The Effects of Oxygen Lack on Peripheral Nerves

Thesis

by

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INTRODUCTION

Peripheral Nerve Metabolism

The metabolic gas exchange of peripheral nerves was not scientifically established until the beginning of the 20th century. Early attempts to measure the oxygen uptake in nerve were unsuccessful (Helmholtz 1868), the very small amount escaping detection by the methods used at that time. The first nerve asphyxiation experiments failed too (Ranke 1868, Ewald 1868), because no suitable physiological solution was to be had at that time, and nerves lost their excitability as quickly in oxygen as they did in hydrogen or nitrogen. However, the oxygen consumption of the central nervous system had been detected, so it became the concensus of opinion that the nerve metabolism was confined to the nerve cell body. The axone activity was considered to be anaerobic, a purely physical phenomenon, and as would be expected from such a conception, it was indefatiguable.

In 1904, the oxygen consumption of frog nerve was actually detected for the first time by Thunberg. He devised a micro-capillary-manometer in which small movements of a kerosene bubble recorded minute decreases in the oxygen volume of the system. This method was too crude for accurate quantitive determinations, but with some modifications (Fenn 1927-8-9) it proved capable of good volumetric measurements.

The CO_2 emanating from a frog nerve was detected for the first time by Tashiro (1913-14-15). He described the formation of a cloudy precipitate of $BaCO_3$ on the surface

of a droplet of $BaCl_2$ solution sealed in an airtight chamber with the nerve. Although he claimed to be able to calculate by this method not only the actual amount of CO_2 given off, but the change in this amount brought on by nervous activity, these data are questionable. Adoption of the Haas indicator method by Moore (1919) and Parker (1925-26) led to much more dependable results. This method was based on the color change of phenosulfanephthalein caused by the production of carbonic acid.

Winterstein (1926) and Chang (1930) have also compiled data on the metabolism of peripheral nerve. The subject has been excellently reviewed by Gerard (1932).

Peripheral Nerve Asphyxiation in Cold Blooded Animals

Mathews, in 1901, was puzzled by the rapidity with which nervous activity disappeared in freshly killed animals. Employing the string galvonometer to record the nerve impulse directly he discovered that if the nerves were removed immediately upon the death of the animal and placed in the proper physiological solution they were able to maintain their excitability for many hours. Delay in the removal impaired the survival of the excised nerve. This reaction suggested to Mathews the possibility of anoxia, so he repeated the experiments of Ranke and Ewald (Mathews 1902). A frog nerve was well washed in saline and then placed on electrodes in a small container which was sealed shut. Hydrogen, well-saturated with water vapor, was made to fill the chamber. Oxygen was used in the control experiments. After several hours the

response from a nerve in hydrogen atmosphere disappeared, whereas nerves in oxygen were electrically as active as ever after the same length of time. Thus frog peripheral nerves were found to be asphyxiable.

Nerve asphyxiation became the subject of a series of intensive investigations by the German school (vonBraeyer, Frohlich, Fillie, Thorner, Gottschalk) under the direction of Vervorn from 1900 to 1920. Further contributions in the field have been offered more recently by Cooper (1923), Koch (1926), Fenn (loc cit), Furusawa (1929), Heinbecker (1929-31), Gerard (1930), Cowan (1934), Thompson (1936), and Lehmann (1937). In all of the older work and in part of the new work the frog nerve was the preparation used. Gerard asphyxiated a few dog nerves, but collected most of his data from frog nerves. Koch and Lehmann worked exclusively with mammalian material, rabbit nerves and cat nerves respectively, while Furusawa and Cowan limited their study to crustacean nerves. Thompson observed anoxia in human nerves.

Frog nerves were found to be able to maintain their activity for about an hour in an atmosphere of nitrogen or hydrogen, but then they began to lose their excitability slowly, finally becoming inactive after 3-5 hours (von Braeyer 1902, Frohlich 1903, Thorner 1911, Gottschalk 1914-18, Cooper, Gerard, and Heinbecker). The original postulation by von Braeyer that the latency in the onset of the anoxia effect was due to an oxygen reserve in the nerve tissue was generally

agreed upon. From results obtained in experiments in which the nerve was stimulated at several places von Braeyer contended that the loss of excitability progressed centrally along the part of the nerve asphyxiated in the chamber. This contention was disputed by Frohlich who argued that the conduction along partially asphyxiated nerve became decremental near the chamber walls, an effect described later by Kato (1924) for narcotized nerve. Frohlich failed to find any directional loss of activity during asphyxia.

Cooper's investigation of the least interval and recovery time of asphyxiated nerve also indicated conduction with decrement. The least interval (Lucas 1917[3]) was the least time between two stimuli, the second 8 times the strength of the first, to cause muscle summation. The recovery time was the least time between two stimuli, the second twice the size of the first, to cause the muscle to summate. The recovery time was observed to remain constant, the least interval increased during the anoxia period. The constant recovery time seen by Cooper instigated an argument between her and Frohlich. Whereas Frohlich had concluded from experiments using stimuli of different frequencies on nerves in nitrogen that the refractory period of the nerve lengthened to as much as 0.1 sec during anoxia, Cooper claimed that the constant recovery time could only mean a constant refractory period throughout asphyxiation. She based her argument for decremental conduction on this claim. Cooper's results were refuted by both Heinbecker and Gerard. The latter repeated Frohlich's

experiments recording the integrated nerve action potentials rather than the muscle twitch and obtained results similar to those reported by Frohlich. Heinbecker could actually observe the broadening of the potential spike during nerve asphyxiation, and noted too the increase in the time interval between two identical stimuli necessary in order to produce two identical spikes. Thus the refractory period definitely varied during anoxia. The question of decrement although "tempting" as Gerard puts it, was not brought up again in connection with nerve asphyxiation. This was probably because of Kato's thorough investigation of the subject using narcotics, the results of which failed to show a decrement in conduction in frog nerve single fibres. Working with whole nerves as Cooper had, the problem of decremental conduction in various numbers of fibres at various places in the chamber and at various times presents a difficulty which can only be eliminated with the elimination of all but one of the fibres.

The blocking of nerve conduction by asphyxiation was reported by both von Braeyer and Frohlich. They distinguished between conduction and excitability by varying the position of the stimulating electrodes relative to the asphyxiated area. For conduction measurements this area was between the stimulus and the muscle. For examination of excitability the stimulus was applied directly in the asphyxiated area. The conduction was found to be normal for at least 2 to 3 hours after the nerve was in an oxygen free medium, then suddenly disappeared. It was very evident that most of the nerve

fibres were blocked all at once. This finding was not confirmed by Gerard who contended that nerve fibres stopped conducting at varying times and at varying places.

Frohlich was able to ascertain that the velocity of conduction was reduced by anoxia, the amount of reduction depending on the length of nerve asphyxiated. No definite relationship between the two was found. Heinbecker describes the velocity reduction also, but does not speak of the effect of the length of the asphyxiated part of the nerve.

Temperature increase decreased the survival time of nerves in nitrogen or hydrogen (von Braeyer, Gerard) as was expected on the basis that such a change would tend to increase the rate of the nerve metabolism. Fenn was able to measure the increase in Oxygen consumption of frog nerve with increasing temperature.

Nerve Membrane Depolariztion During Asphyxiation

Koch, 1926, succeeded in developing a technique whereby certain rabbit nerves were made anemic by arterial occlusion. Before stopping the circulation, in the leg, however, electrodes were placed on the sciatic nerve. Care was taken to first crush the nerve under one of the leads, or cut it, so that the demarcation potential was recorded. Then the circulation was cut off, and the variation of the demarcation potential was observed. At first there was a brief voltage increase, then a steady decrease to a very small constant value. With the resumption of circulation the potential recovered rapidly to its original value, overshooting it in some cases.

The experiments of Koch were as important in the field of nerve anoxia as were those experiments of Mathews, for with these results it was possible to understand for the first time what was happening to the nerve deprived of oxygen. The nerve membrane was depolarized when the nerve was subjected to an oxygen free atmosphere. Koch simply assumed that the nerve became inactive and this drop in injury potential was a measure of the inactivation. He did not realize the significance of his results.

Furusawa (1929) investigated with a ballistic galvanometer the demarcation potential of the nerve from a crab claw noting the changes in this potential during anoxia. With the oxygen removed the voltage between injured and normal tissue (inside and outside of the membrane) slowly became smaller and disappeared. Furusawa realized that the membrane was being depolarized. Gerard confirmed this discovery using frog nerves and Cowan observed the same with Maia nerves. Cowan suggested that the membrane lost its ability to maintain the potassium ion gradient across the membrane, its selective semi-permeability failing to function without oxygen so that potassium leaked out and the concentration gradient potential was reduced.

$$V = \frac{RT}{NF} \log \frac{C_{inside}}{C_{outside}} = 0.058 \log \frac{C_i}{C_o}$$

Immediately the question of decrement was brought up again, but in a modified form termed reduced "all" by Gerard.

Gerard did a rather peculiar thing as regards this problem.

He describes the assumption of linear blocking of nerve fibres, that is "a" fibres are blocked if "A" length of nerve exposed and "2a" fibres block if "2A" length exposed, as unsafe, but adds that if it were true then the following calculation could be made. The reduction of the action potential of the nerve at the end of the chamber to one half its normal value would mean non-conduction in 50% of the fibres at that point. Then only 25% would be non-conducting at the middle of the chamber so the potential should be 3/4 normal there. However he found the potential only 63% normal in the center of the chamber instead of 75% indicating a slightly reduced potential in each fibre. He then concludes that the individual "all" potential of each fibre is reduced during anoxia as might be expected on the basis of the depolarization of the membrane without oxygen. As Gerard stated the method of arriving at the conclusion was not sound. A better method in which whole nerves are used is included in the results of this paper, but for such observations only single nerve fibres should be employed.

