THE PRODUCTION OF CARBON DIOXIDE BY NERVE.

By G. H. PARKER.

(*From the Zoological Laboratory, Harvard University, Cambridge.*)

(Accepted for publication, April 2, 1925.)

I. INTRODUCTION.

The older physiologists, such as Johannes Müller, believed that nerve impulses were transmitted at a rate comparable to that of light, a belief that led to the interpretation of such impulses as essentially physical phenomena. But when Helmholtz in 1850 measured for the first time the rate of transmission of the impulse on frog nerve and found it to be about 29 meters per second, grounds were given for a chemical interpretation of this action and it was afterwards often compared to the burning of a chain of gun-powder. From this standpoint it is natural to expect that nerve activity would be associated with the absorption of oxygen, the generation of heat, exhaustion, and the production of waste products such as the metabolites water and CO_2. Considerable experimental evidence has been amassed in the last few years to show that oxygen is necessary for the continued activity of nerve, but thus far heat has not been found associated with nerve action. Signs of exhaustion have been claimed only on direct evidence, and of possible metabolites, CO_2, the only one readily identifiable, has been declared present by some investigators and absent by others.

The production of CO_2 by nerve was first specifically claimed by Waller (1896, 1897 a, 1897 b, 1910) about three decades ago when he showed that the electrical properties of a nerve changed on immersing it in an atmosphere rich in CO_2 and that a similar change occurred when the nerve in ordinary air was stimulated. He argued from these observations that the stimulation of the nerve produced within it a metabolite which acted on the fibers in the same way as CO_2 did and that this metabolite was very probably CO_2 itself. Waller, however, was never able to demonstrate by any direct means the presence of CO_2 in the stimulated nerve.

The positive identification of this substance as a product of nerve activity was first accomplished by Tashiro (1913 a, 1913 b, 1917) who devised for this purpose a special apparatus which he called a biometer. In a closed glass chamber a nerve in an atmosphere free from CO_2 was suspended above a drop of aqueous barium hydroxide. If such a nerve should give out CO_2, particles of barium carbonate would be expected to form on the surface of the drop and on carrying out this test such proved to be the case. Tashiro believed that the production of these particles was so regular that the method admitted of quantitative results and he devised a somewhat complicated procedure to attain this end. According to his observations 10 mg. of nerve from the spider crab produced 6.7×10^{-7} gms. of CO_2 in 10 minutes. On stimulation this amount was said to increase to 16×10^{-7} grams or nearly threefold that from the quiescent nerve.

Moore (1917, 1918) immersed a piece of living nerve in a solution of phenolsulphonephthalein and demonstrated that the solution became progressively more and more acid the longer the nerve remained in it. The material producing this acidity could be removed by washing the solution freely with pure air, a result which led him to conclude that the acidity was due to CO_2 and not, for instance, to lactic acid. Moore (1919 a, 1919 b, 1921) was not able to detect any increase of CO_2 on stimulation. In this respect his results were in strong contrast with those of Tashiro.

Tashiro's conclusions were sharply criticised by Bayliss (1915, p. 379) who pointed out that a nerve contained not only nervous tissue but also a considerable amount of connective tissue whose respiratory activity might afford the CO_2 identified by Tashiro. Bayliss also called attention to the fact that since the stimulating electrodes used by Tashiro were within the respiratory chamber, the increase of CO_2 on stimulation might result from the electrical heating of the nerve rather than from increased metabolism.

In consequence of the lack of harmony in the results of Tashiro and of Moore and of the criticism of the whole situation by Bayliss I was led to undertake a reinvestigation of the subject. My work was carried out at the Marine Biological Laboratory at Woods Hole, Massachusetts, and I wish to express to the director, Dr. F. R. Lillie, and to his associates my keen appreciation of the very generous opportunities accorded me at their institution.

II. MATERIAL AND METHODS.

The nerve whose production of CO_2 I proposed to test was the lateral-line nerve of the smooth dogfish, *Mustelus canis* (Mitchell). This nerve is one of the few pure nerves in the vertebrate body. It is composed of very uniform, medullated, sensory fibers. It can be quickly and easily dissected from the side of the fish and yields a very clean smooth trunk, thick anteriorly and diminishing posteriorly as it gives off branches to the lateral-line organs. It is easy to obtain stretches of this nerve 10 centimeters in length.

My first attempts at the determination of CO_2 from this nerve were made by methods that involved the conversion of barium hydroxide into carbonate. But neither the procedure devised by Tashiro (1913 a, 1913 b, 1914) nor that used by Lund (1919) yielded satisfactory quantitative results and my inability to carry out an accurate and close calibration of any apparatus led me to abandon these means. My experience with Tashiro's method agreed closely with that of Adam (1921, p. 362) who found it extremely sensitive as a qualitative test for CO_2 but was unable to use it for reliable quantitative work.

As it was necessary for me to obtain accurate quantitative measurements, I turned, as Bodine (1921 a, 1921 b, 1922) had done, from the barium hydroxide methods to indicator methods. One of these had already been well developed by Osterhout (1918) and modified in a convenient way for my purpose by Jacobs (1920). Moreover, I had already had some experience with this method (Parker, 1922 b) and I therefore attempted to apply it to nerve.

The apparatus that I devised (Fig. 1) consisted of a small glass respiratory chamber and two standard tubes mounted on an inclined stage that could be moved up and down by an electric motor. The respiratory chamber used was of either an open or a closed type. The open type is shown in side view in Fig. 2 and is composed of a glass tube (T) about 15 centimeters long with a tightly fitting ground-glass stopper (S). Following the suggestion of Irwin (1920) this apparatus was made entirely of glass which eliminated leakage due to rubber connections. The glass used was pyrex thus preventing the contamination of the contained fluids by dissolved glass products. The lower part of the tube (I) had a uniform diameter of about

15 millimeters and the upper part (A) one of about 28 millimeters. The two parts of the tube were attached to each other quite out of center but so that the outer face of the narrower part was in one region exactly in line with the outer face of the wider part. The

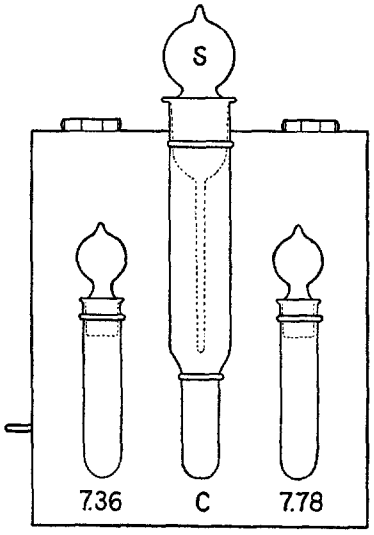

FIG. 1.

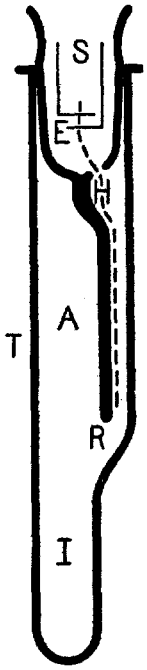

FIG. 2.

FIG. 1. Hinged movable stage carrying a respiratory chamber (C), closed type, and two standard tubes containing solutions of phenolsulphonephthalein made up for pH values 7.78 and 7.36.

