THE RESPONSE OF NERVE TO OXYGEN LACK

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In an earlier paper (Gerard, 1927a) on the effect of asphyxia on the heat production of nerve during activity, some preliminary observations of action potentials were included; and it was proposed to analyze the mechanism of asphyxiation by means of further action potential studies. Other observations I made at that time indicated that the presence or absence of oxygen had a marked influence on injury potentials and on the shape and even the sign (positive or negative after-effects) of the action potentials in frog nerve. In the present experiments the study of these phenomena has been carried further, and in the course of the work a number of unanticipated effects have come to light. In the last two years, Furusama (1929) and Amberson and Downing (1929), in Hill's laboratory, where the above studies were carried out, have elaborated certain phases of these problems, and Gerard and Forbes (1928) have further studied positive and negative after-effects in the isolated nerve. A recent paper by Heinbecker (1929) has also dealt with asphyxia of nerve.

The thermal and electric response of a nerve kept in an oxygen-free atmosphere gradually falls to zero. Although it was calculated (Gerard, 1927a; Hill, 1928) that oxygen dissolved in the nerve should diffuse out or be used up within a few minutes, the fall of activity persists for hours. Since it was certain that the nerve required oxygen for the completion of chemical changes involved in activity (Gerard, 1927b) the existence of an "oxidizing reserve," sufficient to supply its needs during this time, was hypothesized (Gerard, 1927a, b). The chemical behavior of nerve during asphyxiation (Gerard and Meyerhof, 1927) has lent support to this view and it has been possible to construct a series of equations fairly accounting for the known changes of activity, equilibration, and asphyxiation of nerve (Gerard, 1927c).

Of considerable interest from this viewpoint is the question of just how the gradual decrease in activity during asphyxia is brought about. Since the total response to a tetanus is recorded, there appear three ways, not mutually exclusive, in which this response might be gradually lessened.

1 Reported at the XIII International Physiological Congress, Boston, 1929.
1. Individual fibres might become blocked at different times; 2, the refractory period might progressively lengthen so that an increasing number of the stimuli fail to evoke responses, and 3, the single responses of individual fibres might be gradually depressed—as in the early stages of narcotic action. It was the prime purpose of this research to assign a quantitative value to each of these factors, and the results obtained demonstrate that all play a rôle. The appearance of other effects, however, has rendered an exact numerical statement rather dubious.

METHODS.  

a. Nerves. Sciatic nerves of the grass frog and bull frog taken at all seasons, and the unbranched peroneal nerve of the dog have been used. The nerves were rapidly dissected, with the usual precautions against stretch or other mechanical injury, tissue tags or blood clots, and with branches cut far from the main trunk. The isolated nerve was at once laid on electrodes in a moist chamber, and although only moistened with Ringer's solution (unbuffered) sufficiently to insure good contact, it remained moist and usually in excellent condition for 24 hours or longer. Experiments were made at room temperature, which varied at different times between 19° and 28°C.

b. Chamber. For the bull frog and dog nerves a large vulcanite chamber
with five compartments (fig. 1) was used; for grass frog nerves a smaller one with three. Ringer soaked filter paper covered the floor of each compartment and gas inlet and outlet tubes were supplied to each. The dividing partitions were only \( \frac{3}{4} \) mm. thick at the center and were here each cut by a vertical slit half way down, to accommodate the nerve. When the nerve was in place, the slits adjoining the middle compartment were sealed with vaseline or a mixture of vaseline and kaolin and the whole covered by a glass plate on a vaseline seal. A minimum of material was used about the nerve in closing the partitions since a mass of vaseline covering several millimeters length of nerve would cause partial asphyxia of the center of such a stretch; and it was not entirely simple to make the seal airtight. Careful blotting of the nerve before applying the vaseline was necessary, and manipulation was continued in all cases until the compartment would maintain a pressure of 50 mm. or more measured on a water manometer. Compartments 1 and 2 and 4 and 5 were usually left in communication. The whole chamber was thinly coated with paraffin to insure against electrical leaks.

In compartment 1 were two stimulating electrodes \( A \) and lead 1, in the second compartment lead 2, in the third leads 3 and 4, in the fourth lead 5 and an adjacent electrode which, with 5, could be used as stimulating electrodes \( B \), and in the fifth compartment lead 6 and lead 7 from the injured end. Action currents were led from 1-6 by a dial switch and directly from 7. The nerves lay ordinarily with their central end on stimulating electrodes \( A \) and were crushed 2 to 3 mm. above lead 7. All electrodes were of thick silver wire and, whether used with a condenser in circuit or not, were freshly coated with silver chloride for each experiment. A wire with small silver plates soldered at appropriate intervals to fit down between electrodes was very convenient for this coating. No electrode was placed within 15 mm. of another nor within 9 mm. of a partition. The total length of nerve required was over 12 cm.

For the grass frog nerves, the small chamber with three compartments was used. The electrode arrangement was similar but only four leads were taken from the side of the nerve, one in each of the end compartments and two in the center one.

c. Gases. Moist oxygen was kept bubbling slowly through all compartments except when replaced in the center one by nitrogen. It was led in through the outside tube of the end compartments and out through the second and fourth, thus insuring a steady stream of oxygen towards but not into the central compartment. A separate tube led oxygen into the center compartment and a simple adjustment allowed the substitution of nitrogen for oxygen, or the reverse. The outlet dipped into several millimeters of water to prevent back diffusion.

Nitrogen obtained from the Air Reduction Co. containing not more
than 0.3 per cent of oxygen was used. This was regularly further purified by slow passage over heated copper gauze, though experiments without this extra precaution showed no different results. The gas was led through glass tubing with a minimal amount of rubber pressure tubing at the joints, and through several wash bottles to moisten and cool it. The rate of bubbling was kept approximately constant (one bubble every few seconds), as gross changes in gas flow caused variations in action potentials.

d. Stimulation. Tetanizing stimuli delivered by a Harvard coil, even when the buzzer contacts were of platinum, were not sufficiently regular to give uniform responses. Entirely satisfactory results were obtained with steel hack-saw blades vibrating a point into a mercury pool, and under the control of an electro-magnet in the interrupted circuit. (Bernstein interrupter). Over a wide range of adjustment of the contact (such as raising or lowering the point) between point and mercury, constant responses were obtained. The primary of the induction coil was in this circuit, with a two volt storage battery, and the vibrator was kept going through the whole experiment. The nerve was stimulated by closing a two pole key on the leads from the secondary coil. The secondary at 12 to 13 cm. and horizontal usually supplied slightly supramaximal stimuli.

It is necessary to connect a condenser across the point and the mercury into which it vibrates, to prevent sparking and consequent irregularities and oxidation of the mercury. In the past a 10 M.F. condenser has been used, but it was found that the stimulating effect of the secondary shocks is profoundly influenced by the size of the condenser across the primary interrupter. Table 1 shows this effect on one preparation, everything being maintained constant except the condenser across the spark gap. Maximum action potentials were obtained with a 0.2 to 0.5 M.F. condenser, but some sparking appeared when the capacity was reduced below 3 M.F. and became quite marked with only 0.5 M.F. As a final compromise, a 2 M.F. condenser was used, which gave a very faint spark and required only a slight increase in shock strength to give maximal responses.

<table>
<thead>
<tr>
<th>CAPACITY</th>
<th>ACTION POTENTIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>µF.</td>
<td>mm.</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>117</td>
</tr>
<tr>
<td>1</td>
<td>126</td>
</tr>
<tr>
<td>0.5</td>
<td>135</td>
</tr>
<tr>
<td>0.2</td>
<td>137</td>
</tr>
<tr>
<td>0.1</td>
<td>95</td>
</tr>
<tr>
<td>0.05</td>
<td>76</td>
</tr>
</tbody>
</table>
Two such systems were used, one giving 304 (make and break) shocks per second, the other 88. The nerve was tetanized for a second every 30 seconds, this regular repetition being usually rigidly adhered to so as to avoid equilibration effects (Gerard, 1927a, b). Starting with fast tetanization, action potentials were led successively from electrodes 1 to 6, and then again with slow tetanization, so that an observation at one frequency from one lead was repeated every six minutes. The regularity of response when this schedule was adhered to and their scatter when it was violated attest its importance.

e. Recording system. For studying the integrated response of a nerve to a rapid succession of stimuli, a slow recording instrument has many advantages; and in these experiments a sensitive moving coil galvanometer (1 mm. = $10^{-10}$ amp. at 5 meters, period 3 seconds) was used throughout, its deflections being read directly from a scale. In some experiments the injury potentials from the nerve were balanced in the usual fashion, but this involved a readjustment of the balancing potential at each electrode, and temporary polarizations, and in most experiments a 10 M.F. condenser was inserted in the circuit (fig. 1). The galvanometer was shorted when the switch was turned from one electrode to another, while the condenser charged to the new injury potential, (if not shorted the deflection gives a measure of the resting potential between the two electrodes) and the short was then opened, leaving the galvanometer on "open circuit" through the nerve and the condenser of "infinite" resistance. Any change of potential in the system, as when one lead becomes less positive due to activity of the nerve, causes the condenser to charge or discharge and send a momentary current through the galvanometer, which is read ballistically. Since a 10 M.F. capacity is slow in changing its charge, especially through a high nerve resistance, and the galvanometer slow in responding, rapid oscillations of potential will be smoothed out and a deflection result which directly measures the average potential reached. This insures an integration of

* The effectiveness of any quantity of current in producing a deflection is greater the earlier in the deflection period it passes through the galvanometer. Here the greatest current flows early in the deflection period (condenser changes logarithmically) and it is most effective at this time. Through 50,000 $\omega$, a 10 m.F. condenser requires 1.5 seconds to reach 95 per cent of its full charge, through 10,000 $\omega$ only 0.3 second. This means that for a longitudinal resistance of the nerve of 10,000 $\omega$, 95 per cent of the total current will have affected the galvanometer, for a 50,000 $\omega$ resistance only about 60 per cent.