According to Heinbecker the survival time varied directly with fibre size, the larger fibres lasting longer without oxygen. Gerard also came to this conclusion.

Recovery

The effect of anoxia was reversible with the substitution of air or oxygen for the asphyxiating gas, but the extent of the reversibility was cause for debate. Von Braeyer obtained complete recoveries in frog nerves after 3 - 10

minutes in oxygen. He used the muscle response to record the threshold of nerve activity so that the actual spike potential could have been reduced either by partial fibre blocking or by a reduction of the "all" without such a difference being detectable. Gerard also obtained complete recoveries after short periods of oxygen following asphyxiation. He observed this using the integrated nerve action potential as a measure of the nerve activity. At the start of recovery and also at the start of asphyxiation a period characterized by super normal action potentials was noted which he attributed to the broadening of the impulse. With increasing recovery the impulse shortened reducing the integrated action potentials.

Frohlich observed that the threshold of many of the asphyxiated frog nerves never returned to their normal value with oxygen alone. In fact washing with oxygen free saline had more effect on the reversing than did the gas, oxygen, by itself. Aerated physiological solution rarely failed to bring about complete recovery of a nerve washed in it long enough. The conclusion was drawn that during asphyxiation, nerve metabolic end products normally removed by oxidation collected, these metabolites eventually becoming so concentrated as to act toxically on the nerve itself. Oxygen alone was not able to remove them once they had accumulated in high concentrations. Evidently they could be carried off by washing in the saline.

These results and conclusions were confirmed by Fillie.

Besides this he found that when submerged in saline the nerve survival time during anoxia was increased. Thorner, Gottschalk, and Cooper also observed the same effects described by Frohlich and Fillie. Heinbecker reported only partial recoveries with oxygen or air alone after asphyxiation and attempted to determine the postulated toxic substance. He investigated effects of high concentration of normal metabolites on recovery of asphyxiated nerves. Substituting CO_2 for nitrogen did not prevent the complete recovery of the action potentials with oxygen. The same results were obtained when high concentrations, compared to normal, of lactic acid were used. Thus neither of these common end-products of metabolism was the cause of the poisoning of the nerve.

Gerard, having obtained complete recoveries, had discarded the toxic substance idea, but it was brought back again only a few years later by Cowan as an explanation for the partial recoveries of Maia nerves from asphyxiation. Cowan's contention was that the potassium leaking out of the nerve fibre as suggested by the depolarization might reach a concentration outside the nerve which would depress the excitability. Potassium was known to depress nervous activity (Graham 1933). However, no direct tests for potassium were made by Cowan and no further attempts have been made since that time to find just what the cause of the inhibition of recovery is.

Other nerve asphyxiation experiments of interest have

been reported and should be included here in a brief survey. Von Braeyer found that nerves could be asphyxiated by placing them in a brei of iron filings and NaCl solution. The oxygen was drawn from the nerve tissue to the iron to form iron oxide and the nerve lost its excitability. Complete recoveries were observed when the nerve was removed from this medium and oxygen administered to it.

Frohlich found that nerves taken from frogs in the winter had a shorter survival time in nitrogen than those taken from frogs in the summer. He noted too that the survival time of nerves from starved frogs was always less than the survival time of nerves from well fed animals. Frohlich decided that in the winter the hibernating frog was in much the same condition as a starved frog, the lack of nutrition being the cause of the shorter time for asphyxia.

Thorner reported fatigue in frog nerve becoming very prominent in the later stages of asphyxiation and early part of recovery. Gottschalk found that a 25 minute oxygen interval between anoxia periods was necessary to bring the nerve back to its original state as determined by the duplication of the original survival time. If the oxygen "rest" were less than this optimum then a relationship between the time in oxygen and the following survival time was found to hold.

$$\frac{\text{Oxygen (1)}}{\text{survival(1)}} = \frac{\text{Oxygen(2)}}{\text{survival(2)}} \quad \text{etc.}$$

Gottschalk claimed that the nerve recovered its excitability in 5-10 minutes after complete asphyxiation, but that it took another 15-20 minutes for the reserve supply to reach

its normal level. The survival time of the repeated asphyxiations was dependent on how full the reserve was.

Asphyxiation of Peripheral Nerve of Warm Blooded Animals

There have been only five previous investigations in which the mammalian nerve was the object for experimentation. The work of Koch, in 1926, has already been described. Yosomiya in 1927 asphyxiated rabbits, in toto, and found that the nerve sensitivity increased slightly at the start of asphyxiation but soon began to decrease, the reduction continuing until the death of the animal. The effect of oxygen lack on the nerve threshold became noticeable for peripheral nerves at oxygen tensions of 8-9%, for sympathetic nerves at 5-6%. Gerard found that the nerves from dogs were asphyxiable in less than an hour, a shorter survival than that exhibited by the frog nerve. These recordings were made at 23 degrees centigrade so that at normal body temperature it would be expected that the survival time would be still less.

Asphyxiation of human nerves was accomplished (Thompson 1936) by applying a pressure of 160 mms Hg with a cuff around the upper arm. During the first 12 minutes there was a slow lowering of the threshold followed by a sudden rapid rise until no further response was evident. Recovery was rapid with release of the pressure. The threshold stimulating the ulnar and medial nerves was defined by the amount of alternating current necessary to be just felt by the subject.

The most recent work limited exclusively to mammalian

peripheral nerve asphyxiation was carried out by Lehman (1937). It was stated by Lehman at the outset that results previously obtained with nerves in Ringers solution could not necessarily be considered reliable because the state of the nerve in ordinary physiological solution was not comparable to its state when in vivo. (see Grundfest and Gasser 1936). It was found that the hydrogen ion concentration of the solution had a strong effect on some of the electrical responses of the nerves. The threshold of activity and the after-potentials could be easily varied by small pH changes, but the spikes were found to be but little affected. The results of this preliminary survey showed that the threshold rose with increasing acidity and fell with increasing alkalinity even to the point where spontaneous discharges were observed. At pH 7.4 there was excellent correspondence between nerve potentials recorded either from the intact animal or from excised material. In the same survey the $CaCl_2$ and KCl balance in the solution was studied and it was found that removal of the $CaCl_2$ had the same effect as raising the pH, supporting the view that increased pH lowered the calcium ion concentration. Ample data had been gathered previously to do with K ion changes (Graham 1933, 1935 see discussion) and this was confirmed.

The chamber used by Lehman for the asphyxiation work was in the shape of a small cylinder. The nerve was suspended on Silver-Silver chloride electrodes placed in the chamber so that with the chamber half filled with solution

the nerve was just above the surface. A 90 degree rotation would completely immerse the nerve. Ordinary commercial nitrogen was used as the 0.1% oxygen it contained was not enough to make any difference in asphyxiation time. Induction stimuli were used and all records were made with the cathode ray oscillograph. With the onset of asphyxia the threshold was seen to rise slowly after a preliminary drop so that cat nerves (phrenic, peroneal, tibial, and saphenous) were inexcitable in about 25 minutes. Oxygen restored the spike potential in only a few minutes, but the after potential became super sized at first before subsiding to normal. Most extraordinary, however, were the changes exhibited by the threshold in ~~one~~ *the* course of recovery. In the first two minutes it returned almost to normal only to rise again to 3 times its normal value and then slowly settle to its starting value, the whole procedure requiring 50 minutes.

Importance was placed on the after-potential, i.e. the variation reported by Gasser et al as following the spike and only recordable with very high amplification. This after-potential is a questionable phenomenon. It is true that the spike whether monophasic or diphasic approaches its baseline only assymptotically since the membrane possesses a definite capacitance and reactance (Cole 1928), but for this trailing part of the deflection to show properties unique to itself seems doubtful.

These findings of Lehman have been submitted as a possible explanation of the two muscle tones appearing in ani-

mals after spinal cord asphyxiation (van Harreveld 1943). The early threshold drop and then rise could cause the first tone which is very short-lived and then the second threshold drop brings on the second tone. However, since all other threshold recovery curves to be found in the literature are smooth, uniform expotential curves approaching a limit, it was decided that a comparative study of the subject of nerve asphyxiation should be undertaken to confirm or refute the claims of Lehman. The results of this study are described and discussed in the present thesis.

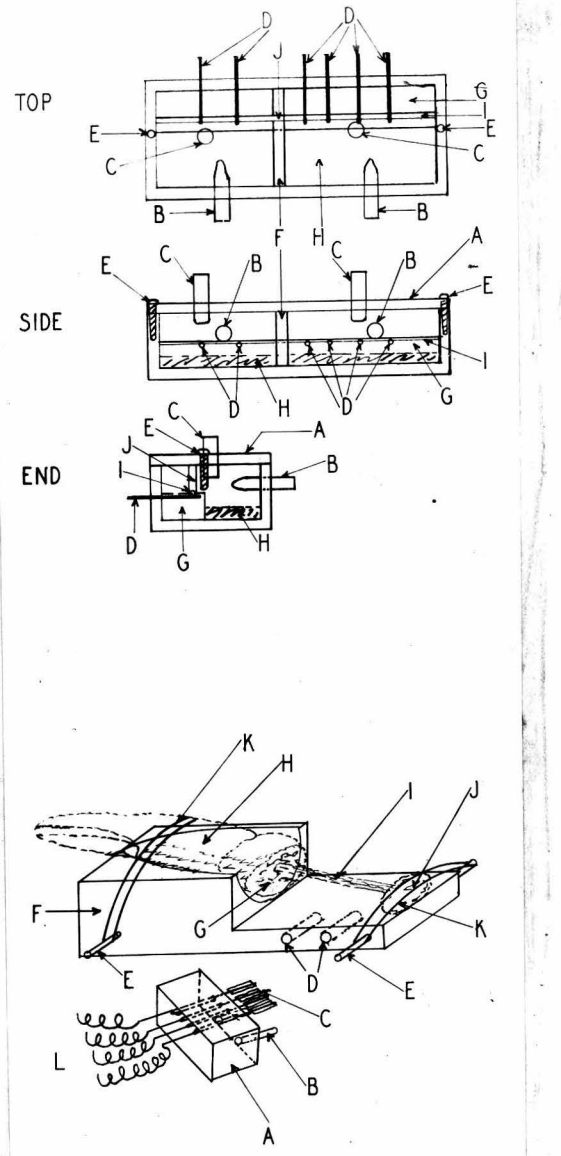
METHODS

Nerve Asphyxiation Chambers

The technique used was similar in many respects to that of previous investigators in the field of nerve asphyxiation. It was found possible to improve upon the type of chamber used for asphyxiation and thus to increase the durability of the nerve. This was done by constructing chambers of lucite a substance reported to have no noticeable toxic effects on nerves no matter how long the nerves are in contact with it (Marmont 1940). Also it is a very workable transparent material and an excellent insulator. It is non-soluble in water, gas tight, and is very rigid at low temperatures. It becomes pliable at about 100°C, which is well above any temperature to be used with living nerve tissue.