FIG. 2. Side view of a respiratory chamber (pyrex glass), open type, × ½, composed of a respiratory tube (T) made up of a narrow part (I) filled with the solution of indicator and a wide part (A) containing air and the nerve. The hollow stopper (S) carries on its inner face a rod (R) on which the piece of nerve (dotted line) is laid. Near the base of the rod the stopper is perforated by a hole (H) through which the end of the nerve may be passed for stimulation by electrodes (E) placed in the cavity of the stopper. After the nerve is in position the hole is closed with a stiff mixture of kaolin and vaseline. The closed type of respiratory chamber is like that just described except that it lacks the hole (H) and the electrodes (E).

stopper carried attached to its inner face a bent glass rod (R) which was so shaped that its principal length was parallel with the wall of the wider part of the tube but to one side of its center. The length of this rod was approximately 5 centimeters and when the stopper was in place, the rod extended through almost the whole length of the wider part of the tube but without coming in contact anywhere with its walls. The cavity of the stopper (S) was freely open to the exterior and the thin wall between this cavity and that of the respiratory chamber was perforated by a hole (H). The closed type of respiratory chamber (Fig. 1) was exactly like the open type except that its stopper (S) was not open or pierced by a hole.

In using the closed type of apparatus the narrower part of the chamber was filled with a solution of the indicator, phenolsulphonephthalein, through which air devoid of CO_2 was freely bubbled thus filling the wider part of the chamber with pure air. The piece of nerve to be tested was then laid on the glass rod to which it adhered through its own stickiness and the stopper with its rod and attached nerve was then quickly inserted into the tube before the pure air in the tube could be contaminated to any great extent by the surrounding atmosphere. By this procedure it was possible to close the tube so that it contained indicator, live nerve, and air with only a minimum of CO_2 accidently included at the moment of closure. By steps to be described presently the effect of this small variable amount of CO_2 was eliminated from the final results.

To facilitate the exposure of the indicator to gases that might be given out by the nerve, the respiratory chamber was attached to the movable stage so that the side of the chamber in which the nerve was lodged was uppermost and the stage was set in motion (Fig. 3). This consisted in shifting the stage in such a way that the respiratory chamber was alternately on an incline with its closed end down and on the horizontal. By this means the indicator solution was flushed back and forth six to eight times a minute under the nerve but without coming in contact with it. Thus if any substance passed from nerve to indicator, it did so through the air contained in the respiratory chamber. In this respect the method was believed to be an improvement over that of Moore (1917) in which the nerve was immersed in the indicator solution itself. In the method used in the present

research it was not necessary to remove by aeration the material that occasioned the acidity and thus prove that it was CO_2 as Moore did, for with the nerve separated always from the indicator by air the only substance at all likely to pass across to the indicator and

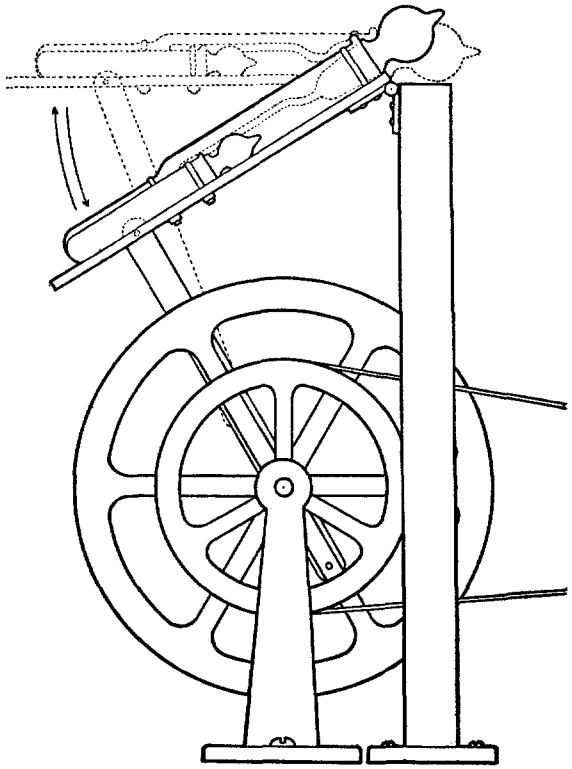

FIG. 3. Side view of the movable hinged stage with its support and a portion of the set of gears by which it was moved up and down. The gears were operated by an electric motor. The extreme positions of the platform are represented in the drawing, the lowest position in full lines, the highest in dotted lines. The gears were so arranged that the stage was lifted and lowered six to eight times a minute.

increase its acidity is CO_2. On grounds that will be presented in a subsequent paper, I believe it can be shown that, contrary to Tashiro's conclusion (1922), ammonia is not given out by a nerve at this stage in its preparation. Hence it is not necessary in experiments such

as are recorded in this paper to take precautions, as suggested by Tashiro (1921), for the removal of this gas.

In the apparatus employed in these tests the washing back and forth of the indicator solution below the nerve had the further advantage of keeping the air in the respiratory chamber well charged with water vapor and thus preventing the nerve from drying, an important precaution in the whole operation.

On testing the nerve in the way described it was found that the indicator gradually changed color in a direction that showed increased acidity. To measure the amount of this change the two standard tubes attached to the stage were used (Fig. 1). Each of these tubes was cut from the same piece of pyrex tubing from which the narrow part of the respiratory chamber had been taken. They were as nearly as possible of the same caliber and thickness of wall as the material in the respiratory tube itself. They were fitted with ground stoppers and filled with standard solutions of the indicator of known pH value (see Osterhout and Haas, 1918, p. 422). The tube on the right contained a solution composed of 8 cc. of 0.2 mol. boracic acid solution, 2 cc. of 0.05 mol. borax solution, and two drops of a saturated aqueous solution of phenolsulphonephthalein. Its color represented a pH value of 7.78. The tube on the left was filled with a solution composed of 9 cc. of 0.2 mol. boracic acid, 1 cc. of 0.05 mol. borax, and two drops of phenolsulphonephthalein solution and represented pH 7.36. The indicator in the respiratory chamber, for reasons that will be given in connection with calibration, was composed of a mixture of 10 cc. of 0.0001 mol. sodium hydrogen carbonate and two drops of a saturated aqueous solution of phenolsulphonephthalein. The solutions in the standard tubes showed a slight deterioration in the course of several weeks; consequently they were set up anew from week to week. The stage on which the three tubes were mounted was covered with white cardboard as a background against which to compare the colors of the several solutions and all such comparisons were made under the illumination of a "daylight" lamp to avoid the uncertainty due to the fluctuating light of the sky.

In beginning a test the respiratory chamber which was in position on the stage was prepared by bubbling through its indicator solution air freed from CO_2 by having been passed over sodium hydroxide.

This air was stored under slight pressure in an appropriate glass reservoir. By this means the small amount of CO_2 contained in the indicator solution was washed out and its color in consequence shifted to a purplish tint well beyond that characteristic for pH 7.78. This operation also incidentally filled the respiratory chamber with air practically free from CO_2. When this point had been reached the pyrex washing tube was withdrawn from the respiratory chamber, the chamber was at once closed by inserting the glass stopper with its rod and attached nerve, and the flushing movements were started. In closing the chamber, as already stated, a small amount of outside air with its contained CO_2 was probably always introduced, but this amount was invariably so slight, as judged by the change in the tint of the indicator, that the whole system still remained well on the alkaline side of pH 7.78. This source of error, therefore, took care of itself, for after the slight readjustment due to this small influx of CO_2 the change in the color of the indicator proceeded in an acid direction with great slowness and regularity. As the tint of the indicator in the respiratory chamber gradually approached that in the more alkaline (pH 7.78) of the standard tubes, it was carefully observed and at coincidence the time was read to seconds by a watch and recorded. The apparatus was kept running continuously and when the color in the respiratory tube agreed with that in the second standard tube (pH 7.36), the time was again read and recorded. The difference between these two times represents the period required by the nerve to produce enough CO_2 to shift the hydrogen ion concentration of the respiratory chamber from 7.78 to 7.36. In ordinary experiments a piece of nerve that was large enough to accomplish this change in from ten to twenty minutes was found most convenient.