In a series of experiments to directly test the system, a constant quantity of electricity was discharged through the galvanometer from a variable capacity through a variable resistance. With 0.1 or 1.0 m.F. the deflection was independent of the series resistance between 10,000 and 100,000 $\omega$. With a 10 m.F. capacity the deflection became less as the resistance rose above 10,000 $\omega$, but even with 50,000 $\omega$ deflections were nearly 80 per cent of the maximum, instead of 60 per cent. When
all potential changes during the ballistic "utilization period" of the galvanometer. Thus ten nerve impulses early in this period would give ten times the deflection of one, a prolonged action potential more than a short one, etc. The use of the condenser makes balancing the injury potential unnecessary, minimizes polarization, and practically eliminates changes in the longitudinal resistance of the nerve as a factor determining the magnitude of the recorded responses. Numerous controls of the reliability of such a system have been made, and it may be pointed out that the results obtained are alike whether the balanced circuit or the condenser is used.

f. Method of analysis. As long as the response at lead 5 or 6 to stimulation at A remains constant, the total number of active fibers and the impulses they carry are not changing; as it falls the ratio of any particular response to the initial ones represents the percent of fibre-impulses still arriving. The comparison can be made more accurate and any gradual failure of the nerve allowed for if the response at lead 6 to A stimulation (above the asphyxiated stretch) is compared to the same for B stimulation (below the asphyxiated stretch). A fall of fibre-impulse value means either blocked fibres or a prolonged refractory phase in the nitrogen stretch with fewer impulses per fibre passing through. This second factor is studied with the aid of fast and slow tetanization. A lengthening refractory period must cause a decreased response to stimuli at 3.3 σ intervals earlier than to those at 11.4 σ intervals.

Changes in the average size and form of the action potential for a single impulse in a single fibre can be estimated by comparing the responses at leads 3 and 4, in nitrogen, with those at 5 and 6 below the asphyxiated stretch. A decreased response in the exposed region with no change or a smaller decrease in the control region below must signify depression of conduction in the exposed region. Such a decreased response means a decrease in the area of the time-potential curve of activity, since it is the integrated potential change that is measured, and the decrease may be in magnitude or duration of the action potentials. An increased response would similarly represent a higher potential or a greater duration of an unaltered potential. Again, a comparison of the responses to rapid and
slow tetanization gives a clue as to which effect is present. An increased potential with no change in time should increase the fast and slow responses proportionately. An increased duration should increase the slow responses relatively more than the fast, unless the “tails” of the action potentials are completely additive (Amberson and Downing, 1929, find only partial addition), since a longer portion of each persistent potential will come to expression before smothered in the next action wave. The decrease of a positive after-effect would, of course, act similarly to an increased duration of negative potential. Reference to figure III may make the matter clearer. The methods and interpretations applied in studying the effect on nerve heat production of changing frequency of excitation (Gerard, Hill and Zotterman, 1927) are also of interest in this connection. From those results it is clear that the intensity of the nerve’s response to a stimulus is highly dependent on the time elapsed since its previous response. A prolongation of refractory period and a decreased impulse response in nitrogen might be, from this viewpoint, hardly more than two expressions of the same change.

RESULTS. 1. Initial values. It is of some interest to compare the responses at different electrodes to different stimuli at varying times and for several types of nerve.

In general, the green frog nerves give deflections over twice as great as those of bull frogs or dogs, table 2. It seems probable that the higher values with the small frogs result from a thinner and less resistant perineurium, though different sized fibres may be a factor. A similar but more marked difference in the two kinds of nerves is shown in the response to oxygen after asphyxia, when the rise in action potential of green frog nerve may be several times that of the bull frog nerve.

The lead near the crushed end (15 mm. away) is regularly depressed to \( \frac{2}{3} \) or \( \frac{1}{2} \) the values of the others (see Davis and Brunswick, 1927) which are all of the same general magnitude. Since the nerve resistances between leads 1 and 7, and leads 5 and 7 are widely different but the galvanometer response to activity is approximately the same for each pair, this affords further evidence that, with the condenser circuit, the deflection directly follows the potential change and is largely independent of nerve resistance.

Although the responses at leads away from a crushed end are roughly
alike, in any one experiment the responses at individual leads often show wide variation. To a certain extent this was found to depend on the special relation of the various branches to the leads. For example, the main thigh branches leave the bull frog nerve between electrodes 2 and 3, and electrode 2 usually gives lower values. Still, variation of responses is found along the dog's peroneal in the absence of branches, and local condi-

<table>
<thead>
<tr>
<th>DATE</th>
<th>NERVE</th>
<th>TEMPERATURE °C.</th>
<th>RESPONSE IN ARBITRARY UNITS</th>
<th>Fast tetanization Lead</th>
<th>Slow tetanization Lead</th>
</tr>
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<tbody>
<tr>
<td>1928-49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 9</td>
<td>Green frog 26 1/2</td>
<td>(a) 130 105 105 75</td>
<td>50 40 45 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) 105 90 90 58</td>
<td>45 45 45 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>December 6</td>
<td>Bull-frog 22 1</td>
<td>50 50 50 45 55</td>
<td>45 40 45 35</td>
<td></td>
<td></td>
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<tr>
<td>December 28</td>
<td>Green frog 24 1</td>
<td>240 220 270 110</td>
<td>50 50 60 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 25</td>
<td>Bull-frog 23 2</td>
<td>27 33 35 48 54</td>
<td>8 11 11 22 22 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 20</td>
<td>Bull-frog 22 2</td>
<td>35 30 26 28 35 24</td>
<td>4 23 24 24 37 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 26</td>
<td>Bull-frog 26 1/2</td>
<td>72 39 65 70 71 10</td>
<td>4 64 42 65 72 65 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 18</td>
<td>Dog 25 2</td>
<td>42 41 34 37 28 26</td>
<td>5 12 10 10 9 11 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 21</td>
<td>Dog 27 4</td>
<td>24 23 24 23 27 22</td>
<td>1 12 10 10 9 11 7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* (a) Stimulation repeated every 20 seconds; (b) stimulation every 30 seconds. Note equilibration effect of frequent repetition is more marked for rapid than for slow tetanization. Other values in table are for repetition at 30 second intervals.

These irregularities make the quantitative evaluation of changes somewhat arbitrary. If electrode 2 gives an initial response of 55 mm. and 3 of 40 mm., and under new conditions they give respectively 110 and 90, which has shown the greater change? The absolute increase of 2 is more than of
3, the percentage increase of 3 is greater than 2. In general the latter is the more satisfactory measure and is the one used here; but if, in the above example, lead 3 is 15 mm. less than 2 by virtue of some local accident and the axone activity is alike under each, the percentage change may give an unfair comparison.

A further interesting variation appears when the responses at one electrode to fast and to slow tetanization are compared. The ratio $\frac{F}{S}$ (fast response) is roughly constant through the series at 2.5–3.0, but may differ irregularly from 2 to 3.5 at different leads from the same nerve at about the same time. It is worth noting that though the ratio of the frequencies used is 3.5, the ratio of responses is definitely less, sometimes only half so great. This figure, like that of Gerard, Hill and Zotterman (1927) ($\frac{F}{S} = 1.6$ for $\frac{300}{90}$) shows that at the higher frequency the nerve has not been able to recover so fully between responses as at the lower one or that considerable potential normally persists for more than 30 after an action wave. Probably both effects occur. The higher temperature at which the present series was carried out accounts for the higher $F/S$ ratio.

The responses for any electrode and stimulus change in the course of time (table 2). Usually all show a regular decrease, rapid at first and then very slow, but not infrequently the values at some electrodes fall and at others rise during the same time. The $F/S$ ratio also tends to fall slowly with advancing age of the preparation.

The rapid early fall of responses is not due to dying of the nerve but rather the reverse, since it depends on the "crush sealing over" and allowing action potentials to reach lead 7 and so partly cancel responses at the upper electrode. A fresh crush or scald proximal but not distal to the original one restores the initial values, and when these again fall a third injury again does so. This point is of some importance in connection with the changes produced during asphyxia. The slow fall, between 1 or 2 hours and 24 or more, seems to be a true dying of the severed nerve cells. This fall is seldom beyond half of the early values and many experiments have shown no fall over night, or even an occasional rise. Even with a fall in action potentials of nearly half, the threshold of excitation has often shown no increase.

The responses at lead 6 for stimulation at A and at B are alike, or are a little less for A stimulation than for B, indicating some blocked fibres between these two points. The usual agreement between these values, even with supramaximal stimulation, indicates also that shock escape is not affecting the galvanometer. This is further certified by two regular observations. 1. As the shock strength is increased steadily from minimal
to strongly supramaximal, the observed deflections at each lead rise rapidly to a maximum, which is reached at all at the same stimulus strength, and are increased not at all or slightly (probably due to sympathetic fibres) with further increase in strength of shock. 2. When a stretch of nerve is blocked by asphyxia (which does not destroy its structure as does a crush) no deflections are obtained from leads below it.

For the other leads, comparison of the responses to tetanization at A and at B shows some interesting variations. When a stimulus is applied to a nerve between the intact and crushed end leads, as for stimulation at B and leads from 1 to 4 and 7, the situation becomes complicated by shock escape. A slow galvanometer leading from two uninjured points of an unbranched nerve should give no response when a region between the leads is rapidly tetanized with make and break shocks, since action currents would be diphasic and the induced currents are equal and opposite. Actually, however, a unidirectional current does flow and the galvanometer deflects, the direction reversing when the stimulating electrodes are reversed. This is the Fleischl effect, observed over half a century ago (1878) and rarely considered since. (See, however, Ebbeke 1922.) It indicates rectification due to unequal polarization in the two directions of flow, probably related to the different forms of make and break currents; and recalls the rectification observed with unequal or dirty electrodes (Hill, 1913).

When the action currents are not fully diphasic, due to injury at one end of the nerve, they produce a given deflection to which the effect of the stimulating current adds or subtracts. Both values are simply determined by taking deflections with the secondary leads first in one direction and then reversed. Halving the difference of the two deflections gives the stimulating current, and the true action current is given by their average. The same discussion applies to potentials when recorded with a condenser in circuit. Ordinarily the Fleischl effect is not over one-fourth of the action potential. Since it is dependent on polarization it affords some measure of the state of the cell membrane, and, as one might anticipate, decreases as the nerve dies. It is also decreased, reversibly, during asphyxia. Ebbeke (1922) reports a decrease of the Fleischl effect in nerve as a result of stimulation with strong shocks and, especially, on tetanization, which he interprets as indicating lowered membrane resistance and polarizability. This sensitiveness to the state of the nerve, incidentally, affords evidence that the effect is a biological one and not an electrode artifact. The nerve sheath may, of course, contribute to the polarization.

Another factor modifying responses to stimulation at B is the branching of the nerves. At A all fibres are stimulated and, although groups successively branch off from the main trunk, all fibres in contact with any electrode become active. At B only the longer fibres are excited, those
having branched off above this level remaining inactive and serving as shunts across the active ones (see Wade, 1924; Osterhout and Harris, 1929). The response at lead 4 is consequently slightly more than 3, with very few fibres leaving between them, and responses are similar for leads 2 and 1. Between 2 and 3, however, the large branches emerge and the anticipated sharp drop in galvanometer response is observed on passing from 3 to 2.