Three chambers were made, one for vertebrate nerves, the other two for crustacean nerve preparations.

The vertebrate chamber is diagramed in Figure 1, upper part. It was made of 1/8 inch lucite, 2 1/8 inches long, 1¼ inches wide, and ½ inch deep. It was divided into 2 sections by a 1/8th inch partition (F). A narrow slot (J) was cut in the partition to allow the passage of the nerve (I). This slot could be sealed with vaseline when it was desired to use the chamber in two sections. Small glass pipettes served as gas inlets (B) through the side wall of the chamber and as gas outlets (C) through the lid (A) of the chamber. A ledge (G) ¼ inch high and ½ inch wide was placed in the chamber to support the nerve. Six silver-silver-chloride



A----------lid

B----------gas inlet

C----------gas outlet

D----------electrodes

E----------lid screw

F----------partition

G----------ledge

H----------fluid

I----------nerve

J----------partition slot

A----------electrode block

B----------plug

C----------electrodes

D----------holes for plugs (B)

E----------wire rod

F----------preparation holder

G----------indenture to fit limb

H----------propodite

I----------nerve

J----------ishiopodite

K----------elastic band

Fig.1. Diagrams of vertebrate nerve asphyxiation chamber (Upper), and crustacean nerve holder (lower). See text for detailed description.

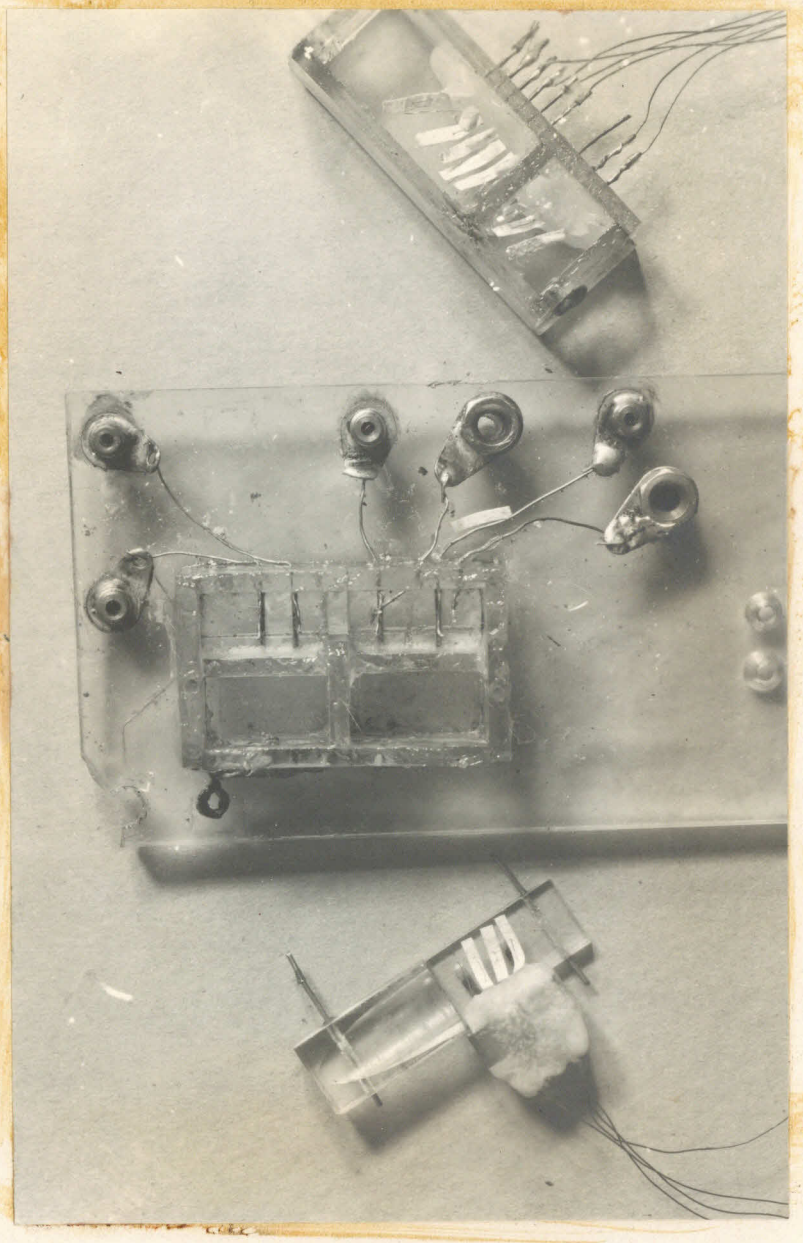


Fig 2. Photograph of crustacean nerve chamber(top),vertebrate nerve chamber (center),and crustacean nerve holder (bottom).The latter with nerve preparation attached was placed in large gastight lucite box(not shown)for anoxia experiments.

electrodes (D) were set in grooves in this ledge so that they were flush with the surface of the ledge. The nerve was thus supported along its whole length, not just by the electrodes. This means of holding the nerve was devised in order to preserve the nerve in its normal state the longest possible time.

Two crustacean nerve chambers were made. First, a smaller replica of the vertebrate chamber was constructed. It was made of 1/8th inch lucite, $2\frac{1}{2}$ inches long, 1 inch wide, and 3/4ths of an inch deep. It was divided into 2 sections by a 1/8th inch partition. 10 platinum wire electrodes connecting with saline saturated filter paper strips (Furasawa 1929) and spaced about 1-2 mms apart were sealed into the side of the box, 4 on one side of the partition and 6 on the other. This chamber was made primarily for the recording of an action potential from a single fibre at various places on that fibre during anoxia. The only differences from the vertebrate chamber were the platinum electrodes and the absence of the ledge. The platinum electrodes were used because silver is toxic to crustacean nerves (Marmont 1940). The ledge was omitted because the filter paper strips provided ample support for the nerve.

For really long anoxia periods it was necessary to leave the nerve attached so that a third chamber was constructed large enough to include the whole limb of the animal if necessary. This chamber consisted of a large lucite box $5\frac{1}{2}$ inches long, $2\frac{1}{2}$ inches wide, and $1\frac{1}{2}$ inches high. A lucite

holder adaptible to either the leg of a lobster or the claw of the crayfish was constructed as shown in the lower diagrams of figure 1. The wire connections (L) ran through small holes sealed with vaseline in the side of the large box. The gas inlets and outlets were the same as in the vertebrate chamber. The claw or leg holder consisted of two parts. The base section (F) was shaped (G) to fit the propodite (H) at least approximately. The limb was held firmly in place by elastics (K) looped over the propodite and the ishiopodite (J) to heavy wire rods (E) fixed in this section. The second part of the holder was made of a small lucite block (A) into which were sealed with parafin 4 platinum fine wire electrodes (C) connected with fine copper wire leads (L). The parafin served to cover the solder joints and the unlaquered copper so that no foreign ions came in contact with the solution. 2 prongs (B) fastened at the bottom of the "electrode holder" fitted into 2 holes (D) bored in the base section. It was found, as others had observed, (Furasawa 1929) that if small strips of filter paper (C) soaked in saline were placed on the platinum wire the nerves lasted longer than if in direct contact with platinum wire. Also in the case of small nerve bundles there was less leading off artifact than with direct wire contact.

The mounting of the preparation on this combined holder-container is shortly to be described. A leg, or claw, was cut from the animal at the ischiopodite. The nerve was exposed (Wiersma and van Harreveld 1936) in the ischiopodite

and the limb fastened to the base of the holder with the elastics. The whole base with limb was held by a jaw clamp to a ring stand and submerged in a dish of saline (sea water in the case of the lobster). The nerve fibre or bundle was then prepared (Wiersma and van Harreveld 1936), and when ready for placing on the electrodes the holder was rotated 90° clockwise so that the limb was on its side and the holes for the prongs of the electrode holder were facing up. With a needle the nerve was held away from the shell while the prongs of the electrode plug were inserted into the holes from above, the filter paper strips being slid in between the nerve and the shell. The needle was then withdrawn and the holder rotated back through 90°. The weight of the nerve brought it slowly down onto the filter paper. The holder was then carefully raised until the preparation and holder were above the solution, whence the holder was removed from the clamp and placed quickly in the lucite box. The four leads were pushed through the vaseline in the holes in the side of the box and the lid put on so that the nerve would not dry out. Saline, about 1/8th inch deep, covered the bottom of the box keeping the enclosed air saturated. With the connecting of the gas tubes to the chamber the experimental set up was complete.