In testing the apparatus one of the first trials that was given it was to run it without nervous tissue. Under such circumstances the shift from pH 7.78 to pH 7.36 was accomplished in from two to three days. These trials were made with a thoroughly sterilized outfit and in a laboratory room freely open to the outer air, but the observer was of course in the room and his breath must have continually contaminated the atmosphere. The very slow change shown by the indicator is believed to have been due to the gradual entrance of minute amounts of atmospheric CO_2 into the respiratory chamber by way of

the narrow space between the glass stopper and the neck of the respiratory tube. This leakage, however, required such a long time (two to three days) as compared with that necessary for an observation (ten to twenty minutes) that no attempt has been made to correct for it.

The closed type of respiratory chamber was used for the study of quiescent, unstimulated nerve. To test stimulated nerve the open type of chamber was used. This type (Fig. 2), as already stated, had a stopper whose external cavity opened freely to the outside and whose wall was perforated by a small hole (H) near the base of the rod. When this type of apparatus was used the nerve to be tested was laid along the rod in the usual manner but the end nearer the stopper, instead of being left within the chamber, was carried through the small hole into the hollow of the stopper as indicated by the dotted line in Fig. 2. Within the stopper it was placed on the platinum electrodes (E) of an induction apparatus. By this means the nerve could be stimulated well outside the respiratory chamber.

To prevent the free leakage of outside air into the respiratory chamber through the hole in the stopper, this opening was plugged with a mixture of kaolin and vaseline. This mixture made a snug fit between the nerve and the glass rim of the hole. It had been previously tested on nerve-muscle preparations and had been found not to interfere with nerve conduction even after twelve hours of application. Of course the open type of respiratory chamber possessed a higher rate of leakage than the closed one did. This rate varied with different experiments. When this type of chamber with the hole completely plugged was run without nerve the change from pH 7.78 to pH 7.36 required from two to three hours. A leakage correction was therefore introduced into all measurements made with the open type of chamber.

This general arrangement is believed to meet the criticism of Bayliss (1915, p. 379) as applied to Tashiro's apparatus in which the electrodes were within the respiratory chamber. In the apparatus just described the electrodes were so far outside the chamber that any heating and consequent liberation of CO_2 could have no effect on the gases in the respiratory chamber because of remoteness. Riggs (1919, p. 401) showed that when a nerve is stimulated not by elec-

tricity but by salts, an increase of CO_2 occurs. This increase, as he pointed out, could in no way be attributed to heat and hence its presence shows that the increase in Tashiro's experiments was not all dependent upon heat. But how much CO_2 may have been discharged in consequence of heat and how much may have been produced by increased metabolism cannot be stated. Hence the desirability of removing in some radical way this objectionable feature. It is believed that the apparatus used in these experiments accomplishes this end.

The calibration of the respiratory outfit employed in these experiments was first attempted by a method that I had previously devised for the Osterhout apparatus (Parker, 1922 a), but in consequence of the small capacity of the respiratory chamber used in these experiments I was unable to introduce into it with the necessary precision small measured quantities of CO_2. This effort, therefore, entirely failed.

Through the kindness of Dr. E. J. Cohn my attention was called to a method of calibration which had been devised by him to meet a similar condition in a research that was being carried on by Dr. G. B. Ray. This method, which has recently been described (Ray, 1924), met the requirements of my apparatus and I therefore adopted it. My thanks are due to Dr. Cohn and to Dr. Ray for their help in this important step.

The application of this method turns in part upon the fact that there is an even distribution of CO_2 throughout the apparatus at any given time irrespective of the proportion of fluid and of air contained in it. If, now, the indicator is dissolved in a weak aqueous solution of sodium hydrogen carbonate of known concentration, the amount of CO_2 per unit volume can be calculated for any pH value. In these experiments the concentration of sodium hydrogen carbonate used was 0.0001 mol. Under such conditions the amount of CO_2 at pH 7.36 was 0.0106 mg. per 10 cc. and at pH 7.78, 0.0040 mg. per 10 cc. Consequently the weight of CO_2 needed per 10 cc. to change from pH 7.78 to pH 7.36 is the difference between these two amounts or 0.0066 mg. (Ray, 1924, p. 510). The capacity of the closed respiratory chamber used in these experiments was found on measurement with mercury to be 30.4 cc. Hence the weight of CO_2 necessary to shift this chamber

from pH 7.78 to pH 7.36 was 0.0066 mg. × 3.04 or 0.020064 mg. The capacity of the open chamber used for the stimulation tests in these experiments measured in the same way was 30.1 cc. and the weight of CO_2 needed to make the appropriate shift in this case was by a similar procedure determined to be 0.019866 mg.

Ignoring the very small but continuous leakage of CO_2 into the apparatus from the outside, it may be said that when a piece of nerve causes the indicator in the closed apparatus to shift from pH 7.78 to pH 7.36 it must do so by discharging 0.020064 mg. of CO_2. As the time for this process is recorded in the experiment, all that is necessary for for the calculation of the rate of production of CO_2 is given. The weight of the nerve is also easily measurable and thus the rate of production of CO_2 can be readily expressed in relation to the amount of nerve concerned. In the following tabulations all the results pertaining to the production of CO_2 by nerve are expressed in milligrams of this gas per gram of nerve per minute.

Before turning to the actual results of the tests, it may be well to make a brief statement as to the sensitiveness of the apparatus. When a nerve tested in the closed chamber is found to discharge the amount of CO_2 requisite for the shift from pH 7.78 to pH 7.36 in 20 minutes, it can be shown that a difference in the tint of the indicator is just observable with certainty in about one minute after coincidence with the standard tube. As under these circumstances there are twenty such intervals in passing from one of the two pH values to the other, it is assumed that the method is sensitive to one-twentieth of the amount of CO_2 necessary for the total change or approximately 0.001 mg. This appears to be the limit of the amount of CO_2 that can be quantitatively detected by a respiratory chamber of the size used in these tests. The use of a smaller chamber would of course reduce in proportion to its size the amount of CO_2 that could be detected. In this way the apparatus is open to increased refinement. If Tashiro's statement (1913 b, p. 140) that the biometer used by him was sensitive to 1.0×10^{-7} grams of CO_2 (= 0.0001 mg.) is correct, his method must have been about ten times as sensitive as the one I used.

III. OBSERVATIONS.

When a freshly prepared lateral-line nerve from the dogfish is tested for CO_2 in the closed type of respiratory apparatus, this gas can be shown to be discharged from the nerve at first in considerable quantities and afterwards in much smaller amounts. The discharge is at first in the nature of a gush after which there follows a steady outflow for a number of hours with only slight diminution. This is well illustrated in nerve I (Fig. 4). As expressed in milligrams of CO_2 per

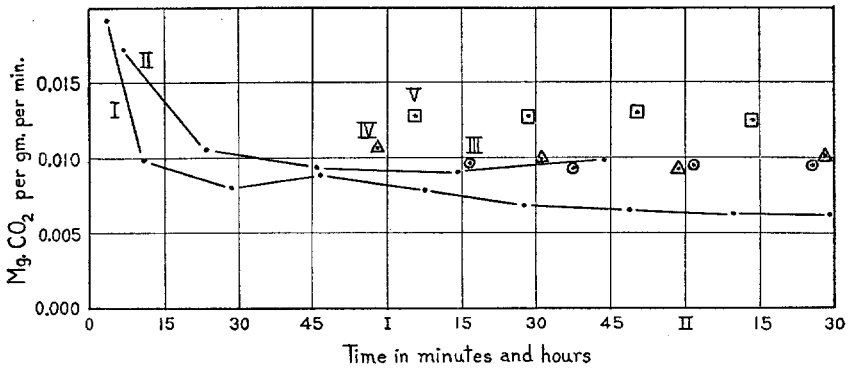

FIG. 4. Plottings of the respiratory rates in milligrams of CO_2 per gram of nerve per minute of five lateral-line nerves from the dogfish. The readings from Nerves I and II are represented by dots connected by lines; those from Nerve III are in circles, from Nerve IV are in triangles, and from Nerve V are in squares. Temperature about 23°C.