For stimulation at either A or B action potentials can be obtained from two leads on normal nerve with no crush between them. The magnitude is greatest when large branches lie between the two leads and the effect probably depends on this branching. Table 3 contains the results of one such experiment and its analysis is given to illustrate several of the points so far discussed.

<table>
<thead>
<tr>
<th>LEADS (LOWER ONE CONSIDERED CONSTANT)</th>
<th>RESPONSE TO STIMULATION AT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>1-2</td>
<td>-25</td>
</tr>
<tr>
<td>2-3</td>
<td>+8</td>
</tr>
<tr>
<td>3-4</td>
<td>-4</td>
</tr>
<tr>
<td>4-5</td>
<td>-7</td>
</tr>
<tr>
<td>5-6</td>
<td>-11</td>
</tr>
<tr>
<td>6-7</td>
<td>-11</td>
</tr>
<tr>
<td>1-7</td>
<td></td>
</tr>
<tr>
<td>sum (1-7)</td>
<td>-60</td>
</tr>
</tbody>
</table>

See text for explanation.

It will be noted, table 3, that the action potentials between any two electrodes are about the same for stimulation at A or at B, except when electrode 2 is one of the leads. It is just below 2 that the large thigh branch leaves the main trunk. Aside from the small action potentials, due possibly to differences in the nerve sheath at various points and independent of the point of stimulation, the following factors account for the findings.

With stimulation at A all fibres are excited. At lead 1 full sized potentials develop in each; at 2, because of proximity to an injury (the cut branch), potentials are much depressed in many fibres and the average change is less than for 1. Lead 1 therefore becomes negative to 2. At lead 3 those fibres which branched at 2 are out of the picture, leaving mainly fibres carrying full sized potentials—so this also shows a greater negativity than 2. Potentials at 5 are greater than at 6, near the crush, and at 6 greater than at 7, beyond the crush.

At B only the long trunk fibres are stimulated. Since there are no sig-
significant branches below lead 3, potentials between leads 3, 4, (5), 6 and 7 are the same as for stimulation at A. At 2 and 1 there are present a large number of inactive fibres that leave in the branch below 2, and which act as shunts across the active fibres. The change at 3 is therefore greater than at 2. Since the same number of uninjured fibres are active at 2 and 1 and are shunted by the same number of inactive ones, there appears no significant potential difference between them.

It may be well to point out here that, with the usual connections of the galvanometer to a distal lead from the injured end and a proximal one from an uninjured portion, the deflection obtained on tetanization indicates a negative change of the proximal lead relative to the distal one. This is called throughout a negative deflection. A positive deflection indicates the reverse. No deflection, of course, means either that no potential change occurs under either lead or the same one under both. Since ordinarily complete absence of response at a crushed end is not attained, a zero deflection via leads from an injured end and intact side is not necessarily a critical value. This is amply illustrated by the records during asphyxiation of nerve.

With the condenser circuit described, a continuous regular tetanization leads to a new average potential on the condenser. The galvanometer gives a ballistic negative throw at the start and then returns to rest as continued tetanization maintains the new potential. On cessation of stimulation, if the nerve potential returns to its initial value, the galvanometer should give a positive deflection equal to the original negative one. It was observed from the start of these experiments, some years ago (see Gerard, 1927a), that the positive deflection was regularly greater than the negative one, often by more than 50 per cent. Control tests with constant or induced potentials through nerve or metal circuits have demonstrated the entire symmetry of the recording system—deflections in both directions are equal on closing or opening or reversing the direction of an applied potential. It seems to follow, then, that the fall of potential of the active electrode at the initiation of activity is less than the rise at cessation. Either there must be, following the initial sharp negativity, with continued stimulation a gradual further increase of negativity occurring too slowly to affect the galvanometer but leaving a greater negativity to rise from at the end of stimulation; or if the negative action potential is maintained constant there must be a positive after-effect on cessation.

A somewhat more direct demonstration of the positive deflection is obtained by short-circuiting the galvanometer during the tetanization so that it remains at rest. If the short circuit is then opened at once (by hand), after stopping tetanization, a positive deflection is obtained larger than the negative one previously determined. If an interval of about a second is allowed to elapse between the stop of tetanization and opening
the short circuit, little or no deflection is obtained. In all probability a marked and cumulative positive after-effect is being recorded. Such positive after-effects have long been known, and recently Gerard and Forbes (1928) have found them often present in the cat's peroneal. Amberson and Downing (1929) studying single impulses with a Downing galvanometer have offered entirely different evidence of their rather regular appearance in frog's nerves. This positive after-effect disappears considerably before the negative action potential does when a nerve is asphyxiated and returns after it on the readmission of oxygen. Table 3a.

It remains to discuss the changes in total action potential on repetition. Gerard (1927a) found the electric response of nerve to a given tetanization (280 shocks per second for 20 seconds) to become greater as the periods of tetanization were repeated at shorter intervals (10 minute intervals to 2 minute intervals). The thermal responses became less as the action potentials rose, and the rise of total action potential was interpreted as due to prolongation rather than increased magnitude of the negative potentials, and thus akin to fatigue. With prolonged tetanization the total action potentials fell, and in fact fell parallel with the fall in oxygen consumption per impulse under similar conditions (Gerard 1927b). This was shown by Gerard and Forbes (1928) (see also Forbes and Rice, 1929) to be due to decreased action potential per impulse and increased refractory period with fewer impulses per second. Decrease in positive and negative after-potentials resulted from slight repetition. Amberson and Downing (1929)

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>DEFLECTION AT</th>
<th>RATIO STOP START</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start of tetanus</td>
<td>Stop of tetanus</td>
</tr>
<tr>
<td>Before $N_2$</td>
<td>-135</td>
<td>-120</td>
</tr>
<tr>
<td>25 minutes in $N_2$</td>
<td>-79</td>
<td>-66</td>
</tr>
<tr>
<td>35 minutes in $N_2$</td>
<td>-28</td>
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</tr>
<tr>
<td>40 minutes in $N_2$</td>
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<td>-7</td>
</tr>
<tr>
<td>45 minutes in $N_2$</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>After $O_2$ 4 minutes</td>
<td>-315</td>
<td>-315</td>
</tr>
<tr>
<td>7 minutes</td>
<td>-335</td>
<td>-335</td>
</tr>
<tr>
<td>22 minutes</td>
<td>-205</td>
<td>-205</td>
</tr>
</tbody>
</table>

The fall of the ratio below 1.0 late in asphyxia indicates that fatigue is gradually reducing action potentials between the start and stop of the tetanization. A similar rapid fatigue was found by Fillie (1906) at the beginning of recovery following a period of asphyxia.
have found marked and long delayed positive and negative potentials which appear only on repetition of activity.

It is apparent from the foregoing that the total action potential is affected by several factors which change with the state of activity or equilibration of the nerve and it may therefore either increase or decrease as one or another predominates. The rise in total potential during equilibration may thus be correlated with increase of the delayed elements and the subsequent fall with decrease of the initial change (and secondarily of the delayed). These effects, as will be seen, are rather strikingly similar to those observed in the course of asphyxia. In the present experiments the usual definite rise of total action potential was noted when regular stimulation was begun after a period of rest in oxygen, but during and after asphyxia the situation was often reversed (see note to table 3a). It was also regularly observed that the rise was relatively greater with rapid tetanization than with slow, as would be anticipated if the effect is an equilibration to a new level of activity. (See table 2, data of Aug. 9.)

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>RESPONSE TO STIMULATION AT</th>
<th>RATIO A/B</th>
<th>PER CENT OF FIBRES BLOCKED</th>
<th>PER CENT OF INITIALLY ACTIVE FIBRES BLOCKED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours in O₂</td>
<td>43</td>
<td>48</td>
<td>0.90</td>
<td>10</td>
</tr>
<tr>
<td>15 minutes in N₂</td>
<td>41</td>
<td>47</td>
<td>0.87</td>
<td>13</td>
</tr>
<tr>
<td>30 minutes in N₂</td>
<td>37</td>
<td>47</td>
<td>0.79</td>
<td>21</td>
</tr>
<tr>
<td>45 minutes in N₂</td>
<td>21</td>
<td>46</td>
<td>0.46</td>
<td>54</td>
</tr>
<tr>
<td>60 minutes in N₂</td>
<td>0</td>
<td>42</td>
<td>0.00</td>
<td>100</td>
</tr>
</tbody>
</table>

2. Changes in and below an asphyxiated region during asphyxia. It is convenient to consider later the changes at leads 1 and 2 above the region of asphyxia, and discuss here the changes at leads 3 and 4 in nitrogen and 5 and 6 below it. The graphs of several complete experiments are shown in figures IV, V, VI.

Results with the unbranched dog's peroneal may be analyzed first. The per cent of fibres blocked in passing through the nitrogen stretch is obtained by the fall in the ratio of A/B responses at lead 6 (see table 4). Thus in one experiment there were 3 per cent of the active fibres blocked after 15 minutes in nitrogen, 13 per cent in 30 minutes, 49 per cent in 40 minutes, and 100 per cent in one hour. At electrodes 3 or 4 at these times not more than the calculated percentage could be blocked but almost certainly less were. Failure of one fibre at any level will, of course, render it inactive distal to that point, and the longer the stretch of asphyxiating nerve above a given electrode the more the chance of such a critical point
being included. Electrode 6 (and 5) records impulses from A that have successfully traversed a 32 mm. stretch while 4 records impulses after 23 mm., and 3 after 9 mm. of travel in the nitrogen partition. Responses at

4 always fall faster and fail before those at 3, and those at 6 presumably do so faster than at 4. It is not safe to extrapolate back from 6 to 3 and 4, assuming a linear falling off with distance in nitrogen, since oxygen may
RESPONSE OF NERVE TO OXYGEN LACK

FIG. VI
Bull Frog sciatic 23°C
A stimulation
Fast
Slow
1
2
3
4
5
6
*Electrodes in Nitrogen

Time in minutes

Fig. VI

FIG. VI-A
Green Frog 28°C

Washed with
Oefree Ringer's solution

Time in hours

Fig. VIa
diffuse in from either end for an undetermined distance; but such a calculation would indicate that at \( \frac{1}{2} \) hour, when half the fibres were blocked at 6, 35 per cent were blocked at 4, and 15 per cent at 3. Since oxygen diffusion from each side and the positions of electrodes 3 and 4 are symmetrical to the center of this stretch, though the value calculated for either one may be wrong (too high for 3 and too low for 4), their average must be essentially correct. At three quarters of an hour in nitrogen, then, the average of fibres blocked at 3 and 4 was 25 per cent. At this time, however, responses at 3 had fallen 58 per cent (from 50 mm. to 21 mm.) and at 4, 68 per cent (from 60 to 19-), with an average fall of 63 per cent. It appears, therefore, that 75 per cent of the fibres were able to yield at this time only 37 per cent of the original response, or that the action potential per impulse per fibre had been reduced to half.