Electrical Equipment

A square wave generator was used for stimulating the nerves. It consisted of a relaxation oscillator feeding into a peaking amplifier. The pulses from the latter tripped

a self-blocking multivibrator circuit which swung one way and remained there for a length of time determined by the time constant of its circuit. The frequency of stimulation was governed by the time constant of the relaxation oscillator. The duration of the square wave was controlled by the time constant of the multivibrator. A potentiometer in the cathode circuit of an output triode determined the amplitude of the output linearly up to 22 volts. The wave shape from this instrument was extremely rectangular.

The action potentials were recorded by a cathode ray osscillograph via a five stage amplifier. The potentials were led to the grids of a push-pull differential amplifier which to a large extent eliminated pick up of stray fields and reduced the stimulus artefact. Electric fields and stimulus artefact affect the input grids at the same time so that the output of the tubes would swing simultaneously in opposition to one another canceling any deflection, while the nerve potential causes the input grids to vary one at a time resulting in a faithful variation. The sweep of the osscillograph was linear when repetitive, but became partly logarithmic when single. Single sweeps were obtained by overbiasing the thyratron tube so that it could not fire unless a pulse was sent in to its grid from the outside. When repeatedly firing the condensor in the circuit discharges quickly to a certain voltage whence the tube cuts off and the condensor recharges again. A very linear part of the discharge curve can thus be obtained because the condensor

can only charge up to a value at which the tube will fire and this may be adjusted to any desirable amount. With the single sweep after the tube cuts off and the condensor starts to charge pulling the electron beam spot across the screen the condensor completes its charge bringing in the logarithmic part at the end of the sweep since the tube cannot fire again because of the overbias. Although the lack of linearilty might cause distortion of the potential wave shape the single sweep was advantageous in that it could be synchronized with the stimulator. This made it possible to record action potentials with only a few stimuli thus eliminating overstimulation of the preparations. A more detailed description of this equipment is included in the thesis by G. Marmont (1940).

For measuring nerve depolarization a slow moving galvanometer of 15000 ohms resistance with relatively low sensitivity was connected through a low-noise push pull amplifier to the preparation. This amplifier was designed by Mr. J.B. Hawes of the National Technical Labs and is diagrammed in Fig. 3. It consisted of 2 input tubes (932's) operating at 21 volt plate voltage with a 15 volt balancing bias. Under these conditions the grid current was practically nil so the apparatus was essentially a true micro-voltmeter. The first stage fed into a 6C8G double triode output tube. The latter was operating at 66 plate volts and 1 volt cathode bias. The apparatus became quite stable after several hours of operation, this stability being insured at sensitivities

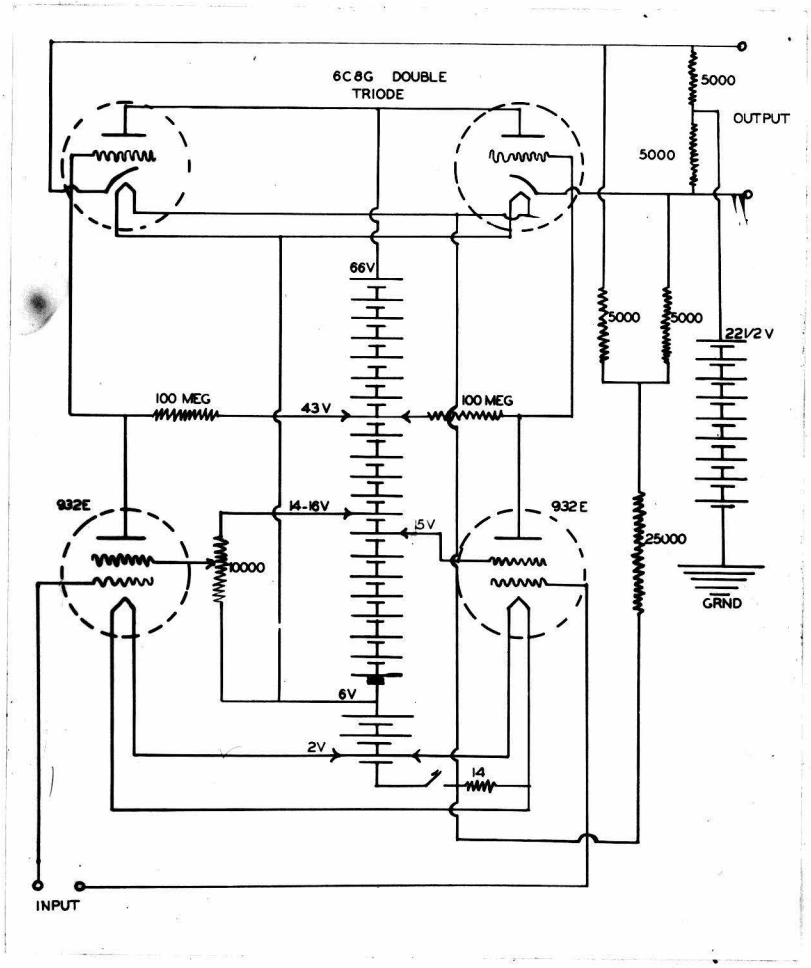


Fig. 3. Diagram of D. C. Amplifer designed by J. B. Hawes of The National Technical Laboratories for depolarization of nerve experiments.

down to 1 millivolt per cm deflection. (Maximum sensitivity with the galvanometer used was 5 mms deflection per microvolt approximately.)

Temperature Control

The nerve chambers were placed in a large metal container which was submerged to its brim in a constant temperature water bath. A thick asbestos cover fitted on the container above the water surface keeping the heat from escaping into the room air. A hole was drilled in the cover to hold a thermometer. The bath was regulated by a thermostat control to $\frac{1}{2}$ degree Centigrade. Only for temperatures higher than room temperature was this control used. At low temperatures ice was melted slowly in the bath. The resulting cooling lasted sufficiently due to the large amount of water, so that experiments could be begun at 18 degrees, for example, and during a two hour period vary a degree to a degree and a half one way or the other. This provided sufficient accuracy, since most experiments with mammalian nerves required shorter times.

Gases

In all experiments nitrogen was used for asphyxiation. It was obtained from the Linde Air Products Co., of Los Angeles, and was rated by the company as containing slightly less than 0.3% oxygen. The nitrogen was led through a two foot metal tube containing heated copper gauze to eliminate the small amount of oxygen. The oxygen free gas then passed through two wash bottles filled with water to saturate the

gas before entering the chamber. At first mixtures were made using 5% CO_2 to keep the pH in the chamber constant, (Lehman, loc cit) but it was soon found that this added detail made no difference on the nerve behavior during asphyxia or recovery therefore it was discontinued. It was also found that passing nitrogen through heated gauze had no noticeable effect on the survival time, thus this procedure was discontinued.

Nerve Preparations

Mammalian Nerves

Nerves from rabbits, cats, and a few from dogs were used, the procedure for their removal from the animal being the same in all cases. The animals were well narcotized with nembutal and the nerves carefully removed and placed in a beaker of physiological solution at room temperature. The tibial and peroneal of the sciatic nerve were separated and removed from the leg in lengths greater than 7 cms. The phrenic nerve also could be excised in great lengths, but this operation took longer and involved killing the animal by pneumothorax before the nerve was accessible. It was found that nerves removed even shortly after death showed a pronounced shortening of the survival time and were generally in worse condition than those taken from a live animal. Use of the phrenic nerve in most instances was avoided for this reason. The saphenous sensory nerve and the radial and ulnar nerves of the foreleg though shorter made excellent preparations.

To remove the tibial and peroneal nerves, the skin was cut and pulled back from the thigh and an incision made separating longitudinally the fibres of the biceps femoris. When this incision was spread apart the sciatic nerve was exposed resting on the adductor femoris. The nerve runs between these two muscle layers mentioned. By moving the prong of a forceps gently along the nerve the sheath was stripped from it and the peroneal and tibial bundles fell away from each other. These were readily tied off and removed.

The saphenous was taken from the medial thigh be removing the skin and very carefully separating the connective tissue underneath with a forceps. The nerve is attached along its length to the saphenous artery and the saphenous vein by connective tissue, and it must be teased away from both.

The radial, medial and ulnar nerves are located in the upper foreleg lying between the biceps and epitrochlearis muscles. Pulling back the epitrochlearis muscle exposed these nerves which could be then teased away from connective tissue and blood vessels. The ulnar nerve lies adjacent to the axillaris artery, so again care must be taken in its removal to avoid rupture of this vessel.

All nerves were placed in Tyrode's solution at room temperature except in those cases described in the Results Section. This same solution was used for washing the incision in the animal during the excision of the nerve whenever necessary.

To asphyxiate the nerve, it was taken from the beaker and placed gently in the trough on the ledge in the chamber. A few drops of Tyrode's solution warmed to 38 degrees were placed in the bottom of the chamber and over the nerve. If it was desired to divide the chamber in two parts the vertical slit in the partition was sealed with vaseline before the cover was screwed down on the chamber. Glass pipettes connecting with the gas hoses were inserted in the holes in the wall of the chamber, whereupon the whole chamber was placed in a metal box to which were fastened shielded cables from the stimulator and amplifier. The ends of the cables were soldered to jacks which could be inserted into sockets to which the Ag-AgCl electrodes were soldered. This way of electrical connection eliminated the moving around of cumbersome long heavy wires. The metal box was then lowered into the larger metal container submerged to its brim in the water bath and the asbestos cover placed on top. A thermometer was inserted through the hole in the top for temperature readings. A test was made by stimulating the nerve briefly and noting whether or not it responded with action potentials of the proper size and shape. The size of the potential disclosed whether the right amount of fluid was around the nerve and whether good electrical contact was being made. If the shape were peculiar, i.e., monophasic instead of diphasic, something more serious was wrong and adjustments made. Usually oxygen was allowed to bubble into the chamber first, passing through the wash bottles immersed

in the bath. This helped speed up the warming of the interior of the nerve chamber to that of the bath. When the appropriate temperature was attained, the oxygen was replaced by nitrogen and asphyxiation begun. The normal nerve recordings, threshold, amplitude of potential and so forth were made during the first oxygen period when the right temperature was reached and just before the nitrogen was turned on. During asphyxia recordings were repeated every 2, 3, or 5 minutes until the nerve no longer responded. The technique was the same during recovery except in many instances the records were noted every 30 seconds. Variations of these methods for special purposes are explained in the Results.