gram of nerve per minute this nerve showed an initial rate of 0.0192 which was followed by a decline first to 0.0098, and then to 0.0080, after which followed a slight increase to 0.0089, and then a gradual decrease to 0.0063. Essentially the same course is shown in Nerve II. This type of discharge was characteristic of all fresh nerves in these tests; an initial gush that lasted through about half an hour or more followed by a steady outflow with very gradual decline that extended in some instances over a period of as much as 8 hours. This decline was ordinarily succeeded by disturbances that were probably due to bacterial invasion; they were quite irregular and obscured the remaining steps of the process. The type of discharge just described has been noted by several previous observers (Tashiro, 1913 a, p.

117; Moore, 1918, p. 35; and Riggs, 1919, p. 401). Its significance is not wholly clear for the source of the CO_2 concerned has not been definitely determined.

There is every reason to believe that a nerve freshly removed from an animal such as a dogfish is taken from surroundings richly impregnated with CO_2 (Bayliss, 1915, p. 379). Such a nerve, therefore, would probably be well saturated with this gas and on exposing the nerve to the air the CO_2 would naturally escape from it at first rapidly and then more slowly till an equilibrium would be reached. In this way the relatively sudden gush of CO_2 followed by the more gradual discharge, as already described, might be explained. But a closer

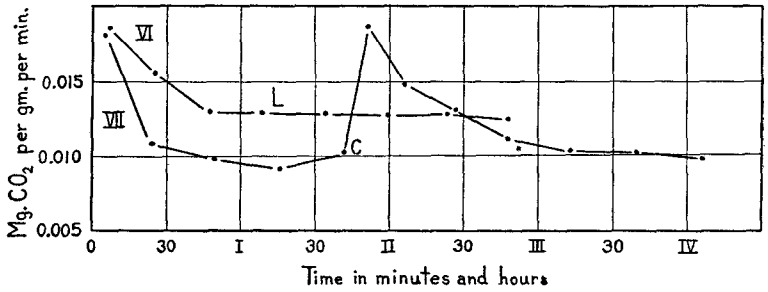

FIG. 5. Plottings of the respiratory rates in milligrams of CO_2 per gram of nerve per minute of two lateral-line nerves from the dogfish. Nerve VI, after its fourth test, was lifted (L) from the rod, returned to it, and then tested further. Nerve VII, after its fifth test, was cut (C) and then tested further. Temperature about 23°C.

inspection of the rates of escape of CO_2, as illustrated, for instance, by nerve I (Fig. 4), shows that though the gush might be considered the result of the exposure of a highly saturated nerve to air, the steady outflow cannot be thus explained. This outflow maintains itself at too high a level and for much too long a time to be interpreted as the final steps in the escape of CO_2 from such a reservoir as a nerve might be. The course of this outflow implies at once a continuous slow production of CO_2 within the nerve itself and thus supports the belief that the CO_2 at this stage of the discharge is not merely gas escaping mechanically from temporary storage, but a true excretion from the nerve.

It is also possible that the gush itself may be of this nature, for it immediately succeeds the preparation and handling of the nerve, operations that may be highly stimulating and hence productive of CO_2. To test the effects of preparation on a nerve several lines of experimentation were followed. Nerves were prepared in the usual way, tested, and, after they had arrived at the period of steady outflow, they were handled in that they were lifted from the rod in the respiratory chamber, returned to it, and then retested. In no case as a result of this treatment was the amount of discharged CO_2 increased to a measurable degree. This is well illustrated in nerve VI (Fig. 5). The rate of this nerve was at the beginning 0.0180 mg.; it then fell through 0.0156 to 0.0131, 0.0130, 0.0129, and so on to 0.0125. After 0.0130 had been reached the nerve was lifted (Fig. 5, L) from the rod and returned to it, but as the subsequent records show, 0.0129 to 0.0125, without any observable effect on the output of CO_2. Such results were regularly obtained and show conclusively that the simple handling of a nerve does not noticeably alter the rate of its CO_2 production. The high resistance of cold-blooded nerve, such as frog nerve, in comparison with mammalian nerve to the ill effects of even such vigorous mechanical action as stretching has already been pointed out by Forbes and Ray (1923, p. 461).

In a second set of tests the dogfish nerve, after it had arrived at the stage of the steady outflow, was cut in many places instead of being merely handled. This treatment yielded a very striking increase of CO_2 as shown by nerve VII (Fig. 5). This nerve began with an initial rate of 0.0176 mg. which soon fell to approximately 0.0100. After the fifth test on this nerve it was cut (Fig. 5, C) in sixteen places with the result that at the next test its rate had risen to 0.0186, higher than its initial rate. Recovery from this state was as rapid as from the initial gush and to approximately the same level (0.0100). Cutting, therefore, unlike mere handling calls forth an excessive discharge of CO_2 which, however, lasts over only a short period, like the initial gush.

Does cutting a live nerve excite metabolism or merely increase the surface through which stored CO_2 may escape? The latter opinion seems to be supported by Moore's observation (1917, p. 18), which I can confirm, that when a nerve is cut and put under a purple solution

of phenolsulphonephthalein, the ends of the nerve become bright yellow showing that they are very acid, probably from CO_2. But this is not the only part of the nerve that gives out CO_2. If a long piece of freshly prepared nerve is put in a respiratory chamber whose stopper is provided with a hole and the nerve is placed in such a way that its two cut ends are outside the respiratory chamber so that only an uncut loop of the nerve lies on the rod within the chamber, the same initial gush of CO_2 occurs as it does when the whole nerve is within the chamber. In one example of this kind the sequence of rates was as follows: 0.0231, 0.0142, 0.0131, 0.0121, 0.0120. Hence it is clear that the initial gush is not entirely through the cut ends of the nerve but must be thought of as resulting from a lateral escape from the nerve as well as from its ends. If, now, such a nerve as that just described is vigorously pressed or almost crushed but without injuring it to the extent of exposing much new surface, a new gush of CO_2 can be evoked. This was seen in the nerve from which the records just given were taken. After this nerve had subsided to the rate 0.0120, it was vigorously manipulated, though not really crushed, with the result that its carbon dioxide output immediately arose to 0.0247 and then fell again to 0.0119. Thus it is evident, as already maintained by Moore (1918, p. 35; 1921, p. 24), that vigorous manipulation and crushing increase the CO_2 output of a nerve to a striking degree. Although it is probable that some of the CO_2 that escapes from a prepared nerve has not been produced in the nerve but may have come from adjacent tissue and been held in the nerve as in a reservoir, it is certain, as the preceding results show, that the amount of such CO_2 must be relatively very small and that the principal volume of this gas in the initial gush and probably all of it during the steady outflow are the products of local metabolism within the nerve.