In the last paragraph the possibility of decremental conduction has not been considered, nor the influence of prolongation of the refractory period discussed. The weight of recent evidence (Kato, 1926; Rice and Davis, 1928) disinclines one from invoking decremental conduction, though these results and other effects are subject to such an interpretation. An increased absolutely refractory phase, barring decrement, should decrease responses at lead 3 and beyond to the same degree rather than progressively more with more distal leads, as in the case of fibre block. This would modify the above calculations in the direction of a smaller decrease per fibre impulse, but even if the fall at 6 be assumed to be entirely due to increased refractory period, which would not account for 4 falling more rapidly than 3, the fibre impulse value in nitrogen would have fallen to less than three-fourths its value in oxygen. The further experimental analysis is made by comparing the responses to fast and slow tetanization.

When responses at 6 to fast tetanization fall sooner or further than to slow tetanization, an increase of the absolutely refractory period of the region in nitrogen is indicated. Increase of the relatively refractory period in the nitrogen stretch would, of course, not affect individual responses beyond it, so a fall in F/S at 6 directly follows a rise in absolutely refractory period in the asphyxiated region. In the exposed stretch, however, since the intensity of the propagated disturbance is decreased during the relatively refractory period, an increase in this period would serve to decrease the fiber-impulse value, and for fast tetanization more than for slow. Table 5 gives the results of an experiment with a dog’s peroneal nerve. It will be seen that the average F/S at 3 and 4 fell from 2.4 to 1.5 during 20 minutes in nitrogen, while that at 5 and 6 fell from 2.7 to 1.5. The absolutely refractory period has increased sufficiently to block 40 per cent of the rapidly arriving impulses. Since the fall of the ratio at the electrodes in nitrogen is no greater than at those below, there is no evidence in this experiment for depression of the fiber-impulse value in nitrogen due
RESPONSE OF NERVE TO OXYGEN LACK 515
to impulses travelling in the less recovered part of the relatively refractory period. Yet the fibre impulse value at 4 had fallen to less than 50 per cent its initial value at 20 minutes.

The 40 per cent more rapid fall of total response to impulses at 3.3 \( \sigma \) intervals than to impulses at 11.2 \( \sigma \) cannot be taken to mean that the absolutely refractory period has increased to about 6 \( \sigma \), and that none of the impulses at 11 \( \sigma \) intervals have been lost. The individual fibres are not all alike and evidence will be presented that they fail in nitrogen in groups, so the 40 per cent extra loss for fast stimulation is a purely statistical one due, possibly, to considerable prolongation of refractory period in some fibres and little in others. Even the responses to the slow rate of excitation may, therefore, have suffered some from prolonged refractory periods, though the effect would probably be but little.

An observation made several times in these experiments further substantiates the above discussion. The response at an electrode in the nitrogen region to fast stimulation falls more rapidly than to slow and the curves may cross near the end of the period, so that the nerve gives no response to fast tetanization while still responding to slow. This Wedensky effect indicates that the oncoming impulse in normal nerve is just about able to excite the asphyxiated stretch when both are at "rest." At intervals of 11 \( \sigma \) the normal stretch will just complete its relatively refractory phase between impulses and a full sized disturbance follow each stimulus; at intervals of 3.3 \( \sigma \) the nerve, early in its relatively refractory phase, transmits subnormal impulses which are insufficient to excite the asphyxiated portion with its increased threshold. A further factor would operate when an increased interval between stimuli allows the oncoming impulses in the normal nerve to attain the threshold intensity for exciting the asphyxiated stretch. This latter has a prolonged total refractory period, or prolonged rise of threshold after conduction, so that after one response its threshold will still be high when the next one or several impulses reach

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>LEAD 3</th>
<th>LEAD 4</th>
<th>LEAD 5</th>
<th>LEAD 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>Slow</td>
<td>F/S</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>4 hours in ( O_2 )</td>
<td>24.5 10.5 2.3</td>
<td>23.5 9.5 2.4</td>
<td>27 11 2.5</td>
<td>22 7.5 2.9</td>
</tr>
<tr>
<td>10 minutes in ( N_2 )</td>
<td>15.5 7.0 2.3</td>
<td>11.0 5.0 2.2</td>
<td>25 9.5 2.6</td>
<td>19.5 7.0 2.8</td>
</tr>
<tr>
<td>15 minutes in ( N_2 )</td>
<td>10.5 5.5 1.9</td>
<td>5.0 3.0 1.7</td>
<td>13 7.5 1.9</td>
<td>11.5 5.5 2.1</td>
</tr>
<tr>
<td>20 minutes in ( N_2 )</td>
<td>5.0 3.0 1.7</td>
<td>1.5 1.0 (1.5)</td>
<td>3 2.0 1.5</td>
<td>2.5 1.5 1.7</td>
</tr>
<tr>
<td>30 minutes in ( N_2 )</td>
<td>1.0 0.5+</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

June 21. Dog peroneal. \( T = 27.5^\circ \). Animal under ether 3 hours before nerve removed.

Table 5

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it from the normal stretch, and they will be blocked. The fact mentioned above, that the F/S ratio below the region of asphyxia falls as much as in it, indicates that this complete blockage of impulses as they impinge on a high threshold region is the main result of prolongation of the absolutely and relatively refractory periods. Figure VII illustrates these points.3

It may be noted that by study of the conditions of block of a nerve it should be possible to evaluate quantitatively the separate factors, for conduction, of 1, the exciting strength of an active region upon an adjacent resting one, and 2, the excitability of the resting region. We hope to pursue this matter. The mechanism of cold block is probably also very similar to that here outlined; and Heinbecker's (1929) finding that the refractory period of each fibre type rises to about the same value before failure occurs is also in harmony with these considerations.

FIG. VII

To sum up the results so far, the data indicate that during asphyxia the intensity of a single impulse for a single fibre may be reduced to half, that the refractory period increases enough to cut out an average of one-third of the impulses arriving at 3.3 σ intervals, and that the ultimate complete failure is due to blocking of all the fibres. Different fibres block at different times, probably each one as the lowered exciting power of its own re-

3 It may be well to point out that the tests used for absolute refractoriness have depended on a response at a distance from the point stimulated, and therefore involve conduction. The absolutely refractory period, then, probably is not a measure of the time during which a nerve region is inexcitable but of the time during which activity of this region is unable to excite adjacent ones; i.e., the time of blocked conduction.
### TABLE 6

Bull frog sciatic, $T^\circ = 25^\circ$, condenser circuit. March 25

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours rest n $O_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 7+</td>
<td>3.7 33-</td>
<td>10 3.3</td>
<td>35+ 11-</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>$N_2$ in middle partition</td>
<td>26 7</td>
<td>3.7 30-</td>
<td>8 3.7</td>
<td>34 11</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>45 minutes</td>
<td>32 7</td>
<td>4.6 33-</td>
<td>9 3.7</td>
<td>40* 17*</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>80 minutes</td>
<td>44 10</td>
<td>4.4 40-</td>
<td>11 3.6</td>
<td>29 8</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>120 minutes</td>
<td>57 19</td>
<td>3.0 49-</td>
<td>16 3.1</td>
<td>18 6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>150 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 180 minutes $O_2$ admitted</td>
<td>78* 53</td>
<td>1.5 65*</td>
<td>48* 1.4</td>
<td>168* 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>41 19</td>
<td>2.2 37</td>
<td>16 2.3</td>
<td>89 57</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>150 minutes</td>
<td>31 10</td>
<td>3.1 29</td>
<td>9 2.3</td>
<td>50 21</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Values as fractions of initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum in $N_2$</td>
<td>2.1 2.6</td>
<td>1.5 1.4</td>
<td>1.15 1.6</td>
<td>1.35 1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hours in $N_2$</td>
<td>1.6 1.4</td>
<td>1.2 0.95</td>
<td>0.8 0.75</td>
<td>0.61 0.95</td>
<td>0.3 0.4</td>
<td>0.30 0.35</td>
</tr>
<tr>
<td>Maximum in $O_2$</td>
<td>2.9 7.2</td>
<td>2.04 8.4</td>
<td>4.8 13.0</td>
<td>2.0 4.5</td>
<td>0.95 1.0</td>
<td>0.80 0.75</td>
</tr>
<tr>
<td>2½ hours in $O_2$</td>
<td>1.15 1.4</td>
<td>0.9 0.8</td>
<td>1.4 2.0</td>
<td>1.2 1.7</td>
<td>0.9 0.9</td>
<td>0.80 0.7</td>
</tr>
</tbody>
</table>

* Indicates a maximum value.
response falls below its increased threshold. The relation of oxygen exclusion to these changes will be considered later.

The experiments with green-frog and bull-frog nerve have yielded similar results. Representative data are included in table 6. In most cases, responses at the lower electrode in nitrogen did not fall lower than those at the electrode below nitrogen but they approached zero together. In several instances, however, the response in nitrogen was depressed to as low as half that of a more distal electrode in oxygen. A lowered fibre-impulse response is, therefore, present in these nerves though to a less striking degree than in the case of the dog nerves.

In two experiments it was noted near the end of an asphyxial period that electrodes five and six still gave small negative responses while responses at four had passed through zero and were weakly positive. A few minutes later all responses were zero. Current escape appears to have been excluded, so the positive effect must have represented a positive action wave at four or a negative change at seven, leading from the "dead" end. The latter is probably the case. It has been pointed out that with activity of a nerve the negative change at the crushed end is rarely absent, actually it may be two-thirds as great as along the intact side (see later). If impulses in the fibres still active in nitrogen were depressed to less than half their normal intensity and returned to their usual value on entering the oxygen supplied stretch beyond, the negativity at lead seven would be greater than at lead four. Four would then appear to become positive until complete block brought all responses to zero.

In all experiments, the F/S ratio fell markedly before the end of asphyxia. For the three best, for example, the average ratio at the electrodes in nitrogen fell from 3.1 to 1.7 and at the distal electrodes in oxygen from 3.1 to 2.0.

