidity and completeness of this discharge, the quickest way of obtaining a muscle which shall have lost all power of the spontaneous production of CO_2. No evidence of any kind has been found to support the alleged 'fixing' effect at 70° C. A muscle placed in water at this temperature undergoes extremely rapid clotting and yields a large quantity of CO_2, which appears more rapidly and at a greater initial rate than the discharge due to 40° C., but the total amount of which is only slightly greater than that occurring at this lower temperature. A muscle having undergone heat rigor at 70° C. is of course unable to exhibit the same phenomena again when subsequently heated to 40° C. The results of all the experiments have shown that the effects produced at any temperature (above 40° C.) include all the effects which might have been produced at a lower temperature.

The rapidity with which the discharge of CO_2 during the onset of heat rigor appears, reaches its maximum, and declines, provides an explanation of the variable results obtained by Tissot[1]. He found no constant relation between the CO_2 output from muscle and the degree of temperature at which it had been caused, unless the heating was conducted whilst the muscle was in the collecting tube. It is obvious that in his other series of experiments, in which the heating operation was concluded before the muscle was arranged for analysis, the special CO_2 discharge must in nearly all cases have escaped his observation.

I have obtained results similar to those of the very important experiments of Hermann[2] by which he showed that the chemical processes of both contraction and rigor draw upon one and the same store of material. Tetanus of the muscle previous to excision has always markedly diminished the yield of CO_2 obtained during heat rigor. The following is a typical experiment.

Of two frogs (brains destroyed, circulation intact), the right leg of each was strongly tetanised by stimulation of the right lumbar plexus, at intervals, for 20 minutes. The two right legs were then excised and arranged in one experiment chamber (*A*) the two left legs together in a

[1] *op. cit.* [2] *op. cit.*

second (*B*). The course of survival respiration of each crossed pair was then followed.

In the first half-hour,

the rate of discharge from *A* was ·19 c.c. CO_2 per ½ hour.
„ „ „ *B* „ ·18 c.c. „ „

During the second half-hour, both chambers were immersed in water kept at 42° C.; when

the rate of discharge from *A* was ·40 c.c. CO_2 per ½ hour (fatigued).
„ „ „ *B* „ ·58 c.c. „ „ (control).

The heat-rigor discharge is also diminished in proportion as natural rigor, giving the 'plateau' of CO_2 production in the natural curve, has proceeded. Of two 'crossed' pairs of legs, *A* and *B*, *A* during the second hour after excision yielded in half-an-hour at 42° C. ·65 c.c. CO_2. *B* during the 16th hour after excision, when the rigor plateau had been entered upon for 10 hours, yielded in half-an-hour at 42° C. ·38 c.c. CO_2 In a similar comparison one 'crossed' pair during the second hour after excision yielded in half-an-hour at 42° C. ·61 c.c. CO_2. The other exactly similar pair during the 10th hour after excision yielded in half-an-hour at 42° C. ·52 c.c. CO_2.

It has been similarly shown that the rise of the survival rate of discharge caused by chloroform vapour (§ 6 above) is due to a demand made on the same material as that breaking down in heat-rigor. By shorter or longer application of chloroform the yield of CO_2 during subsequent heat-rigor may be less or more markedly diminished. And a muscle, on the other hand, which has undergone heat-rigor, is entirely unaffected so far as its output of CO_2 is concerned by the vapour of chloroform in any dose applied subsequently.

I have not yet investigated the changes in rate of CO_2 output which might be expected to accompany the coagulation of the constituents of the muscle occurring at definite degrees of temperature between 40° C. and 100° C. For such an investigation the method described appears admirably suited, but for the statement of any results in which the coagulation effects are clearly distinguished from one another if they exist, and from the effects of heat-rigor, many more experiments are required.

APPENDIX A.

Tissot[1] has published the account of some observations on the excitability of rigid muscles, which are connected with some points already noticed in this paper. He has found that rigid muscles both of Mammalia and of the frog may be excitable by electrical, chemical, and mechanical stimuli. His experiments show that after the appearance of general stiffness in a muscle, irritability to stimuli may still be shown by more or less badly marked contraction responses. In the experiments described the only criterion of the fact that the muscle in all its parts had undergone rigor, was the initial appearance of general stiffness. As they stand therefore, it may be contended, they only illustrate the fact that the various parts of a muscle undergo rigor at different times and that the onset of rigor takes place in progressive stages. They hardly support the author's conclusion "that cadaveric rigidity is not a phenomenon incompatible with the life of the muscles and that its appearance is not a proof of their death," if by rigidity is meant rigor complete in all its stages. Upon our present knowledge, the completion of 'rigor' coincides with the completion of the destructive decomposition of the material on which the occurrence of contraction depends.

One of the chemical irritants used in these experiments was chloroform, and the results obtained by its means are interesting in relation to the effects of chloroform upon the survival respiration of muscle described at § 6 above. Tissot found that the effect of the application of chloroform vapour to an excised muscle increased after death (*i.e.* excision). He gives curves illustrating the prolonged contraction or permanent shortening caused by this poison. The same dose which produces an almost imperceptible shortening in a fresh muscle, causes a very marked shortening at the time when rigor is beginning. This effect declines in intensity as the rigor proceeds. Such results as these harmonise with the effects of the vapour upon the survival respiration which I have described. A small dose of chloroform was found to depress the rate of CO_2 discharge from a freshly excised muscle, but to increase it when applied at a later survival period. The effect diminished as the rigor 'plateau' approached its completion, and eventually the strongest doses of chloroform were without effect. The circumstance that chloroform should have

[1] *op. cit.*

such a slight irritating effect upon fresh muscle is possibly to be explained as due to the counterbalancing tendency already referred to as its 'anæsthetic' effect during the earliest survival periods.