In this connection the CO_2 output of a nerve killed in boiling water is not without significance. If a freshly prepared nerve is steeped in boiling water one minute and immediately thereafter tested, it will be found to give out CO_2 at first at a low rate, approximately 0.0025 mg., and then at diminishing rates for about two hours after which no more CO_2 can be detected. These observations agree with those of Tashiro (1913 a, p. 116) who was unable to discover any striking amount of CO_2 from nerve killed by boiling. Moore (1917, p. 18)

also noted that a nerve killed by chloroform or by heat did not increase the acidity of the indicator in which it was temporarily immersed. These results show clearly that relatively soon after the death of the nerve the production of CO_2 falls very low and soon ceases altogether. The living nerve of a dogfish, however, will continue to produce this gas for at least eight hours after its removal from the fish's body, and this steady outflow, as already indicated, may be regarded as a fair measure of the metabolism of a quiescent nerve.

Such being the case it would be natural to suppose that the amount of CO_2 given out by a nerve would be in proportion to its weight. This relation has from the beginning been implied in the present discussion and, as might be expected, holds true on experimental test. Thus two nerves, A and B, were measured together and apart. nerve A was 10.2 cm. long and weighed 118 mg.; nerve B was 9.4 cm. long and weighed 82 mg. The nerves were tested in the closed respiratory chamber. To produce the amount of carbon dioxide for the necessary shift in this chamber (0.020064 mg.) required 1430 seconds for nerve A, 1990 seconds for nerve B, and 817 seconds for the two nerves together. Multiplying the weights of the nerves by the time required by each the following numbers are obtained: for nerve A 168,740, for nerve B 160,180 and for both 165,400 numbers that are in approximate agreement and that show that the amount of CO_2 discharged by a nerve is in proportion to its weight.

To obtain some quantitative statement of the rate of CO_2 production in quiescent dogfish nerve, records have been taken from nerves during the first two hours after the disappearance of gush. These records were obtained at a temperature of approximately 23°C. and are included in Fig. 4; they come from five nerves. Two of these nerves are numbers I and II already referred to and are represented by graphs in Fig. 4. The three remaining nerves, III, IV, and V, are also shown in Fig. 4 but merely by points, those of nerve III within circles, of nerve IV within triangles, and of nerve V within squares. I have assumed that all the records that lie between the 45 minute line and the 2 hour and 30 minute line in Fig. 4 may be reasonably regarded as representing the metabolism of quiescent nerve. Within this limit those from nerve I range from 0.0089 mg. to 0.0063 mg. and average 0.0071 mg.; from nerve II they average 0.0094 mg.; from

nerve III 0.0095 mg.; from nerve IV 0.0100 mg.; and from nerve V, the highest of all, 0.0128 mg. The general average for all five nerves is 0.0095 mg. Hence it may be stated that the quiescent lateral-line nerve of the dogfish discharges CO_2 at the average rate of 0.0095 mg. per gram of nerve per minute.

This determination stands somewhat higher than those reported by Tashiro (1913 a, pp. 112, 127). According to this investigator the nerve of the spider crab produces 6.7×10^{-7} gms. of CO_2 per 10 mgs. of nerve per 10 minutes or, expressed in the terms used in this paper, 0.0067 mg. of CO_2 per gram of nerve per minute. Frog nerve, according to Tashiro, produces 5.5×10^{-7} gms. of CO_2 per 10 gms. of nerve per 10 minutes, or 0.0055 mg. of CO_2 per gram of nerve per minute. Although both these determinations lie somewhat below mine, all three are obviously so close that they may be regarded as of the same order of magnitude and undoubtedly represent a close approximation to the true metabolic rate of quiescent cold-blooded nerve.

Is the rate of CO_2 production in the quiescent lateral-line nerve of the dogfish changed by stimulation? To answer this question the following tests were carried out. Long stretches of nerve were set up in the open type of respiratory chamber and, after the period of gush had passed, they were alternately stimulated by an induction apparatus and allowed to remain quiescent. As already stated the electrodes used for such operations were situated outside the respiratory chamber in the hollow stopper of that apparatus (Fig. 2). In all, four nerves were tested and in each instance a reading was first taken for the resting state after which electric stimulation was applied and a reading was taken while this was in progress. The nerve was then allowed to remain quiescent for from ten to fifteen minutes after which another reading, assumed to represent the quiescent state, was taken and again on stimulation a last reading. Thus four readings were obtained from each of the four nerves. These readings and the resulting averages are given in Table I.

As is shown in Table I all four nerves gave evidence of increased rates of CO_2 production during stimulation as compared with quiescence. The average rate of CO_2 production during quiescence was 0.01043 mg. CO_2 per gram of nerve per minute, and during stimulation

0.01208 mg., an increase in rate of 0.00165 mg. In percents the increase varied on the average from 14.3 to 16.7 of the rate in quiescence and gave a general average of 15.8 per cent. The increase though slight in amount, was characteristic of all the nerves tested. It cannot, I believe, be attributed to the heating of the nerve by the stimulating current, as suggested by Bayliss in his criticism of Tashiro's results, for, as already stated, the part of the nerve to which the current was applied was well outside the respiratory chamber.

As a check on the tests just recorded I steeped a nerve in boiling water for one minute and then subjected it to the kind of test just described for living nerve. During about two hours this nerve continued to give out a very small amount of CO_2 which beginning at 0.0020

TABLE I.

Rates of CO_2 Production in Milligrams of CO_2 per Gram of Nerve per Minute of Four Lateral-Line Nerves of the Dogfish Alternately Unstimulated and Stimulated.

No. of nerve.	State of nerve.				Averages.		Diff.	Diff. %.
	Unst.	Stim.	Unst.	Stim.	Unst.	Stim.		
1	.0116	.0138	.0114	.0125	.01150	.01315	.00165	14.3
2	.0096	.0115	.0092	.0103	.00940	.01090	.00150	16.0
3	.0118	.0130	.0112	.0138	.01150	.01340	.00190	16.5
4	.0090	.0113	.0096	.0104	.00930	.01085	.00155	16.7
General averages.....					.01043	.01208	.00165	15.8

mg. per gram of nerve per minute dropped eventually to nothing. This amount was never increased on stimulation but showed a steady decline. The contrast between the responses on stimulation of a living nerve and of a nerve killed by boiling is well shown in Fig. 6 in which are plotted the records of nerve 4 in Table I and those of the nerve killed in boiling water. The solid lines represent periods of quiescence, the dotted lines those of stimulation. As already stated, with living nerve each period of stimulation was accompanied with an increase in respiratory rate, but with the boiled nerve the periods of stimulation showed no such increase but the CO_2 steadily fell off till none could be detected. From these observations I conclude that the increase in the rate of production of CO_2 on stimulating a living

nerve is due to increased metabolism and is not occasioned, as Bayliss suggested, by experimental error.

The increased rate of CO_2 production on stimulation as detailed in the present account is not in agreement with the results of Tashiro or of Moore. Moore (1919 a, p. 66; 1919 b, p. 617; 1921, p. 24), using the method of immersion on frog nerve, was unable after 30 minutes of stimulation to observe any increase of rate in the output of CO_2. But Moore states that his method was capable of detecting only about a 10 percent difference. The increase that I have found, 15.8 percent, is so near this that perhaps even this increase failed to show itself in Moore's procedure. It is also possible, though I think

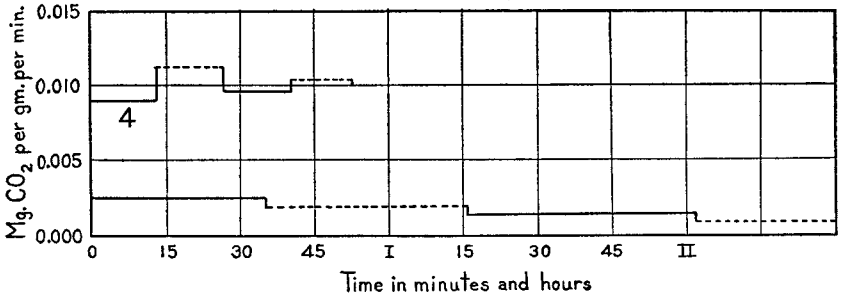

FIG. 6. Plottings of the respiratory rates in milligrams of CO_2 per gram of nerve per minute of two lateral-line nerves from the dogfish. The upper plotting (4) is from Nerve 4 in Table I and the lower one from a nerve killed by immersion in boiling water for one minute. Both nerves were alternately quiescent, represented by the full line, and stimulated, represented by the dotted line. Temperature about 23°C.

it not likely, that the rate of CO_2 production on stimulation in the frog nerve, which was used by Moore, is lower than that in the dogfish nerve, which I used. This question, however, calls for further investigation.