The conditions toward the end of an asphyxial period have been discussed so far. In the earlier stages the situation is quite different. The responses in nitrogen show a marked increase at a time when the electrodes in oxygen below are still constant. The fibre-impulse total action potential in nitrogen has therefore increased in amplitude, duration or both. An increase in amplitude of potential, especially if due to changes of electrical impedance of the nerve, should involve the fast and slow responses proportionally and leave the F/S ratio unchanged. A prolongation of negative potential or decrease in positive after-effect should be more effective for the slow than the fast and cause the ratio to fall. The fall in F/S is quite marked during the rise of total responses and may reach an even lower value than later during asphyxia. This is evidence of a marked prolongation, or shift in the negative direction, of the action potential in nitrogen, though impedance changes may also be present. This is in harmony with the earlier finding (Gerard, 1927) that the total action
potential of a given nerve falls much less rapidly in nitrogen than does its heat production. Heinbecker (1929), using the cathode-ray oscillograph, finds no change in the shape of the action potential during asphyxia. He was not recording, however, delayed potential changes, where the greatest modification probably occurred. A similar discrepancy between the findings with the Braun tube (Heinbecker) and with a slow galvanometer (Amberson and Downing, 1929; Necheles and Gerard, 1929) in the case of carbon dioxide action on nerve also seems to depend on the delayed potential changes. The changed form of the electric disturbance in nitrogen makes it impossible to make accurate comparisons of refractory period effect in and below nitrogen. Since, however, any impulse emerging from the asphyxiated stretch must resume its normal form, the F/S values at leads 5 and 6 do give valid information as to refractoriness, as already discussed.

This early rise in nitrogen is usually but not invariably present. It is nearly always seen in the bull-frog nerve, often absent in the green-frog. When failure progresses rapidly it may be represented by only one or two readings. In previous experiments on R. esculenta (Gerard, 1927a) a prompt rise in nitrogen was attributed to a changed rate of stimulation, since it disappeared when stimulation was repeated at constant intervals. In this series the less abrupt rise has appeared despite this precaution.

The responses in nitrogen showed an average increase at their maximum to 1.55 times the initial value. This was reached soon after responses below the nitrogen stretch had begun to fall. That is, responses at 5 and 6 began to decrease just before those at 4 did, and those at 3 began to fall a little later. The form of these curves gives the impression that the rise of response due to prolongation of potentials is continuing but is cut into by rapidly appearing fibre block. Later, apparently, decreased amplitude of each action potential more than offsets its prolongation and the area of the potential-time curve is again diminished.

After responses at 4 began to fall they sometimes dropped very rapidly and largely by fibre block, as if some factor necessary to conduction reached a critical value. Usually, however, they fell more slowly and showed definite waves of decrease (fig. IV). Identical waves were seen in the fall of responses at 5 and 6 and less markedly at 3, so that fibres were apparently failing in groups. There were commonly two waves, sometimes three, probably representing a differential susceptibility of large and small fibres to asphyxia. Heinbecker (1929) similarly found that asphyxia blocked the fibres in groups, the smaller fibres failing before the larger.

The responses at various electrodes to stimulation at B, have been in harmony with the results for stimulation at A. For the latter, responses at 4 fall more rapidly during asphyxia than those at 3; for stimulation at B responses at 3 fall before those at 4. In either case responses at the ele-
trode reached by the impulses over a long asphyxiated stretch fall faster than at the nearer electrode. Any possible pre-existing polarity of the nerve, effect of branches, or distance between active and injured leads is excluded as a factor; and the effect must be due to fibre block at different points (or decrement), as previously discussed.

In all cases with B stimulation, as asphyxia proceeded, the responses at 4 and 3 in nitrogen, and 2 and 1 beyond it, fell to zero and continued to "fall" until strongly positive. This was not due to the Fleischl effect, since reversing the coil leads left the deflections in the positive direction. No local current spread was involved, the positive throw was equally great at electrodes 30 and 70 mm. away from the stimulated region. Arising at B, impulses pass in one direction to 6 and 7 (the crushed end) and in the other through the region of asphyxia to 2 and 1. A reduced response at 7 is regularly present and the normally greater response at 1 or 2 leads to negative action potentials from the two usual leads. But the progressive asphyxial block in no way affects the impulses travelling from B to 7, while decreasing to zero the impulses reaching 2 or 1. The crushed end thus becomes negative to the normal nerve beyond an asphyxiated stretch. The maximum positive deflection obtained measures the activity at 7; the total change from minus to plus, the normal activity at 2 or 1. This evidence, as that given elsewhere, shows that after some hours' standing, activity at 7 may be over two-thirds as great as at an uninjured point.

It has been assumed in the discussion so far that asphyxial block renders the responses fully monophasic. Even if the block by asphyxia did not completely stabilize the potential at electrodes beyond it, their potential changes would be less than previously appeared at the crushed end; the latter having undergone recovery changes for some hours. Reasons for the presence of activity beyond a crush have been indicated by Bishop, Erlanger and Gasser (1926). Essentially, the nerve beyond the crush serves as an inert lead from some point at the crush which has a potential intermediate between the positive surface and the negative core. Fall in potential of the surface must give a proportionate fall of all points along the potential gradient between surface and core, and hence a fall at the lead involved.

The conditions at a crush may be analyzed somewhat further. Figure VIIIa represents schematically the electrical situation along a stretch of nerve near a crush. Points on the surface of the membrane separated from each other by the low resistance, \( r \), are maintained at a potential above the core by batteries, giving potential \( e \), in parallel. There is a high resistance leak, \( R_c \), across the membrane. At the point of injury to the membrane this transverse resistance is greatly lowered to \( R' \). It is readily shown that current will then flow from points away from the injury along the surface.
to the injured point and back along the core, producing a potential drop along the nerve. The current flowing from any point will be less as its distance from the injured point, and so the longitudinal resistance, increases. The core-surface potential must, then, begin to decrease at some distance from the point of injury and become steadily less as the injured region is approached. If $R_1$ is zero then this potential must fall to zero at the crush (fig. VIIIb). The closer $R_1$ approaches $R$, the less the potential drop across it. When $R_1$ is not zero the conditions are those considered by Bishop, Erlanger and Gasser; that is, the nerve beyond the crush serves as a simple lead from some point at a potential, not zero, which is a constant...
fraction of \( e \). Since the membrane potential, \( e \), falls during activity, the lead from the crush also shows a smaller fall in potential. As pointed out, the action potentials at the injured end increase for some time after a crush is made and are again reduced by a fresh crush. At the moment of injury the cell membrane is destroyed, which reduces the transverse resistance, \( R_1 \), and the action potentials at the crush, possibly to a practical zero. Later the naked protoplasm has formed some “surface-interior” membrane (see Chambers, 1924); a definite transverse resistance is reestablished, and with it a potential change during activity (fig. V111c).
RESPONSE OF NERVE TO OXYGEN LACK

The nerve membrane potential falls markedly during oxygen deprivation (see later), probably due to lowering of the transverse resistance and to decrease of the potential developing reactions. Either factor would lead to a decline of potential on passing from normal to asphyxiated nerve. Whether the potential in the nitrogen stretch falls to zero is uncertain, but it apparently does fall to lower values than at an old crush.

It is worth noting that the above considerations account for the coexistence of depressed responses and heightened irritability near an injured region and as a result of catelectrotonus (Bishop and Erlanger, 1926), since a partially depolarized membrane would require less current to complete its depolarization and give less potential change on depolarization than a normally polarized one—other factors remaining unchanged.

### TABLE 8

<table>
<thead>
<tr>
<th>NERVE</th>
<th>DATE</th>
<th>TEMPERATURE</th>
<th>UPPER ELECTRODE IN N₂ TIME TO</th>
<th>LOWER ELECTRODE IN N₂ TIME TO</th>
<th>ELECTRODE BELOW N₂ TIME TO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maximum</td>
<td>1</td>
<td>Maximum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.</td>
<td>minutes</td>
<td>minutes</td>
<td>minutes</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td>25</td>
<td>40</td>
<td>35</td>
<td>67</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td>28</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Bull frog</td>
<td>December 6</td>
<td>22</td>
<td>108</td>
<td>87</td>
<td>235</td>
</tr>
<tr>
<td>Bull frog</td>
<td>April 20</td>
<td>22</td>
<td>87</td>
<td>68</td>
<td>156</td>
</tr>
<tr>
<td>Bull frog</td>
<td>March 25</td>
<td>23</td>
<td>78</td>
<td>80</td>
<td>123</td>
</tr>
<tr>
<td>Bull frog</td>
<td>April 26</td>
<td>26</td>
<td>56</td>
<td>51</td>
<td>95</td>
</tr>
<tr>
<td>Green frog</td>
<td>December 28</td>
<td>24</td>
<td>67</td>
<td>45</td>
<td>150</td>
</tr>
<tr>
<td>Green frog</td>
<td>August 11</td>
<td>26</td>
<td>55</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>Green frog</td>
<td>August 7</td>
<td>28</td>
<td>20</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Green frog</td>
<td>July 14</td>
<td>27</td>
<td>34</td>
<td>28</td>
<td>35</td>
</tr>
</tbody>
</table>

### 3. Time course in N₂.

The time required for a nerve to fail in N₂ varies with the species of nerve and the temperature during the asphyxia (table 8), also with the amount of activity. For each kind of nerve asphyxia proceeds more rapidly at higher temperatures and, though the data do not justify the calculation of a temperature coefficient, it would appear to be over 2. At a given temperature dog nerve asphyxiates most rapidly, green frog nerve definitely slower, and bull frog nerve considerably more slowly. The ability of a nerve to withstand oxygen lack depends in large part on the amount of oxidizing reserve it possesses and the rate at which this is used up. A higher temperature causes a more rapid utilization (the temperature coefficient of oxygen consumption is over 2.0) and hence more rapid asphyxia. The sciatic of a rabbit consumes oxygen almost twice as rapidly as a grass frog sciatic at the same temperature (Gerard, unpublished), and presumably the dog nerve would resemble the rabbit's.
The bull frog sciatic consumes only half as much oxygen as the green frog nerve (Gerard, unpublished). The above observations are, therefore, what would be expected on the basis of differences in the rate of utilizing a reserve; though, of course, differences in the initial reserve may exist. The present data are not sufficient to confirm or refute Gottschalk's (1919) claim that nerves from cooled frogs have a greater oxygen store than from warmed ones, but his results are equally susceptible to interpretation on the basis of differences in metabolic rate.