APPENDIX B. *Protocols.*

It has not been thought necessary to give the experimental details of all the observations which have been described. Full protocols are here appended in the case of four of the experiments, from which the diagrams have been derived. They provide examples of successive estimations by use of one aspirator and absorption chamber, of comparative estimations where both aspirators and chambers are used, and of the method of continuous estimation by the use of one aspirator, and of both absorption chambers alternately. They also contain the methods of investigating the action of poisons, in the form of vapour or solution, upon the rate of CO_2 discharge.

The other diagrams, not represented here, have been constructed according to the method described, and fully express the result and duration of each period of estimation as well as the number of estimations made; they exhibit more graphically than a row of figures the course followed by the rate of survival discharge.

EXPERIMENT I. (Fig. 4.) Frog killed 11.30 a.m. and hung from feet for bleeding. Legs excised and arranged in simple tubular chamber. Chamber air-tight 11.50. Volume of empty chamber 15 c.c. Current started through resistance bottles at 11.50. Switched to absorption chamber B at 12 noon. Temperature 15° C. All absorptions in this experiment in chamber B.

Time.	CO_2 absorbed.	Vol. of water leaving Aspirator. (Arranged for 120 c.c. per hour.)	Rate of CO_2 discharge per ½ hour.
p.m. 12.0 —12.30	·26 c.c.	58 c.c.	·26 c.c.
12.37— 1.12	·21	69	·18
1.26— 1.52	·14	53	·16
2.32— 3.12	·19	80	·14
3.30— 4.40	·31	143	·13
4.55— 5.35	·23	79	·17
5.43— 6.14	·21	61	·20
6.25— 7.5	·23	80	·17
7.15— 8.1	·25	92	·16
8.40— 9.47	·35	135	·16
10.2 —10.22	·12	40	·18
p.m. 10.30—10.30 a.m.	2·88	1430	·12
p.m. 12.30— 1.0	·10	59	·10
2.31— 3.6	·12	70	·10
4.0 — 5.0	·16	122	·08
6.30— 7.15	·11	88	·07
8.30— 9.0	·10	60	·10
10.0 —10.20	·14	41	·21
11.5 —11.25	·22	40	·33

Discontinued. Distinct slight smell of putrefaction on removing the muscle preparation.

EXPERIMENT 54. (Fig. 18.) Two frogs killed 10.55 a.m. Legs arranged in 'crossed pairs' in two simple tubular chambers A and B. Chambers air-tight and current started at 11.5. Nitrogen furnace (see Fig. 17) had been heated from 9.30 and current had been drawn by accessory aspirator since that time through chamber A and its moistening chamber.

Both experiment chambers A and B immersed in cool water kept at 15°—17° C. during whole experiment. Room temperature 21°—23° C. Current through A passed to absorption chamber A, that through B to absorption chamber B, during periods of determination.

	A (in nitrogen current).		B (control in air current).	
Time.	Water from Aspirator.	Rate of CO_2 discharge per ½ hour.	Water from Aspirator.	Rate of CO_2 discharge per ½ hour.
a.m. 11.29—11.49	40 c.c.	·22 c.c.	42 c.c.	·27 c.c.
p.m. 12.5 —12.30	50	·14	50	·19
12.45— 1.10	50	·13	50	·17
1.40— 2.30	99	·12	101	·13
2.47— 3.17	59	·11	59	·12
3.30— 4.0	60	·11	60	·11
4.15— 4.45	59	·08	60	·11
5.0 — 6.11	142	·07	145	·11
6.30— 7.25	110	.07	110	·10
10.7 —10.37	59	·08	60	·10

Discontinued.

EXPERIMENT 38. (Fig. 23.) Frog killed 11.25 a.m. Legs in chamber, air-tight and current started 11.40 a.m. Absorption chambers A and B used alternately. Temperature 17° C.

Time.	CO_2 absorbed.	Aspirator flow.	Rate per ½ hour.
a.m. 11.50—12.10 p.m.	·13 c.c. (in A)	39 c.c.	·19 c.c.
p.m. 12.17—12.32	·08 c.c. (in B)	32	·16

Chloroform vapour passed over muscle from 12.27—12.32, by switching current over chloroform tube arranged between moistening chamber and experiment chamber.

p.m. 12.32—12.47	·09 (in A)	30 c.c.	·18 c.c.
12.47— 1.2	·12 (in B)	30	·24
1.2 — 1.17	·125 (in A)	31	·25
1.17— 1.32	·12 (in B)	31	·24
3.30— 3.50	·16 (in B)	40	·24
6.8 — 6.28	·14 (in B)	40	·21

Discontinued.

EXPERIMENT 67. (Fig. 28.) A ·05 per cent. solution of lactic acid in 8 per cent. NaCl solution, arranged in chamber (see Fig. 42). Legs of exceptionally large frog, fresh caught in chamber and current started 3.10 p.m. Temperature 19·5° C.

Time.	CO_2 absorbed.	Aspirator flow.	Rate per ½ hour.
p.m. 12.50—1.5	·16	30	·32
2.30—2.45	·12	30	·24
4.18—4.33	·11	30	·22
4.45—5.25	·28	80	·21

At 5.25 chamber tilted so that muscle immersed in acid. Flow of current uninterrupted.

p.m. 5.35—6.5	·34	60	·34
6.20—6.40	·16	40	·24

Discontinued.

The initial rate from this muscle preparation is the highest I have observed.