With Tashiro's conclusions the case is quite different. According to this investigator the increase of rate in CO_2 on stimulation is very high, with the nerve of the spider crab 100 to 200 percent and with that of the frog 200 to 300 percent. These increases are relatively so enormous that I feel sure that unless nerves are prodigiously diverse, I should have easily discovered by the method I used differences of this order of magnitude. But of such increases I have seen in the dogfish nerve not the least evidence. How the disparities between

Tashiro's results and mine are to be reconciled is not easy to state. So far as I can judge the method I have used is thoroughly sound. It enabled me to distinguish at least 0.001 mg. of CO_2, and the amount involved in determining an increase of rate of 15.8 percent is a little over 0.0025 mg. or about two and a half times this minimum. Had there been an increase of even 100 percent, not to mention 200 or 300 percent, I am sure I should have observed it, but as this investigation shows no increase of this proportion was ever met with. I do not believe that the nerves tested by Tashiro differ in so fundamental a way from the one studied by me. I suspect that the difficulty lies in the quantitative side of the biometer. The success of this apparatus as a means of measuring CO_2 turns on the formation of barium carbonate particles. In my experience these particles are formed much too irregularly to give a sound basis for quantitative results. Their production probably depends upon the presence of foreign nuclei and these or some other like factors vary so much that uniformity of production never seemed obtainable. In this respect, as already stated, my results agree with those of Adam (1921, p. 362) who has declared the method to be remarkably sensitive from a qualitative standpoint but insufficient quantitatively. I am therefore forced to set down the excessive increases of CO_2 attributed by Tashiro to nerve stimulation as due to an insufficient method. It is nevertheless remarkable that his determination of the CO_2 output for quiescent nerve should fall so close to mine.

As a result of this study on the lateral-line nerve of the dogfish I concluded that this nerve produces when quiescent approximately 0.0095 mg. of CO_2 per gram of nerve per minute and that this amount is increased by about 15.8 percent on stimulation.

What tissue in the nerve is the source of this CO_2 remains to be ascertained. Bayliss (1915, p. 379) pointed out the obvious fact that a nerve contains, beside nerve fibers, connective tissue and the like and that it is evident that this non-nervous tissue must respire. Consequently some, it not all, of the CO_2 given out by a quiescent nerve must come from this source. What proportion in a quietly respiring nerve is derived from its non-nervous elements and what from its nervous components cannot be stated. It is a common belief that the nucleated part of a cell is necessary for its respiration (Meyer-

hof, 1924, p. 11). Since the strictly nervous portions of a nerve trunk are the axis cylinders and since these represent in preparations such as were used in this research, parts of nerve cells separated from their nuclei, there has grown up an opinion that such axis cylinders cannot respire. Those who hold such a view would probably regard all the CO_2 that emanates from a quiescent nerve as due to the activity of its non-nervous components, connective-tissue cells, etc. I frankly admit that I have no evidence to show that such is not the case. But however this may be for quiescent nerve, it must be admitted, I believe, that the increase of CO_2 due to the stimulation of a nerve is from a strictly nervous source, for it is not remotely probable that stimulation as carried out in this research could influence connective tissue, blood vessels, or the like, in such a way as to produce at a distance of a few centimeters the increased CO_2 output recorded in this investigation. Hence I conclude that the 15.8 percent increase of CO_2 in the stimulated nerve of the dogfish is from strictly nervous sources and not from non-nervous elements. This amount, in my opinion, represents CO_2 that is the product of functionally active axis cylinders and their appended parts, such as the medullary sheaths, and is not due to the increased activity of the Schwann sheath-cells, the connective tissue elements, blood vessels, or other non-nervous elements. If this view is admitted, and I can see no reason why it should not be, it is also probably true that some at least of the CO_2 given out by a quiescent nerve comes from the same strictly nervous source as the excess excreted by the stimulated nerve does and represents the normal metabolism of this tissue in its quiescent state.

What proportion the excess CO_2 from stimulated nerve bears to the total CO_2 of strictly nervous origin cannot of course be stated. Tashiro has apparently assumed that all the CO_2 from a nerve was from nervous sources and from this standpoint he has estimated an increase of 200 to 300 percent on stimulation. This estimate led Mathews (1915, p. 590) to declare that nerve is perhaps the most actively metabolic tissue in the body. If, however, the rate of CO_2 increase is relatively low, as my determinations seem to indicate, nerve may not prove to be so exceptional. In a rough way I have tested in my apparatus pieces of fresh muscle and of fresh brain from the dogfish and I have found that the rate of CO_2 production in quiescent muscle

is almost three times that of quiescent nerve and in pieces of brain it is over four times that of nerve. From the observations of Moore (1918, p. 39) frog muscle appears to give out CO_2 at a rate of from three to seven times that of frog nerve and frog medulla from five to ten times that of frog nerve. Although these determinations, as well as my own, are avowedly rough and yield no certain criteria as to the respiration of pure tissue, they are in fair agreement and indicate that nerve has not an exceptionally high rate of respiration but on the contrary exhibits perhaps a lower one than that of many other tissues.

IV. GENERAL DISCUSSION.

Although it is not my intention to discuss in any detail the nature of the nervous impulse, it is nevertheless true that the observations set forth in this paper point to certain conclusions concerning this process and call for some comments particularly in its relation to oxygen, fatigue, and heat.

If active nerve excretes CO_2, it should be expected to absorb oxygen. Over two decades ago von Baeyer (1902) observed that frog nerve in an atmosphere of hydrogen or of nitrogen became inexcitable in from 3 to 5 hours and recovered excitability in air after from 3 to 10 minutes. This condition was shown to be dependent upon oxygen and has commonly been regarded as a kind of asphyxiation. Since its discovery the asphyxiation of nerve has been confirmed by many workers and has afforded the starting point within recent years of a number of important investigations (Tashiro and Adams, 1914; MacArthur and Jones, 1917; Gottschalk, 1920; Adam, 1921; Sheaff, 1922; Thörner, 1922 a, 1922 b; Cooper, 1923).

Assuming oxygen to be an essential element in nerve activity, the minimum amount of this gas needed in the case of any given nerve may be easily calculated on the basis of the CO_2 discharged by that nerve. Thus to produce 0.0095 mg. CO_2, the amount of this gas discharged by a gram of resting dogfish nerve in a minute, would require an oxygen supply of 0.0070 mg. The oxygen consumption of frog nerve, which is probably very close to that of dogfish nerve, has already been determined by Adam (1921) and by Sheaff (1922). Sheaff working with an apparatus, which was essentially a biometer modified

to meet the needs of oxygen determination, found that 10 mg. of quiescent frog nerve absorbed in 10 minutes from 0.434 to 0.76 $\times 10^{-5}$ grams of oxygen, or from 0.0434 to 0.076 mg. oxygen per gram of nerve per minute. This implies a rate of oxygen consumption approximately five to ten times that necessitated by the corresponding CO_2 discharge reported in this paper. Why Sheaff's figures should be so high is not wholly clear. In his description of his method he (1922, p. 47) states that in preparing and placing the nerve he worked *speedily*. Under such circumstances the nerve must have been in the period of CO_2 gush. If CO_2 and oxygen are related as might be expected, this period would also be one of excessive oxygen absorption which may possibly explain in part at least Sheaff's relatively high determinations.