It may also be pointed out that, given a sufficient time for recovery in oxygen following a complete asphyxiation, a second asphyxiation progresses hardly more rapidly than the first. After short periods of recovery in oxygen, the second asphyxiation was found by Fillie (1908) (and more fully studied by Gottschalk, 1914, 1919) to progress more rapidly. Since a nerve takes up an excess of oxygen for an hour or more after an asphyxial period (Gerard, 1927b) it appears that the oxidizing reserve is entirely exhausted during complete asphyxia, is slowly regenerated in oxygen and when fully restored permits the nerve to withstand the same duration of oxygen lack as at first. The exhaustion of such a reserve must, then, be the critical factor in asphyxia. The higher the metabolic rate and the lower the reserve of each fibre or group the sooner does it asphyxiate. If the store of oxidizer is a function of the volume of a fibre, and the energy used in conduction a function of its surface, the smaller fibres should asphyxiate before the larger ones, as Heinbecker (1929) finds to be the case.

4. Changes in and below an asphyxiated stretch following readmission of oxygen. At the end of a sufficiently long period of asphyxia, responses to stimulation at the electrodes in and below nitrogen have fallen to zero. On now admitting oxygen to the nitrogen partition, there was a rapid and spectacular return of action-potentials at these electrodes to high values. Activity appeared in about a minute at all electrodes. At those leading from the nitrogen stretch, responses increased to a maximum in 5 to 10 minutes, usually, and then declined, rapidly at first and then more slowly, along a roughly exponential curve. With either A or B stimulation, that electrode in the nitrogen partition which was more distal from the stimulus showed a later maximum than the other and a longer persistence of the increased responses. The maximum responses obtained were not merely to the height of those before asphyxia but were very much greater, even six times as great for the fast tetanization and twice this figure for slow. Such a great response was obtainable only at the "peak" of the curve, lasting a few seconds. The subsequent falling off did not bring the responses at the more distal electrode back to "normal" values for as long as five hours or more. In a few instances, the maximum responses did not appear in the time mentioned but were reached in half an hour or more.

Responses at electrodes 5 and 6, beyond the asphyxial block, returned
usually to some maximum value which was then maintained. The maximum reached was usually somewhat below the values at these electrodes before asphyxiation. In several cases, however, where the initial decrease in responses of the freshly mounted nerve had been largely completed before asphyxia was begun, the action potentials returned to these original values after complete asphyxia, and practically maintained them over night. In two cases at least the nerves were mounted after dissection without having been moistened with Ringer's solution, except for a small drop at each electrode. They were asphyxiated and showed good recovery without application of Ringer's solution, and were moist and active the next morning. Further, nerves have been several times asphyxiated in nitrogen and at once washed in oxygen-free Ringer's solution with no return of activity. See figure VIA.

It has been often reported (Fillie, 1908; Gottschalk, 1919; Cooper, 1923) that, following asphyxia, partial recovery follows admission of oxygen, or oxygen-free Ringer, but full recovery is possible only with both. The inference has been drawn that in asphyxia part of the failure is due to the accumulation of diffusible but not oxidizable break-down products. It has been shown (Gerard and Meyerhof, 1927) however, that lactic acid accumulation does not interfere with its further formation and that nerves in gas or in Ringer's solution behave alike during asphyxia.

Nerve does form lactic acid under asphyxial conditions as a product of its resting metabolism, and it is not able subsequently to oxidize a significant amount of it (Gerard and Meyerhof, 1927; Holmes and Gerard, 1929). Heinbecker (1929) finds that a high concentration of lactic acid applied to a nerve depresses its activity. This may be due to acidity, which develops after the nerve buffers are exhausted. In this case smaller amounts would have little effect. Under the usual conditions of asphyxia there is not more than 20 to 30 mgm. per cent of lactic acid formed during the time to complete block, whereas Heinbecker's experiments were performed with concentrations of 250 mgm. per cent. It is, of course, impossible to deny any importance to the accumulation of unoxidized metabolites in the course of nerve asphyxiation, but to date the direct interference with oxidations serves adequately to account for the changes observed when nerve is subjected to oxygen lack.

The return of responses at leads 5 and 6 was seldom complete before a quarter of an hour, occasionally not before three-quarters. Probably lower temperature and relatively longer deprivation of oxygen slowed recovery, though no detailed analysis of these data has been made. The peak of the responses at 3 or 4 was reached regularly many minutes before 5 and 6 had come to maximum responses; that is, when many fibre-impulses were still missing at 4 the responses were higher than later when they were present. In the experiment of May 21st, previously analyzed,
the maximum responses at 3 and 4 were obtained at about 5 minutes, when those at 5 were less than 70 per cent of the value reached 10 minutes later.

The gradual return at electrode 5 seems to be a measure, primarily, of the reappearance of activity in successive fibres. A variation in time of recovery for different fibres is entirely comparable to the variation in time of failure; and in fact most of the changes following admission of oxygen mirror, on an accelerated time scale, the changes during asphyxia. This is true for the F/S ratios at leads 5 and 6. These were seen to fall steadily in nitrogen; on admission of oxygen they showed a similar but more rapid rise to, often, the original value (table 6). In cases where a Wedensky effect had appeared at the end of asphyxia and F/S had fallen to less than 1.0, the same condition was seen at the start of recovery and the curves at lead 5 for fast and slow tetanization crossed. This is evidence, then, for the gradual (though relatively rapid) shortening of the absolutely refractory period, and the successive return to function of the various blocked fibres when oxygen is made available.

The responses at leads 3 and 4, reaching such high values even when some fibres are still inactive, show that the time-potential value for a single fibre-impulse has increased enormously. Again, the comparison of fast and slow tetanization permits analysis into increased amplitude and duration. The F/S ratio at the peak in oxygen has fallen to values considerably lower than those recorded during asphyxia (of course toward the end of asphyxia no ratios are obtainable), and then rapidly rises to values close to the original ones before asphyxia. The peak responses for slow tetanization may be almost as great as those for fast; and the conclusion follows that the action potentials are greatly prolonged. An increased amplitude may also be present, probably as a result of breakdown during asphyxia of the transverse resistances in the path of the current from axones to leads, but this cannot be the sole effect since it would not explain a changed F/S ratio.

The later peak at the more distal nitrogen electrode than at the nearer one in the present results is easily understood in terms of fibre recovery. Just as block appears earlier at 4 than 3 because of the longer exposed stretch, so recovery of fibres would be slightly later. It is more difficult to understand why the fall after the peak is often much slower at 4 than 3. The peaks for slow tetanization are similarly reached before those for fast because early during the recovery in oxygen the return of fibre-impulses, as the refractory period becomes less than the stimulus interval, is relatively greater for less frequent stimuli.

It may be well, before proceeding, to consider the factors influencing the total action potential as recorded. The amplitude depends on the following. 1. The fraction of total fibres active and the number of impulses carried by each. These do not affect the fibre-impulse values. 2. The physical
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conditions of the circuit. A decrease in the transverse sheath resistance between the site of the axone potentials and the leads, or an increase of the longitudinal resistance (tissue fluid or Ringer solution) between adjacent active and inactive points on the axones would cause a greater fraction of the potential developed to reach the galvanometer. Such changes are also unrelated to true functional modification of the active fibres. But the sheath resistance probably depends largely on living polarizable surfaces which might be rendered more permeable during asphyxia, in much the same way as the nerve fibres proper. 3. The physical condition of the axone membranes. A decreased transverse resistance allows a greater leakage of current and so tends to lower the resting membrane potential and, secondarily, the action potential. It might also, however, depending on the exact site and means of production of the action potential, tend to increase the recorded change in a manner similar to that considered in (2). 4. The chemical condition of the axone (membrane?). The basic reactions supplying the energy and conditions (special substances?) for maintaining the membrane potential may be decreased, leading directly to a fall in resting and active potentials. It is of course arbitrary, though convenient, to distinguish (3) and (4) since a complex physico-chemical situation is at the basis of both. Changes in the delayed potentials are subject to these factors but are more conveniently considered in the next group.

The duration of the action potential depends also on several factors. 1. The rising phase of the main potential wave. This appears to be of an explosive character and little influenced by the metabolic condition of the nerve. 2. The falling phase of the main wave. This has a higher temperature coefficient than the rise (Adrian, 1921, Bishop and Erlanger, 1926) and has been reasonably correlated with the early stage of recovery. Adrian (1921) found the duration of the fall of potential and of the absolutely refractory period to vary approximately together with changes in temperature. Bishop and Erlanger (1926) found the absolutely refractory period prolonged by cathodal polarization and the time of fall of potential shortened. The slopes of their potential curves show, however, that the rule of fall of potential in cathodal polarization is, if anything, slowed. They end sooner than normally because the fall begins from a lower level. Presumably the absolutely refractory period represents the time required for a certain amount of recovery (substances formed, membrane rebuilt, etc.), as indicated by the findings of Gerard, Hill and Zottermann (1927). If the falling potential is a measure of this same recovery, which is zero at the end of activity, then the refractory period would not vary as the total duration of the falling potential but as the time required for a definite amount of fall. Choosing some arbitrary amount of fall (10 mm.) it appears from Bishop and Erlanger's figures that the time from the start of the response to this point is actually increased by cathodal polarization.
In the case of asphyxia, however, Heinbocker (1929) reported no change of duration of the main axone potential wave despite a prolonged refractory period. If this is correct it would indicate that the falling action potential does not measure recovery in the same sense that the refractory period does. 3. The negative after-effect (remainder, delayed potential). This is presumably related to the delayed heat production and oxygen consumption associated with an oxidative recovery. Its magnitude and duration might be expected to show great variations with the functional state of the fibres, as will be discussed. 4. The positive after-effect. This also appears to be closely related to metabolic changes in the nerve. It is tempting to regard it as an “over repair” phenomenon, especially as it disappears early in asphyxia, but its real nature is little understood. It can hardly represent the negative after effect at the injured end, for then it would increase rather than disappear as the side is asphyxiated. 5. The degree of summation of the delayed potentials of successive impulses. This is certainly not complete but may vary with the condition of the nerve.

During asphyxiation the region of nerve involved exhibits first an increased response—associated with lengthening of the action potential and probably increased amplitude due to lowered transverse resistance, and then a fall—associated with lowered potentials due to depressed chemical reactions and greater membrane “leakage,” lengthened refractory period, fibre failure and ultimate complete block. In recovery there appears first a steep rise as fibres again become active and responses are greater than at any previous time, due in large part to great lengthening of the action potentials. Following this there is a fall of response despite continued
and improving fibre activity, due to the return to more normal durations of the action potentials.

The picture is clearly that of two groups of antagonistic influences. During oxygen lack the return to resting potential after activity is progressively delayed, tending to give ever greater responses. This delay is rapidly reversed when oxygen becomes available and responses decrease to their original level. Were it not for fibre block (and feeble potentials), the curve for responses during asphyxia and recovery would always be above normal, reaching or maintaining its maximum value at the time of reoxygenation, and this maximum would be considerably greater even than that actually reached during recovery (see fig. IX). Fibre block and low potentials do supervene, however, later during asphyxia than the appearance of delayed potentials, and rapidly abolish all response. During recovery, block is relieved more rapidly than the changes causing delayed potentials—whence the sharp peak of responses. Both prolonged action potential and lowered action potential (increased refractory period and block) are expressions of a delay in one or more chemical changes necessary to restore the nerve to its resting activable state following an activation.