Amphibian Nerves

The sciatic nerves of Rana pipiens and Rana catesbiana were used. Both young and old frogs were used, 10 cms of nerve being easily removable from old and large bullfrogs. When removed from the animal these nerves were placed in Frog Ringers at room temperature. To be asphyxiated they were placed in the chamber used for mammalian nerves and the procedure described above employed. It was not necessary except in special cases to use the temperature control bath.

Crustacean Nerves

Nerves taken from the rock lobster, Panulirus interruptus, and from the crayfish, Cambarus clarkii, were used, the same technique applying to both. The leg (Panulirus) or claw (Cambarus) was removed from the animal by pinching the ischiopodite. The medial half of the chitin of the meropodite was chipped off with a pair of snippers and the tendon

of the flexor muscle was removed with forceps exposing the nerve lying along the extensor muscle. The extensor muscle was cut and pulled gently away leaving the nerve isolated. The limb was then fastened to the holder and submerged in sea water (Panulirus) or van Harreveld solution (Cambarus). Under a binocular dissecting microscrope the small nerve bundles were teased apart using fine needles (Wiersma and van Hareveld 1936). Although it has been reported that single fibres left attached to the muscle could last 6-8 hours (Marmont 1940) it was not possible to keep them that long in saturated gas surroundings on filter paper supports. Long durability of single fibres depends on their being supplied with a fluid medium of the proper ionic concentration. On account of the large stimulation current escape especially with high thresholds during anoxia such a medium was impractical. However, much longer survival times could be obtained from small bundles of only a few fibres of which 4 or 5 functioned. Presumably the fluid between the fibres of such a preparation acts as a proper medium for the few active fibres. Although somewhat shortcircuited, the individual fibre potentials were still distinguishable and the stimulus artefact was not too great. These preparations lasted 5 hours or more. When the nerve was prepared the electrode contacts were made as already described elsewhere. It was found that the lobster nerves remained in much better condition for greater periods if held at about 19 degrees.

RESULTS A

Asphyxiation

Survival Time

The survival times of 28 rabbit nerves, 10 cat nerves, and 3 dog nerves are presented in Table I. Each of these nerves was placed in nitrogen immediately upon removal from the animal.

Rabbit

The average survival time of the rabbit nerves was 23.4 minutes. 3 rabbit nerves required a period of 35 minutes without oxygen before becoming completely inactive. One rabbit nerve lasted only 15 minutes without oxygen before losing all signs of response to a stimulus. The survival times of the remaining 24 rabbit nerves are between these two extremes, 15 and 35 minutes. 50% of them are 20 minutes or less, 25% of them are 30 minutes or more.

The specific nerve used is also listed in Table I. It is clear that no specific survival time can be correlated with a specific nerve type. The saphenous nerve might be an exception (as reported for cat saphenous nerves by Lehman, see below) but since only 2 were used no conclusion is possible at present.

All the nerves removed from one animal for experimentation were removed from that animal in one large operation and were placed in physiological solution all at about the same time. Thus only the first nerve to be asphyxiated of such a group could be considered fresh since the others remained in the Tyrode solution for 45 minutes or more while

TABLE I

The survival time of mammalian nerves asphyxiated immediately after removal from the animal.

Nerve Type	Rabbit Nerve Survival Time	Cat Nerve Survival Time	Dog Nerve Survival Time
Tibial	18 mins	45 mins	
"	16	30	
"	30		
"	20		
"	28		
"	20		
"	16		
Peroneal	28	25	26 mins
"	15	45	35
"	35	33	
"	28	43	
"	35		
"	23		
"	22		
"	22		
"	17		
"	20		
"	30		
"	30		
"	16		
"	16		
Saphenous	18	35	35
"	16		
Medial	23	20	
"	15		
Radial	25	phrenic 18	
Name not recorded	32	35	
"	35		
	ave 24	ave 33	33

this asphyxiation was going on. Therefore only 1 fresh nerve per animal was asphyxiated so that specific survival times for different nerves from one animal cannot be correlated.

Cat

The survival times of 10 fresh cat nerves ranged between 18 and 45 minutes and averaged 32.9 minutes. Lehmann reported an average of 30 minutes for cat nerve. He also pointed out that the saphenous nerve survival time was always shorter than the survival times of the other nerves of the cat (16-18 min.). The 1 saphenous nerve used in the present work had a survival time of 35 minutes. Only 2 nerves lost their activity in less than 20 minutes in nitrogen, while 7 of the 10 used were excitable for 30 minutes or over.

Dog

The survival times of the 3 fresh dog nerves listed averaged 33 minutes. Gerard gives 30 minutes as an average survival time for dog nerve.

Lobster

The survival times of 20 lobster nerves are illustrated graphically in Fig. 4. They ranged from 30 to 120 minutes with more than 50% having values between 60 and 90 minutes. 3 of these 20 nerves were not prepared beyond being exposed by removal of flexor and extensor muscles in the meropodite. Each of these 3 nerves became completely inactive after 75 minutes in nitrogen. The average survival time of the other 17 nerves, which were prepared to fine bundles, was 77 minutes.

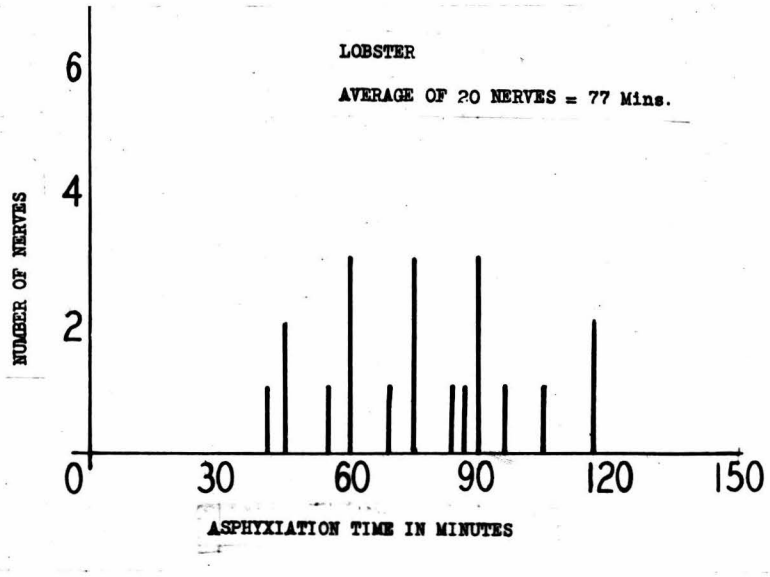


Fig. 4. The survival time of Lobster nerves. Ordinate is number of nerves, abscissa is time (minutes) for complete extinction of the spike action potential of a single fibre.

Frog

Frog nerves could endure anoxia much longer. The nerves of Rana pipiens of which 3 fresh ones were used had survival times of about 2 hours. They are tabulated in Table II. Nerve 118 was bathed in ringers for 2 hours before being asphyxiated and the resulting effect is described elsewhere.

Only 2 fresh nerves of Rana catesbiana were asphyxiated, numbers 155 and 186 in Table III. These maintained their activity in nitrogen for $2\frac{1}{2}$ and $3\frac{1}{2}$ hours respectively. The other nerves listed in this table were aged in physiological solution and are included in another discussion. Since almost all the previous anoxia work on peripheral nerve has been done using frog nerves and since the results reported are identical to those above it was deemed sufficient to limit the present work to the 5 experiments described.

Crayfish

Crayfish nerves subjected to a nitrogen atmosphere remained excitable to the end of the experiment in the majority of cases. The irritability of a prepared opener bundle was found to be as great after 5 hours in nitrogen as at the start. The activity of 3 closer bundles did not show the least sign of change in 100, 120, and 150 minutes anoxia. In control experiments both claws were taken from the same animal and placed one in nitrogen and the other in oxygen. After 5 hours the activity of both was identical. This was repeated with 2 more claws from another animal for 9 hours

TABLE II

Sciatic nerves of Rana pipiens (Leopard Frog) asphyxiated in nitrogen. Stimulation by direct current of 0.1 millisecond duration.

Nerve	Time	Temp.	Minutes in N_2	Threshold	Remarks
63	0	23	0	11	large, diphasic
			15	13	
			37	15	
			65	15	becoming monophasic
			95	20	1/3 original size monophasic
65	0	23	0	11	large, diphasic
			5	13	
			10	14	
			15	14	
			20	-	
			30	18	
			50	22	
			60	22	
			75	22	monophasic
			90	22	
			105	22	
			120	25	at 2-5 shocks/sec.
				30	at 20-30 shocks/sec. not responding to every shock.
119	0	24	0	14	large, diphasic
			5	14	
			10	14	
			45	18	
			85	25	monophasic, small
			100	x	
118	2 hrs.	26	0	5	large, diphasic
			30	5	
			60	5	
			75	5	
			90	5	
			105	6	less diphasic
			120	8	
			135	10	
			150	12	
			160	15	
			180	18	Wedensky block at 60 cycles
			190	20	
			200	-	
			210	23	monophasic, very small, rapid fatigue

TABLE III

Sciatic nerves of Rana catesbiana (Bullfrog) asphyxiated in nitrogen. Stimulation with direct current of 0.1 millisecond duration.