According to Adam (1921) resting frog nerve absorbs only from 0.05 to 0.08 c.c. of oxygen per gram of nerve per hour, which when transposed to the form of expression used in this paper amounts to 0.012 mg. to 0.019 mg. of oxygen per gram of nerve per minute. These determinations are so close to what has been reported in this paper (0.007 mg.), that they afford a reasonable confirmation of the belief that the oxygen absorption and the CO_2 production in nerve are steps in one and the same general process (Lucas, 1917, p. 26).

With this conclusion in mind it is not surprising to find that both Sheaff and Adam record an increase of oxygen absorption on the stimulation of nerve. In Sheaff's studies the determinations are high compared with what would be anticipated from the CO_2 increase recorded in this paper. Adam's determinations, on the other hand, fall very close to the results herein reported. According to him a stimulated frog nerve absorbs an excess of oxygen amounting to 0.02 c.c. per gram of nerve per 15 minutes which is equivalent to 0.0019 mg. oxygen per gram of nerve per minute. This is so near to 0.0011 mg. oxygen, the weight calculated from the excess CO_2 determination given in the present paper as to amount almost to agreement and I therefore conclude that both in quiescent nerve and in stimulated nerve the weight of oxygen absorbed is directly related to the weight of CO_2 excreted.

If nerve exhibits a metabolism in which oxygen is absorbed and CO_2 discharged, why does it not show signs of exhaustion, as, for

instance, muscle does? The ability of nerve to respond continuously to stimulation has long been known and this fact has been urged repeatedly by those who favor a physical conception of transmission. It is, however, quite probable that nerve does tire but that the period of its exhaustion is extremely brief in that its recovery is very rapid. The refractory period of a nerve immediately follows the passage of an impulse and the striking peculiarity of this period is the well known inability of the nerve to respond to a renewed stimulus. During this period the nerve changes from the condition in which it cannot respond to stimulation to one in which it can; in other words, the refractory period is a period of recovery. It begins with all the signs of full exhaustion and passes on quickly to a condition of full recovery. This momentary exhaustion is in my opinion the sign of real nerve exhaustion. It is not a cumulative exhaustion like that of muscle but a momentary one. From this standpoint the activity of nerve with its brief period of tiring and its rapid recovery has been compared to the action of insect muscles in flight (Tashiro, 1915, p. 111) or better to the heart (Lasareff, 1924), in which what appears to be a process of continuous activity is made up of steps of momentary exhaustion and of quick recovery. Viewed from this standpoint active nerve as a consequence of its activity does tire, but its recovery is so quick that the tiring is shown only over a very brief period. This view of nerve exhaustion has already been expressed by Bayliss (1915, p. 390).

Whether the CO_2 liberated from stimulated nerve is produced in the passage of the impulse or during the immediately following process of recovery has not been ascertained. Judged from the standpoint of muscle physiology, the recovery process in nerve may be the one concerned with the production of CO_2 rather than the process represented by the impulse itself (Cooper, 1923), but on this problem I have no critical observation to offer.

If active nerve absorbs oxygen, discharges CO_2, and exhibits momentary exhaustion, its operations would appear to depend in part at least upon oxidation and might therefore be expected to be accompanied with an evolution of heat. As is well known Hill (1911) on tetanizing a nerve for 25 seconds failed to get evidence of an increase of temperature in the nerve by an apparatus that was sensitive to

about six millionths of a degree centigrade. How is the amount of heat that may be assumed in the production of 0.00165 mg. of excess CO_2 per gram of nerve per minute related to this very low limit set by Hill? If we assume the heat in the nerve to have been generated by the oxidation of some such substance as glucose, it can be shown by calculation that the production of 0.00165 mg. CO_2 may be expected to liberate approximately 0.0042 gram-calories of heat. This implies that any piece of nerve on being stimulated would produce in a period of one minute enough heat to raise its temperature about 0.0042°C. provided that the nerve was like water and that none of the heat produced in it escaped. Nerve doubtless requires about as much heat as water does to bring up its temperature a given amount and of course all the heat produced in nerve would eventually escape, but its dissipation would be relatively slow so that beginning with such a comparatively large amount as this reaction implies, a temperature difference capable of being measured ought to be within the possibility of observation. It is, however, conceivable that the chemistry of the nervous impulse may not involve the oxidation of such a substance as glucose, but may depend for its final step upon the oxidation of some almost completely oxidized compound which step would then liberate CO_2 with the least possible evolution of heat and thus greatly reduce this factor. It can be easily shown, for instance, that while the production of 0.00165 mg. CO_2 from glucose is accompanied by an evolution of 0.0042 gram-calories of heat, the same amount of CO_2 can be obtained by burning the much more completely oxidized oxalic acid with an evolution of only about 0.0011 gram-calories of heat or about one-fourth the former amount. In this way it is conceivable that the production of a given quantity of CO_2 may be accomplished under one set of circumstances with a much lower output of heat than under other circumstances. But although the nerve impulse may depend upon a much lower heat-producing process than the oxidation of glucose, it is highly improbable that an oxidation could be found that would reduce this operation to a level consistent with Hill's determination. Harvey (1919, p. 142), however, seems to imply such reduced processes in his discussion of heat production in bioluminescence. Here not enough heat was evolved to make a change of 0.0005°C. in the solution used. Harvey concludes,

not that heat is absent, but that the oxidation of the light producing substance, luciferin, is accomplished by a process that involves only a minimum of heat. But whether this is the true explanation of such conditions or not, there must be a limit to this kind of reduction and this limit in the case of nerve seems to have been exceeded by what Hill has shown to be the requirements of his own determination. In a recent paper by this author (Hill, 1921), however, in the course of a very illuminating discussion of the temperature coefficient of the nerve impulse he concludes that in nerve transmission "a chemical change interposes somewhere in the process." The bearing of this conclusion on the possibility of heat production in nerve action is not discussed probably because the whole subject is unsettled and ripe for reinvestigation rather than for speculation. In such reinvestigation it may be shown that nerve, like muscle, can store carbonates from which with a minimum of heat production CO_2 may be liberated by an acid formed in the nerve.

The comparison of a nerve impulse to the burning of a chain of gun-powder, though not very close, is nevertheless in many ways suggestive. In both oxygen is used, CO_2 discharged, and a progressive activity exhibited. But in nerve the oxidation is probably a step in restoration rather than part of the impulse proper, for the fresh nerve seems rather like a suspended weight which on being loosened by some agent corresponding to the stimulus falls, whereupon work must be done to return it to its initial elevation. It is this operation of return that involves in the nerve oxidation and the output of CO_2. Further, the nerve impulse is a change that progresses over the substance of nerve not by the spread of heat but probably by a change in electrical potential, as indicated by Lillie (1923), and in this respect also it differs from a chain of burning gun-powder. But however we may analyze such examples, it seems quite clear, as stated by Forbes (1923, p. 49), that the purely physical conception of the nerve impulse has ceased to have much weight and we must agree with Hill (1921, p. 334) that chemical change interposes somewhere in it.