Assuming the delayed potentials to correspond to the early part of the delayed recovery process, their behavior is easily understood if the amount of potential is an index of the recovery still to be completed. In fresh nerve they are absent but they appear on activity as a state of equilibration develops. At the same time heat production and oxygen consumption fall. They appear during asphyxia when heat production is decreased and probably prolonged (Gerard, 1927a). They appear under treatment by CO₂ which seems to depress recovery processes. Interference, by any of these means, with the membrane restoration following activity should lead to the persistence of lower (negative) potentials. Levin (1927) likewise related delayed potentials to incomplete recovery in the case of crab nerve though he assumed a lactic acid mechanism.

According to the scheme suggested previously (Gerard, 1927c) oxygen lack causes gradual depletion of the oxidizing reserve (probably intermediate substances in the chain of normal oxidation reactions). This progressively shows and diminishes the oxidative recovery reaction (= prolonged potentials) and decreases one or more of its necessary end products. Decrease of these slows the immediate recovery processes (= prolonged refractory period) and finally diminishes and stops the initial reaction of conduction (= decreased initial potential and block). On readmission of oxygen the reactions again get under way, but since the delayed one is relatively slow some minutes are required before recovery of the immediate ones is sufficient for conduction to reappear. Also, since an hour or more is required for the replenishment of the oxygen reserve, the delayed potential persists for some time.
The picture here sketched in is obviously too simple to represent the full effects of asphyxia on nerve activity, but it may serve as a first approximation. It is obvious, if this analysis is correct, that the peak in oxygen depends on the separation in time of two antagonistic changes. If disappearance of delay were more rapid than or simultaneous with disappearance of block, then no peak would appear, and responses at 3 and 4 would return along the same smooth curve as at 5 and 6. Altered time relations might be possible if the two effects did not depend on the same chemical reaction, and if obtained would constitute evidence of such independence. Change in temperature (reactions with different temperature coefficients) in duration of asphyxia, etc., might thus modify the appearance of the peak. So far no direct experiments have been made to test the effect of temperature. In the work reported previously (Gerard 1927a) the return of heat production and of action potentials following asphyxia gave no evidence of a peak in any experiment. The main differences in conditions were 1, the nerves were kept at an average temperature about 10°C. lower; 2, the duration of the asphyxial period was longer (but not longer in relation to the lower temperature); and 3, the entire nerve, including the stimulated region, was subjected to asphyxia. Since experiments were not completed for many hours, even a day, after the crush, responses must have been largely diphasic; and as the changes due to asphyxia occurred under both leads the effect of prolongation was largely cancelled. This, rather than the temperature difference, is probably the reason that the peak did not appear.

5. Changes at electrodes proximal to an experimental region. A priori, one would hardly anticipate any change in responses led from normal nerve when the region subjected to experimental modification lies beyond it; that is, when impulses arise and travel in unaltered nerve up to the active lead. So generally has this been assumed that such proximal control leads have regularly been dispensed with. In the present series, one was included to permit (by comparison with a lead below the experimental stretch) an estimate of blocked fibre-impulses, but it was rapidly found to be useless for this purpose since responses from it suffered striking changes during the development of asphyxia below. A second electrode was added above the nitrogen stretch to secure further evidence of the nature of these changes.

In all cases leads 1 and 2 varied together, and if there was any difference in the magnitude of the change it was greater at 1 than 2 (table 9). The typical picture, seen in most cases, is that of figure 6 (see also fig. XII). One and 2 show no change while 5 and 6 remain constant, even though 3 and 4 are rising. As 5 begins to fall, 1 and 2 start to rise; and in general the curves at these two leads are fairly the inverse of that at 5. This is true, in the best experiments, even to a duplication of the waves in the fall of 5 with waves in the rise of 1 and 2.
The interpretation of this particular change seems clear cut. The partial activity at "killed" lead 7 normally diminishes potentials led from 1 and 2. As fibres block in nitrogen this activity is lessened or abolished and the recorded potentials at 2 increase. This increase would then start as impulses reaching 7, and therefore 5, begin to decrease, and should mirror

The changes shown. In one experiment, action potentials at 1 rose from 34 mm. before asphyxia to 134 mm. when asphyxial block was complete, the largest rise observed. After a night in oxygen the action potential was 41 mm. A fresh crush was then made above the old one and action potentials at once increased to 134 mm., over three times. Activity at 7 must have been at least $\frac{2}{3}$ that at 1. (See tables 7a and b.)
Further, the F/S ratio at 1 and 2 rises as the curves rise and later falls to its initial value or somewhat lower (table 9); while at all other electrodes F/S falls from the start. The more complete monophasicness of response will also account for the changed ratio, since responses at 5 (and 7) fall off more rapidly for fast than for slow stimulation, and a temporary increase in F/S at 1 and 2 must result. Ultimately both are completely abolished and the ratio returns towards its original value.

Unfortunately for the simplicity of this interpretation, it does not account for other effects. In several experiments (especially with green frog nerves) the proximal electrode responses fell along with those in nitrogen, though less fully, probably because of proximity to the asphyxiated stretch; and in two cases the responses fell and then rose above the original values or rose and then partly fell, although the lower electrodes were responding in the usual manner.

A more serious difficulty is met in understanding the changes at 1 and 2 on readmission of oxygen to the asphyxiated stretch (figs. IV, VI, table 9). Responses at 1 and 2, as at 3 and 4, show definite “peaks” within a few minutes after the admission of oxygen, though these rises are much less than within the experimental stretch. The peaks at 1 and 2 have been absent or small when, during a long period of asphyxia, responses at 1 and 2 have been maintained at high values. Whether the peaks were large or small, there was always a very sharp falling off of responses following the maximum. This falling off was at first more rapid than the fall of responses at 3 or 4 but tailed off and stopped when values equal to or less than those at the start of asphyxia were reached. The new level was not attained until after recovery at 5 and 6 was complete, but usually some time before 3 and 4 had returned to their base line.

A number of experiments were made in attempting to account for the peak at 1 and 2—the subsequent fall to original levels is largely the reverse of the rise during asphyxia, and need not especially concern us. Diffusion of substances along the nerve producing local changes of the upper electrodes can easily be ruled out. An “oxygen lack” could hardly spread to nerve in oxygen, and unoxidized products diffusing from the asphyxiated region, even if not oxidized, should affect electrode 2 more than 1. Also, diffusion should be equal to 5 and to 2, but the former does not show this behavior. Finally, the changes are too rapid to represent ordinary diffusion and occur simultaneously at 1 and 2. The only other way apparent in which the nerve above the nitrogen region could be locally modified involves “distance action” via electrical circuits.

The injury potential of asphyxiated nerve falls greatly (see later), so that in effect the “killed end” is moved to the proximal portion of the nitrogen region. Local currents must flow from here through the axone “cores” and back on the outside of the fibres or sheath, as previously discussed.
Responses near the region of asphyxia should then be depressed, as near a crush, and the rise at 2 during the asphyxiation period should be less than normal. Restoration of membrane potentials in the nitrogen stretch would then increase responses at 2 until returning activity at the end lead, 7, again cut them down. This factor may play a rôle, but on this basis the changes at 2 should again be more pronounced than at 1, which is not the case. Polarizing the nerve at lead 2 (and 3) with potentials of the same strength as injury potentials also failed to affect the action potentials at 2, either during passage of the polarizing currents or at the time of closing or opening.

If the nerve at leads 1 and 2 is not locally changed, the electrical response may still be modified in at least two ways. The path of local current flow in the nerve during conduction of an impulse could be modified, or the physical conditions of the recording circuit might be changed. As regards the former, several possibilities can be conceived, but are simply excluded by sending impulses along the nerve in the reverse direction. If, for A stimulation, these effects appear at 1 and 2 but not at 5 and 6, then for B stimulation they should not appear at 1 and 2 on the far side of the asphyxiated stretch. Actually, however, they still appear at 1 and 2 when stimulation is carried on at B.

This leaves as the necessary condition for the appearance of a peak outside of an asphyxiated region, that such a region must be interposed between the lead on normal nerve and the "dead" lead. The position of the stimulating electrodes and the direction of the impulse are not crucial. The interposed asphyxiated stretch could not produce effects by any change in longitudinal resistance, as previously pointed out. Since the peaks above it do not occur during the asphyxiated period but afterwards, when the asphyxiated nerve is showing its own peak response, the former appears to be somehow dependent on the latter. It is possible that the idle electrodes on the nitrogen stretch are acting as secondary leads back to those above, as found to occur by Bishop, Erlanger and Gasser (1926), though even here it is not clear why 1 should be as much affected as 2. This effect depends on local lowering of the transverse resistance and it could well occur even with no electrode contacts if asphyxia interferes with the membrane's integrity. On this interpretation, the high responses at 1 and 2 depend on increased responses and a persisting low membrane resistance in the previously asphyxiated stretch, and since both factors are changing after the peak to lessen this effect, the falling off of responses after the peak at 1 and 2 should be more rapid than at 3 or 4, as it is. A further indication that responses at the proximal leads are due in part to back currents from the asphyxiated region comes from the F/S ratios. These fall during the peak at 1 and 2 nearly as low as at 3 and 4, as should occur on this assumption, but could hardly result if the responses at 2 measured the
nerve activity at 2. On the other hand, the peaks at 1 and 2 have not always been synchronous with those at 3 and 4, and 1 and 2 have even been well past their peak responses before those at 3 have been reached. It is not clear how far this "back leading" can be invoked to explain the peaks in the unasphyxiated stretch. It may be added that a similar effect is seen with CO₂ in place of N₂, and it appears even when no metal leads are in contact with the nerve in the exposed region.