Nerve	Time	Temp.	Minutes in N_2	Threshold	Remarks
121	2 hrs.	25	0	6	large, diphasic
			25	6	
			75	8	
			100	10	
			125	10	
			155	10	
			185	10	becoming monophasic
			213	11	
			245	11	
			275	12	1/4 original size monophasic
154	3 hrs.	26	0	5	see fig. 2
			15	-	1.2 normal size
			45	-	1.3 " "
			120	-	1.0 " "
			180	-	0.7 " "
			210	-	no measurement
			240	15	0.1 normal size
155	½ hr.	26	0	10	large, diphasic
			15	10	
			45	11	0.9 normal size
			90	-	0.8 " monophasic
			130	16	0.2 " "
			150	24	0.01 " "
186	0	25	0	6	large, diphasic
			60	11	
			120	13	monophasic
			160	16	
			200	20	almost gone
187	4 hrs.	25	0	6	large, diphasic
			180	-	1/4 normal size
			300	25	small potential left, Immersed throughout
188	24 hrs.	25	0	11	large, diphasic
			60	-	
			180	-	
			240	23	1/2 normal size
			330	x	gone
189	48 hrs.	25	0	29	large, diphasic
			120	-	
			240	54	monophasic
			360	-	almost gone
			390	-	gone

with the same result. At the end of the 5 hour period the nerve action potentials were somewhat larger and both closer and bender muscles contracted, whereas only the bender contracted after 9 hours.

To check the apparatus for possible leaks etc., 6 legs from the same lobster were tested, 3 in oxygen and 3 in nitrogen for $3\frac{1}{2}$ hours. All those in oxygen retained their excitability, but those in nitrogen were completely inactive after the $3\frac{1}{2}$ hour period. Thus the chamber is gas tight. The conclusion is therefore that it is not possible to sustain Cambarus nerves long enough to show any effect of anoxia.

All mammalian nerves were asphyxiated at 38 degrees centigrade. Frog and crayfish nerves were held at room temperature which varied from 23-26 degrees. Lobster nerves were asphyxiated at 19 degrees.

Effect of Time in Solution Before Asphyxiation on Survival Time

It has been emphasized in the preceding paragraphs that the survival time determinations were made with fresh nerves, i.e. nerves just removed from the animal. This was done because it has been reported that the metabolic rate of excised nerve in physiological solution changes rapidly. The oxygen uptake rate of mammalian nerve is quite reduced after bathing the nerve in saline for an hour or so. The same change occurs more slowly in frog nerve. Such a variation might affect the survival time of a nerve deprived of oxygen.

A study of such a survival time effect is not to be found in the literature therefore it was undertaken in the present work.

The results from mammalian nerves are collected in Table IV, from frog nerves in Tables II and III. The survival time of frog nerve, both species, increased with 2 to 8 hours washing in physiological solution. Since it takes approximately 2-4 hours to asphyxiate a frog nerve it is not surprising that frog nerves asphyxiated immersed in solution endure longer than those asphyxiated in gaseous media (Fillie et al) for the washing effect would become significant in that lenth of time. Washing before asphyxiation causes the survival time to rise toward a limit of about double the value for fresh nerve.

In direct contrast to this, the survival time of the mammalian nerve decreased with washing in physiological solution. This decrease is not continuous, for it approaches a limiting value approximately 70% of the value for fresh nerve.

Effect of time in serum on survival time

Gerard reported in 1932 that the metabolic rates of excised peripheral nerve remained more constant if the nerve was bathed in serum from the blood of the animal than when bathed in physiological solution. A brief study was therefore made to compare the survival times of asphyxiated nerves in serum to those bathed in physiological solution. The data is recorded in Table V.

TABLE IV

Effect of aging in physiological solution. The survival times are listed under the number of hours the nerve was left in saline before being asphyxiated.

hours in saline	1	2	3	4	5	6	7	8
Rabbit nerves	30 mins 12 21 18 17 17 17 16 25 15 20 ave 19		15 mins 25 25 22 15 12 12 19 17 20 17 26 12 18		17 mins. 18 17 14 25 18	18 min.	13 mins	
Cat nerves		21 17 20 22 27 33 ave 23	10 27 25 10 18	20	10			
Dog nerves		30	20	23				

TABLE V

Asphyxiation times of nerves placed in saline compared with asphyxiation times of nerves placed in blood serum.

Nerve	Solution	Asphyxiation Time	Remarks
158 Rabbit radial	serum	35 minutes	
159 "	saline	24 "	158 and 159 from same animal.
160 "	serum	32 "	30 minutes recovery in saline.
160 "	saline	20 "	
161 "	saline	22 "	30 min. recovery in serum.
161 "	serum	20 "	160 and 161 from same animal.
166 Rabbit Medial	saline	26 "	1 hr. recovery in saline
166 "	"	16 "	2 hrs. recovery in saline.
166 "	"	16 "	
167 "	serum	27 "	1 hr. recovery in serum.
167 "	"	21 "	2 hrs. recovery in serum.
167 "	"	22 "	166 and 167 from same animal.
168 "	"	26 "	washed in serum $3\frac{1}{2}$ hrs. after removal from animal before experiment.
169 "	saline	17 "	washed in saline $3\frac{1}{2}$ hrs. after removal from animal before experiment. 168 and 169 from same animal.
178 Cat Radial	serum	36 "	washed in serum $\frac{1}{2}$ hr. after removal from animal.
179 "	saline	21 "	washed in saline $\frac{1}{2}$ hr. after removal from animal.
180 "	serum	30 "	washed in serum 1 hr. after removal from animal. 178, 179, 180 from same cat.

Nerve 158 was bathed in aerated serum 30 minutes before asphyxiation was begun, 159 was in aerated saline for about 1 hour and then asphyxiated. Nerve 160 was treated the same as 158 except that after being asphyxiated it was placed in aerated saline for 30 minutes and then asphyxiated a second time. This procedure was reversed with nerve 161. Nerves 166 and 167 were bathed only 5 minutes in serum and saline respectively and then asphyxiated. They were then placed in aerated serum and saline respectively for 1 hour and asphyxiated again. They were then given 2 hours washing in their solutions and asphyxiated a third time. The procedures for nerves 168, 169, 178, 179, 180 are clearly outlined in the remarks column of the table. It is evident that the survival time suffers a lesser change with washing of the nerve in serum compared to washing of the nerve in saline. For example, the survival time of nerve 166 is reduced 39% in saline whereas the survival time of nerve 167 is reduced 23% in serum in the same period of time. The difference is demonstrated clearly by the survival times of nerves 158, 159 and 168, 169 and 178, 179.

Effect of ionic concentration (Calcium) on survival time

Chang and Gerard (1936) describe a depression of the oxygen uptake rate in peripheral nerves bathed in physiological solution containing excess calcium ion concentration. This is reversed with calcium lack. Therefore a study was made of the calcium (and potassium) ion concentration effect on the survival time of peripheral nerves in nitrogen. The re-

sults are seen in Table VI. The bracketed number indicate the asphyxiation number. Thus nerve 35 was asphyxiated first in normal ringers requiring 33 minutes in nitrogen. It was then allowed to "rest" 30 minutes in aerated normal ringers, placed in aerated calcium free ringers for a few minutes and then asphyxiated a second time requiring only 24 minutes. This procedure was reversed with nerve 36. The survival times were observed to be consistently longer than normal when the nerve was bathed in solution with excess calcium ions and shorter than normal when this ion was lacking from the solution.

Temperature Effect on Survival Time

14 frog nerves were asphyxiated, 5 at room temperature (25 degrees) and 9 at temperatures of about 30 degrees. All 5 nerves asphyxiated at 25 degrees withstood 150 minutes in nitrogen before losing their activity completely, whereas those asphyxiated at 30 degrees or more survived only 70 to 135 minutes in nitrogen. The results are illustrated graphically in figure 5, upper half.

The lower half of the figure shows the survival time data of 9 rabbit nerves asphyxiated while at various temperatures. 3 nerves were asphyxiated at 38 degrees, controls, the others at lower temperatures. The survival times at lower temperatures were longer than those at higher temperatures.

It is known that the metabolic rate of peripheral nerve varies directly with temperature.

TABLE VI

The effects of variation of Calcium and Potassium ion concentrations upon asphyxiation times of nerves.