V. SUMMARY.

1. A modified Osterhout respiratory apparatus for the detection of CO_2 from nerve is described.

2. The lateral-line nerve from the dogfish discharges CO_2 at first with a gush for half an hour or so and then steadily at a lower rate for several hours.

3. Simple handling of the nerve does not increase the output of CO_2; cutting it revives gush.

4. The CO_2 produced by nerve is not escaping simply from a reservoir but is a true nervous metabolite.

5. The rate of discharge of CO_2 from a quiescent nerve varied from 0.0071 to 0.0128 mg. per gram of nerve per minute and averaged 0.0095 mg.

6. Stimulated nerve showed an increased rate of CO_2 production of 15.8 percent over that of quiescent nerve.

7. The results of these studies indicate that chemical change is a factor in nerve transmission.

VI. LITERATURE.

Adam, N. K. 1921. Note on the Oxygen Consumption of Amphibian Muscle and Nerve. Biochem. J., **15,** 358.

von Baeyer, H. 1902. Das Sauerstoffbedürfniss des Nerven. Zeit. allg. Physiol., **2,** 169.

Bayliss, W. M. 1915. Principles of General Physiology. London.

Bodine, J. H. 1921 a. Factors Influencing the Water Content and the Rate of Metabolism of Certain Orthoptera. J. Exp. Zoöl., **32,** 137.

1921 b. Some Factors Influencing the Catalase Content of Organism. J. Exp. Zoöl., **34,** 143.

1922. The Effect of Light and Decapitation on the Rate of CO_2 Output of Certain Orthoptera. J. Exp. Zoöl., **35,** 47.

Cooper, S. 1923. The Rate of Recovery of Nerve in Asphyxia. J. Physiol., **58,** 41.

Forbes, A. 1923. The Physico-Chemical Basis of Psychic Phenomena. Science, **58,** 49.

Forbes, A., and L. H. Ray. 1923. The Conditions of Survival of Mammalian Nerve Trunks. Amer. J. Physiol., **64,** 435.

Gottschalk, A. 1920. Über den Mechanismus der Erstickung und Erholung des markhaltigen Kaltblüternerven. Zeit. allg. Physiol., **18,** 341.

Harvey, E. N. 1919. Studies on Bioluminescence. XI. Heat Production During Luminescence of Cypridina Luciferin. J. Gen. Physiol., **2,** 137.

Hill, A. V. 1911. The Absence of Temperature Changes during the Transmission of a Nervous Impulse. J. Physiol., **43,** 433.

1921. The Temperature Coefficient of the Velocity of a Nervous Impulse. J. Physiol., **54,** 332.

Hughes, J. S., and H. H. King. 1923. Physico-Chemical Basis of Psychic Phenomena. Science, **57**, 590.

Irwin, M. 1920. An Apparatus for Measuring the Production of Minute Quantities of Carbon Dioxide by Organisms. J. Gen. Physiol., **3**, 203.

Jacobs, M. H. 1920. A Simple Method of Measuring the Rate of Respiration of Small Organisms. Amer. Nat., **54**, 91.

Lasareff, P. 1924. Sur la cause physico-chimique de l'absence de la fatigue dans les centres nerveux au cours de leur action. Compt. rend. Acad. Sci., Paris, **178**, 1100.

Lillie, R. S. 1923. Protoplasmic Action and Nervous Action. Chicago.

Lucas, K. 1917. The Conduction of the Nervous Impulse. London.

Lund, E. J. 1919. A Simple Method for Measuring Carbon Dioxide Produced by Small Organisms. Biol. Bull., **36**, 105.

MacArthur, C. G., and O. C. Jones. 1917. Some Factors Influencing the Respiration of Ground Nervous Tissue. J. Biol. Chem., **32**, 259.

Mathews, A. P. 1915. Physiological Chemistry. New York.

Meyerhof, O. 1924. Chemical Dynamics of Life Phaenomena. Philadelphia.

Moore, A. R. 1917. Acid Development as the Result of Injury in Nervous Tissue. Proc. Soc. Exp. Biol. Med., **15**, 18.

1918. The Carbon Dioxide of Injury and of Respiration in Nervous Tissue. Proc. Soc. Exp. Biol. Med., **16**, 35.

1919 a. Electrical Stimulation and CO_2 Production in Nervous Tissue. Proc. Soc. Exp. Biol. Med., **16**, 66.

1919 b. The Respiratory Rate of the Sciatic Nerve of the Frog in Rest and Activity. J. Gen. Physiol., **1**, 613.

1921. Respiratory Rate in Nervous Tissue. Proc. Pathol. Soc., Philadelphia, **41**, 24.

Osterhout, W. J. V. 1918. A Method of Studying Respiration. J. Gen. Physiol., **1**, 17.

Osterhout, W. J. V., and A. R. C. Haas. 1918. A Simple Method of Measuring Photosynthesis. Science, **47**, 420.

Parker, G. H. 1922 a. The Calibration of the Osterhout Respiratory Apparatus for Absolute Quantities of Carbon Dioxide. J. Gen. Physiol., **4**, 689.

1922 b. The Excretion of Carbon Dioxide by Relaxed and Contracted Sea Anemones. J. Gen. Physiol., **5**, 45.

Ray, G. B. 1924. The Oxidation of Sodium Lactate by Hydrogen Peroxide. J. Gen. Physiol., **6**, 509.

Riggs, L. K. 1919. Action of Salts upon the Metabolism of Nerves. J. Biol. Chem., **39**, 385.

Sheaff, H. M. 1922. A Method for the Quantitative Estimation of Minute Amounts of Gaseous Oxygen and its Application to Respiratory Air. J. Biol. Chem., **52**, 35.

Tashiro, S., 1913 a. Carbon dioxide production from Nerve Fibres when Resting and when Stimulated. Amer. J. Physiol., **32**, 107.

1913 b. A New Method and Apparatus for the Estimation of Exceedingly Minute Quantities of Carbon dioxide. Amer. J. Physiol., **32,** 137.

1914. Carbon Dioxide Apparatus. III. J. Biol. Chem., **16,** 485.

1915. On the Nature of the Nerve Impulse. Proc. Nat. Acad. Sci., **1,** 110.

1917. A Chemical Sign of Life. Chicago.

1921. Can an Indicator Method be used for Measurement of CO_2 Production from Isolated Nerve? J. Biol. Chem., **46,** XV.

1922. Studies on Alkaligenesis in Tissues. I. Ammonia Production in the Nerve Fiber during Excitation. Amer. J. Physiol., **60,** 519.

Tashiro, S., and H. S. Adams. 1914. Carbon dioxide Production from the Nerve Fiber in a Hydrogen Atmosphere. Amer. J. Physiol., **34,** 405.

Thörner, W. 1922 a. Elektrophysiologische Untersuchungen am alterierten Nerven: I. Mitteilung. Die überlegene Erregungswirkung der aufsteigenden konstanten und Induktions-Ströme infolge Zunahme der anodischen Öffnungserregbarkeit und kathodischen Depression während der Erstickung. Arch. ges. Physiol., **197,** 159.

1922 b. Elektrophysiologische Untersuchungen am alterierten Nerven. II. Mitteilung. Sauerstoffentziehung und physiologischer Elektrotonus. Arch. ges. Physiol., **197,** 187.

Waller, A. D. 1896. Croonian Lecture. Observations on Isolated Nerve. (Abstract.) Proc. Roy. Soc., London, **59,** 308.

1897 a. Croonian Lecture. Observations on Isolated Nerve (with particular reference to Carbon Dioxide). Philos. Trans. Roy. Soc., London, B, **188,** 1.

1897 b. Lectures on Physiology. First Series. On Animal Electricity. London.

1910. Physiology the Servant of Medicine. London.