One experiment with a bull frog nerve mounted in the reverse direction so that only a portion of the fibres were stimulated at A yielded unique results (fig. XI). There can be little doubt here that responses at 1 and 2 varied intimately with those at 3 and 4 but this time in the opposite direction. The curve at 2 is an almost perfect mirror image of 4, including a fall in nitrogen during the rise of 4, a subsequent greater rise as 4 fell, an inverted peak with positive action potentials when oxygen was readmitted and eventually a return to the initial values. It is difficult to fully interpret this curve, but it might be surmised that the apparent positive peak at 2 is really a negative peak at 7. As usually mounted, many fibres run past leads 1 and 2 to leave the nerve as a branch as it enters the nitrogen compartment. With the reversed nerve, this is the case for lead 7. The nerve branch thus appears to have some relation to the outside peak. The peaks outside of the asphyxiated region occur, however, in the unbranched dog peroneal, so branching is not the essential factor.
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Whatever the true basis for this distal effect of asphyxia (and the same appears with carbon dioxide), it is of considerable interest. It may seriously complicate the picture in experimental studies. The present set of observations has been carefully scrutinized for possible errors of interpretation due to it, and I believe they do not occur. Further, it may be a clue to a mechanism, other than the passage of nerve impulses, whereby one region of the nervous system can affect another. The work of the Pavlov school, the Gestalt psychologists (as Lashley, 1929) etc. strongly suggests that such an additional mechanism does exist.

6. Resting potentials. These were measured directly by the balancing potential required between any two electrodes or indirectly by the galvanometer throw on changing from one electrode to another, with the condenser circuit. The results were similar with either method, though

<table>
<thead>
<tr>
<th>LEADS</th>
<th>CRUSH ABOVE 7—24 HOURS PREVIOUSLY</th>
<th>FRESH CRUSH BELOW 3</th>
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<tr>
<td></td>
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<td>Action potential</td>
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<tr>
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</table>

Bull frog nerve, 24 hours after dissection. T° = 22°C. First observation 12 hours after a five hour asphyxiation of the region on leads 3 and 4.

The resting potentials at different points along the side of an uninjured, unbranched nerve (measured against any one point) are seldom equal and may vary widely. The potential difference between two leads from the intact side may, in fact, be considerably greater than between side and end (table 10). Presumably local injury, tissue tags, relation to injured end, etc., are responsible for these differences, though following a careful dissection there are no gross irregularities and test may show nearly all fibres able to conduct their whole length. The magnitude of the action potential from any pair of leads has no relation to the resting potential between them. Weak action potentials may be obtained from two leads on the side of an unbranched nerve showing large or small resting potential differences, and action potentials of the usual strength may appear on leading from side and end even though the resting or injury potential between them is practically zero. Since in
these experiments the nerve was crushed several millimeters above the lower electrode, the portion lying on it was not severely injured, and this independence has not the full significance it would otherwise have. A crush does, however, influence the potentials at nearby points, for lead 7, near the crush, regularly is at a lower voltage than the other leads, and crushing at any point markedly alters the resting potentials of regions near it.

![Fig. XII](image_url)

These resting differences are small compared to those appearing regularly (but not invariably) in the course of asphyxia. The resting potentials at the electrodes in the nitrogen stretch fall steadily (become more negative) during the course of asphyxia and rise, more rapidly, on the readmission of oxygen (fig. XII). The potentials reached after full recovery in oxygen may be definitely higher than at the start of the asphyxia, but they
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are then maintained with no evidence of a "peak" effect. The fall and return of resting potential parallels the changes in action potential rather strikingly in some experiments; but in one case, despite a typical failure of action potentials, the resting potentials at 3 and 4 (against 7) showed a steady slow rise unaffected by the asphyxial period. I have no explanation for this anomalous result.

The fall in resting potential during asphyxia is in harmony with current views of nerve activity, and with Furusawa's findings on crab nerve (1929). The resting fibre is supposed to possess a longitudinal membrane polarized as an electrical double layer, positive outside and negative within. The injured region is negative to the intact one because this insulating membrane is destroyed, and an active region becomes negative for the same reason. The polarized membrane is a living structure and presumably maintains its integrity by the continual expenditure of energy obtained by oxidations. This has been suggested by Warburg (1912) in the case of nucleated erythrocytes and by Hill (1928) for the case of muscle. For nerve, Gerard (1927) found an extra oxygen consumption after a period of asphyxia, part of which reappears promptly as CO₂ (Penn, 1928) and represents increased oxidations. Since it has not been possible to demonstrate an accumulation in nitrogen of metabolites which are subsequently oxidized (Gerard and Meyerhof 1927; Holmes and Gerard, 1929), it seems probable that much of the extra oxidation goes to the rebuilding of the cell membrane. Evidence of a decreased membrane resistance during asphyxia has been presented earlier in this paper. The resting potential, then, measures a dynamic equilibrium point of disintegrative and restoring reactions.

The fall of injury potential in nitrogen is thus additional evidence that the normal membrane potentials (and other properties) are dependent on oxidations. That they arise at the intact portion of the cell rather than at the injury is further evidenced by the finding that the asphyxial fall is the same whether the injured portion is included in nitrogen or is left in oxygen.

If activity represents membranc destruction (Lillie, 1923) and the delayed potentials signal its incomplete restoration, the progressive failure of recovery in nitrogen must lead to ever greater delayed potentials or, in other words, failure of recovery of the resting potential. Fibre block may then be one expression of the failure of membrane restoration due to lack of oxygen, the fall of resting potential being another. A similar view has been expressed by Furusawa (1929) in interpreting the "total depolarization" of crab nerve when fatigued to inactivity. This interpretation

4 Greater in the sense of farther from the initial value to which the potential normally would return. This would be true for the summed delayed potentials. The added effect of a single impulse must ultimately become less as the resting potential falls and so reduces the change possible on activity.
does not imply that the chemical reactions of rest and of activity are
the same, and in fact they are not (Gerard and Meyerhof, 1927; Holmes,
Solomon and Gerard, 1930).

The thesis developed in this paper is simply an effort to satisfactorily
correlate recently acquired facts concerning thermal, chemical and elec-
trical events of nerve function. It is in no sense a new theory of nerve
activity but does serve to indicate certain further experiments.

SUMMARY

1. Resting and action potentials of several types of nerve have been
studied by means of a slow galvanometer, using multiple leads and two
frequencies of stimulation applied at two regions. The use of a condenser
in the galvanometer circuit was convenient and allowed reliable measure-
ments. A condenser was also used across the primary of the stimulating
circuit, and its capacity found to have a marked influence on the effective-
ness of the shocks.

By comparison of responses at electrodes in and below nitrogen to stimuli
at high or low frequency it is possible to obtain information as to: total
action potential per fibre-impulse, per cent of fibres blocked, prolongation
of refractory period, changes in form and intensity of action-potential.

2. The action potentials led from nerves before asphyxiation are con-
siderably greater for the green frog than for the bull frog or dog. All
leads from the intact side, even with an unbranched nerve, do not give
equal responses. Potentials from the lead nearest the crush (15 mm.)
are regularly depressed, even to half the values at the other leads.

Responses to rapid tetanization (300 per sec.) compared to slow tetani-
zation (90 per sec.) are not as much greater as the frequency ratio of the
stimuli \( F/S = 3.5 \), but definitely less, even by half \( F/S = 2.0-3.0 \).

Responses fall rapidly for a short time after a crush is made and then
very slowly. After 24 hours, a fresh crush will often restore action poten-
tials to their original value. Evidence is presented that the early rapid
fall is due to increasing activity at the crush and consequent greater di-
phasicness. Due to this activity at the crush, which may reach three-
fourths of the normal, depression of activity at other electrodes may give
reversed responses from leads on the “intact” side and “dead” end.

When make and break stimuli are applied to a nerve between the
leads, unidirectional potentials develop. This effect (Fleischl) apparently
depends on rectification by the nerve membranes and is some measure of
their physical state. The Fleischl effect is greatly reduced in asphyxia.

There is regularly present a positive potential change following the
negative action potential; a positive after-effect. This is abolished dur-
ing asphyxia.

The rise of total action potentials (equilibration) occurring early with
frequently repeated tetanization is greater for fast than for slow tetanization and may be abolished or reversed during asphyxia.

3. In the course of asphyxia, action potentials led from the exposed region first rise and then fall to zero. Potentials led from nerve in oxygen beyond the asphyxiated stretch remain constant during the rise of the others and then fall with them. The fall usually shows definite waves. During the fall, responses at the distal electrode in nitrogen fall faster than at the proximal and may reach values only half as great as in the control stretch below. Responses to fast tetanization fall faster than to slow and a Wedensky effect may appear, in which the nerve below the asphyxiated region still responds when slow tetani are delivered above the nitrogen stretch, but shows no response to fast tetanization. The mechanism of nerve block is discussed.

These observations are interpreted to mean that asphyxia causes: first a prolongation of action potentials, later a depression of potential magnitude, a prolongation of refractory period, and fibre block. Fibres do not block in a homogeneous manner but fall in groups, probably those of each diameter forming a group.

Asphyxia blocks impulses and abolishes potential changes at the point of block—like a fresh crush; but activity at the block (diphasicness) does not gradually return as in the case of a crush.

4. Resting potentials are steadily lowered (side less positive) in an asphyxiated stretch and return in oxygen. The relation of the membrane potentials to action potentials, and the rôle of oxygen in maintaining them is discussed; and an interpretation is offered for activity at a crush and depressed resting and action potentials and increased irritability near it.

5. The time required for asphyxiation of a nerve depends on the temperature and the type of nerve. Dog nerve asphyxiates more rapidly than green frog and this more rapidly than bull frog. The resting oxygen consumption of these nerves is in relation to the time for asphyxiation—the higher the metabolism the faster the block. This is interpreted in terms of exhaustion of an oxidizing reserve. The failure of fibres in groups is similarly interpreted.

6. When oxygen is readmitted to an asphyxiated nerve the action potentials in the exposed region rise rapidly (5 to 10 minutes) to very high values. For fast stimulation, the maximum response may be 5 or 6 times as great as the pre-asphyxial ones, for slow stimulation even 12 times as great. The maximum is a “peak” value and is followed by a less rapid fall to or towards the initial values.

Responses below the nitrogen region show no peak but return in about 15 minutes to their original values.

Nerves kept practically free of Ringer’s solution from the time of their dissection often recover fully in oxygen and remain in good condition over
night. Asphyxiated nerves bathed in oxygen free Ringer usually show no recovery. The theory that asphyxial block results from the accumulation of metabolites is considered and discarded. The changes in nitrogen and oxygen are interpreted in terms of two opposed effects due, possibly, to separate chemical reactions.

7. Electrodes above the asphyxiated region, reached by impulses travelling only in normal nerve, record increased responses as the asphyxia below progresses. The rise starts simultaneously with the fall of the electrodes below nitrogen, and at first the ratio of fast over slow increases. This effect is due to the more perfect monophasicness of nitrogen block than at a crush.

The upper electrodes also show a further peak in responses paralleling the peak in the previously asphyxiated region. Its causation is uncertain, but may represent a back lead from the recovering stretch.

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