	Nerves	Time in Normal Ringer	Time in CA Free Ringer	Time in CA Excess Ringer	Time in K Excess Ringer
35	Cat Saphenous	33 (1)	24 (2)		
36	"	31 (2)	21 (1)		
39	Cat Peroneal	24 (1) 26 (3)	18 (2)		
40	"	17 (1) 19 (3)		30 (2)	
41	Rabbit Saphenous	25 (1)	16 (2)		
43	Rabbit Medial	15 (1)			15 (2)
44	"	14 (1)		15 (2) 14 (3) 12 (4)	
45	"	10 (1)		13 (2)	
46	Rabbit Peroneal	36 (1) 35 (3)	20 (2)		
47	"	20 (1)		27 (2)	
48	"	28 (1)			23 (2)
49	"	17 (1)			8 (2)
50	Dog Saphenous	35 (1)		30 (2) 35 (3)	

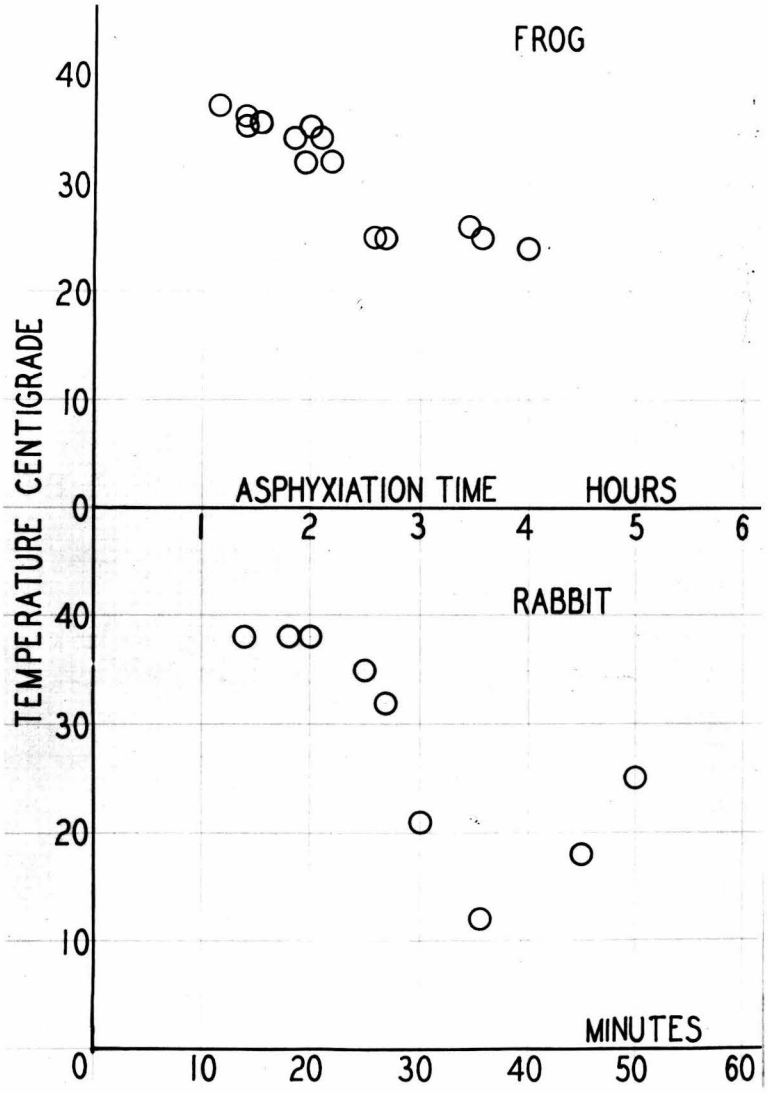


Fig. 5. Graph showing change of survival time in nitrogen with change in temperature. Frog nerve (upper), rabbit nerve (lower).

Stimulation and Fatigue Effect on Survival Time

It has been shown that the metabolic rate of frog nerve increases with stimulation (Fenn 1928). It was expected that the survival time would be reduced by stimulation during anoxia compared to a similar period sans stimulus. This was investigated by using continuous faradic stimulation throughout the asphyxiation and by waiting until the nerve showed signs of losing its excitability and then beginning the faradic stimulation. The survival times of nerves stimulated with frequencies of 0, 60, 180, and 240 shocks per second were compared. Rabbit nerve was used.

The results showed no significant difference in the survival times. At first glance this was puzzling, but the metabolic rate increase from continuous activity as measured by oxygen uptake was less than 15% in frog nerve, any change in the survival time of a rabbit nerve on that basis would be of the order of a minute which would not be significant. Fig. 12 shows a plot of two experiments on nerve 76, a rabbit peroneal. The decrease in amplitude of the action potential was actually more rapid with 15 shocks per second continuous stimuli than with 60 shocks per second and since the 15 shocks per second test was the second asphyxiation of the nerve it is probable that the effect of aging smothered any small change due to the stimulation.

Excitability Threshold and Action Potential Amplitude Changes During Anoxia

When a peripheral nerve is subjected to an oxygen free atmosphere, two changes occur in the electrical activity of

the nerve. The threshold for electrical excitation rises and at the same time the amplitude of the action potential diminishes. Neither of these two changes, however, begins immediately upon commencing asphyxiation. There is always an initial period the length of which varies among the different nerves, during which normal excitability and electrical responses are maintained. Indeed, in some instances the threshold has been observed to decrease slightly during the very early stages of anoxia. Lehmann, Gerard and Heinbecker have reported this super-normal stage, and it was observed in the course of the present work.

In many experiments undertaken to investigate the threshold and response changes in asphyxiated nerve, it was noted that a diphasic action potential became monophasic quite some time before disappearing altogether. This finding was important because it meant that the change in activity was not uniform along the nerve. An example of the phenomenon is illustrated in Fig. 6. The large negative spike was mostly off the cathode ray screen for the first 14 minutes of asphyxiation. In the same interval the positive phase disappeared altogether. Thus the region over the distal lead near the end of the nerve became inactive long before areas further in from the end lost their activity. This effect was called, for convenience, the end-in effect. It was important to know whether this was merely a local occurrence, restricted to a small area around the cut end, or whether it progressed along the nerve. A frog nerve was asphyxiated

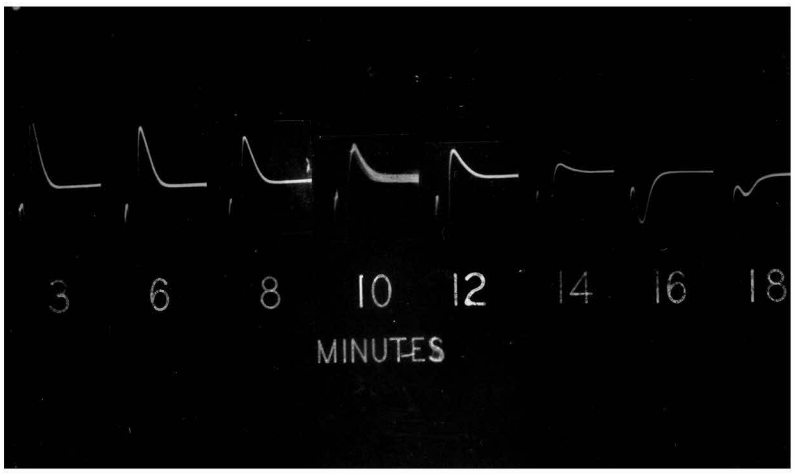


Fig. 6. Positive phase of diphasic action potential of cat phrenic nerve at various stages of asphyxia. The positive phase has disappeared by 16 minutes, the negative phase lasted 23 minutes.

with 3 lead off electrodes, no.1 contacting the middle of the nerve, no.3 contacting the end, and no.2 contacting the area between the other two. The frog nerve was used because the changes were slower and better records were obtainable. The results of this experiment are illustrated in Fig. 7. The upper row of pictures, reading left to right, were taken using leads 1 and 3 after 30, 60, and 90 minutes in nitrogen. The lower row were taken at the same times using leads 1 and 2. The ratio of the positive (down) phases at these times, leads 1 and 3/leads 1, 2, were 1.0, 0.6, 0.43, and 0.4. Thus lead 3 was becoming inactive more rapidly than lead 2. The ratio of positive to negative phase of the 1, 2 lead potential showed no significant change during the 90 minute asphyxiation. Thus the action potential in that area of the nerve was as diphasic at the end of the experiment as at the beginning whereas the action potential from leads 1, 3 became almost completely monophasic.

It was concluded that the effect was limited to the region immediately surrounding the cut end of the nerve. Experiments by F.O. Schmidt (1939) have indicated a higher oxygen uptake rate at the end of the nerve.

This conclusion suggested that the threshold rise too might be influenced by the end-in effect unless the stimulating electrodes were placed well in from the cut end of the nerve. The results of several experiments undertaken to test for this influence are collected in Table VII. The left hand column shows the change in threshold during anoxia with stimulating electrodes near the cut end of the nerve. The right

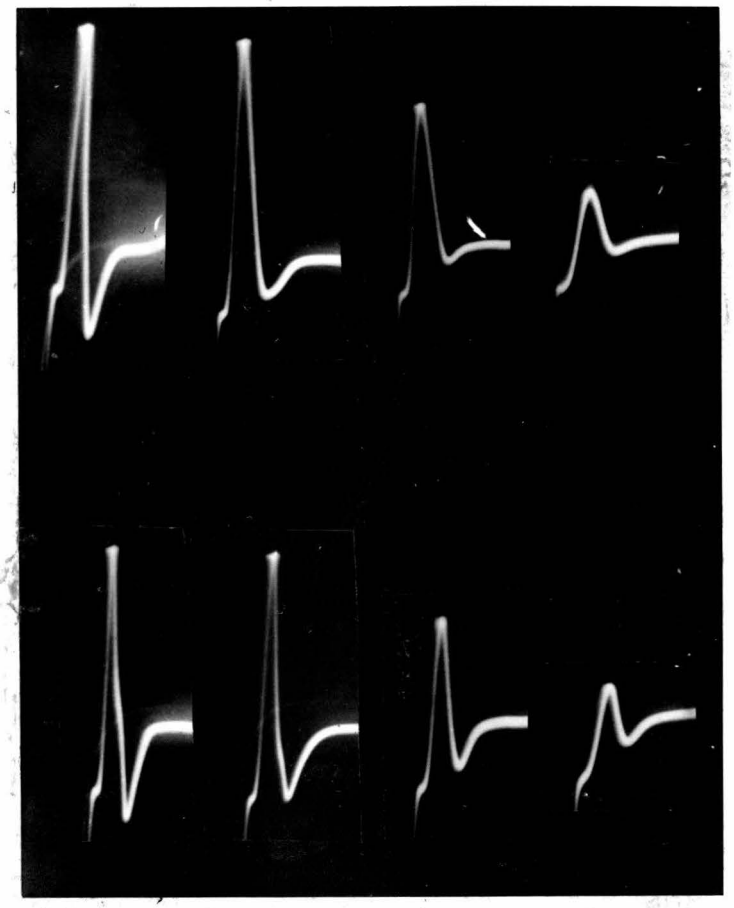


Fig. 7. - Action potential of frog nerve combining common lead near middle of nerve with one near the end (upper), and one nearer the middle (lower). As asphyxia progresses (left to right) note that the distal lead becomes monophasic, the medial lead does not, showing the end-in effect from the cut end.

TABLE VII

Threshold change during anoxia with stimulating electrodes placed near the nerve end listed in columns on left; with stimulating electrodes placed well in, 1 to 2 cms, from nerve end listed in columns on right. All nerves aged ½-1 hour in saline.

	Time in N_2	Threshold		Time in N_2	Threshold
Rabbit Saphenous	0 mins	6	Rabbit Peroneal	0 mins	10
	3	6			
	5	8		6P	10
	8	9			
	10	10		10P	10
	12	10			
	15	11		15P	11
	19	23		20P	12
	25	infinite		25	infinite
Dog Saphenous	0 mins	2	Dog Tibial	0 mins	2
	2	2			
	5	2		5P	3
	8	2			
	10	3		10	3
	13	3			
	16	3		17P	4
	20	5		20	4
	24	7		25P	4
	28	9			
	30	10		30P	4
	32	infinite		35	infinite

hand column shows the threshold change when the stimulating electrodes were placed 1 to 2 cms away from the cut end. The threshold units are 1/200ths of a volt. It is clear that the threshold of excitability begins to change near the cut end of the nerve sooner than it does well away from this injured area. Photographs of action potentials of a rabbit nerve taken after 5, 10, 15, and 20 minutes in nitrogen are reproduced in Fig. 8a. The action potential of a dog nerve taken after 5, 17, 25 and 30 minutes in nitrogen are seen in Fig. 8b. In both cases the threshold remained very constant (see table figures with suffix P for the threshold reading when picture of action potential was taken) while the action potential decreased. The lead off electrodes were near the end of the nerve, the stimulating electrodes were well in from the end. Thus it can be concluded that nerve activity in general is more susceptible to asphyxiation near the cut end of the nerve than it is further in along the nerve on uninjured tissue. The end in effect was also observed with single nerve fibre preparations from lobster nerve. A poor example of the effect is illustrated in Fig.9. The single action potential (middle photographs) is somewhat more diphasic after 20 minutes in nitrogen than after 115 minutes, but the distinction in this record is not as clear as was observed with other preparations.

It is not likely that the effect would influence survival times very much, because survival time was determined uding strong enough stimuli to excite nerve tissue quite

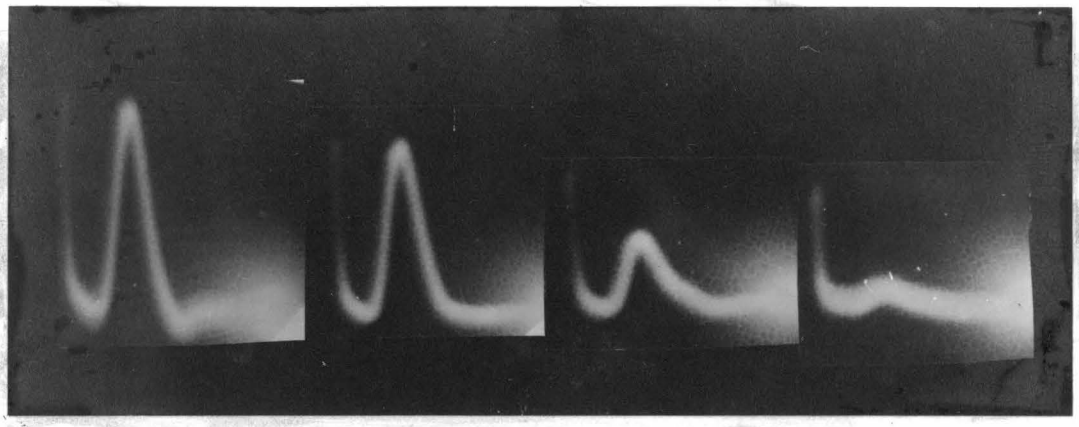


Fig. 8a. Action potential of rabbit tibial nerve after 5, 10, 15, and 20 minutes in nitrogen (left to right). Note absence of diphasic tip seen at 5 minutes in 10-minute picture. See Table VII.

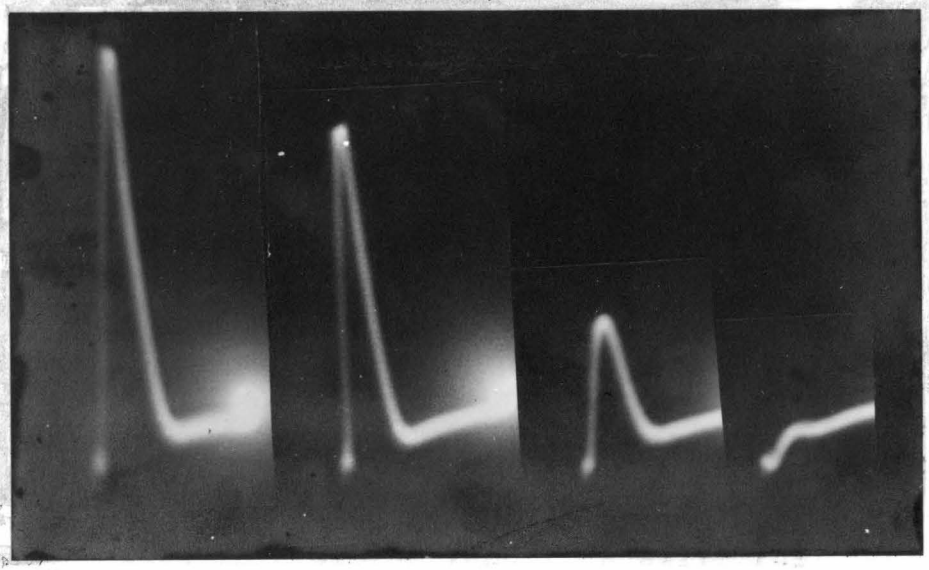


Fig. 8b. Action potential of dog tibial after 5, 17, 25, and 30 minutes in nitrogen. See Table VII.

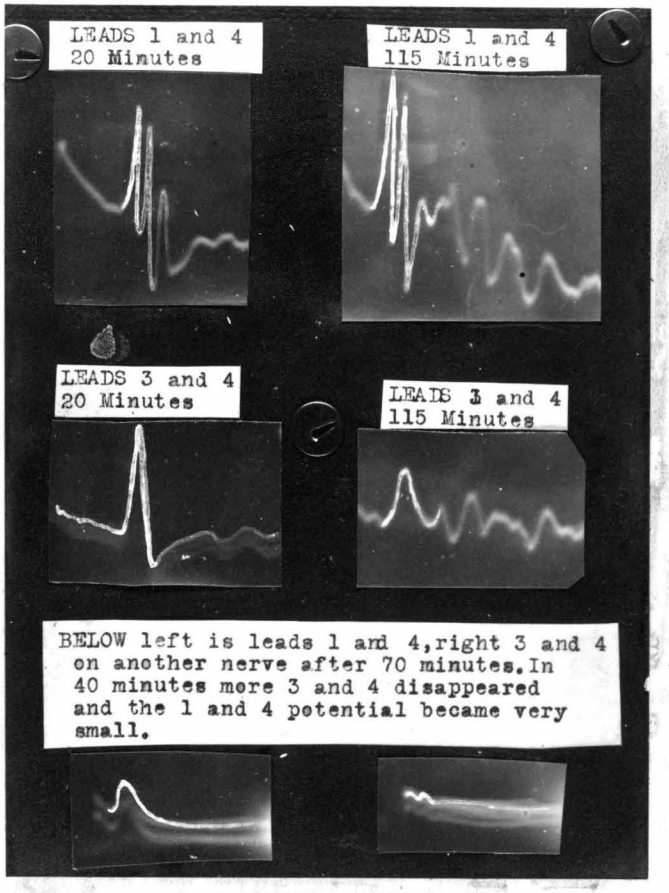


Fig. 9. Potential of single fibre of lobster leg using four leads numbered 1 to 4 from middle toward cut end of nerve. Lead 4 was common and relatively inactive. After 115 minutes in nitrogen lead 1 is still full size, but 3 is definitely smaller showing end-in effect or decremental conduction (?).

some distance along the nerve from the stimulating electrodes. However, it was clear that electrode position on the nerve was important for accurate threshold and potential studies on asphyxiated nerve. Typical curves of threshold change during anoxia are presented in Fig. 10A. Curves of action potential amplitude change during anoxia are in Fig. 10B. A curve for the nerve of each kind of animal used is included. (See legend). These curves were made from data taken with the necessary precautions to eliminate the influence of the end-in effect. The action potential amplitude is measured in percent of initial value, the threshold in 0.02 volt units.

The threshold of the rabbit nerve remained constant for about 10 minutes and then began to increase rather rapidly. The threshold of the cat nerve remained constant for 13 minutes and then rose but more slowly at first than rabbit nerve threshold. The lobster nerve threshold remained constant for 50 minutes and then rose moderately fast, and frog nerve threshold began a slow rise after 2 hours asphyxiation. The threshold of crayfish nerve did not change significantly.

It will be noted that the early drop in threshold value is absent from these curves. This suggests that the drop was due to the adding of the demarcation potential from the end-in effect to the stimulus voltage when the stimulating leads were close to the nerve end. By placing the electrodes well in from the cut end, the demarcation potential was negligible and the threshold did not decrease.

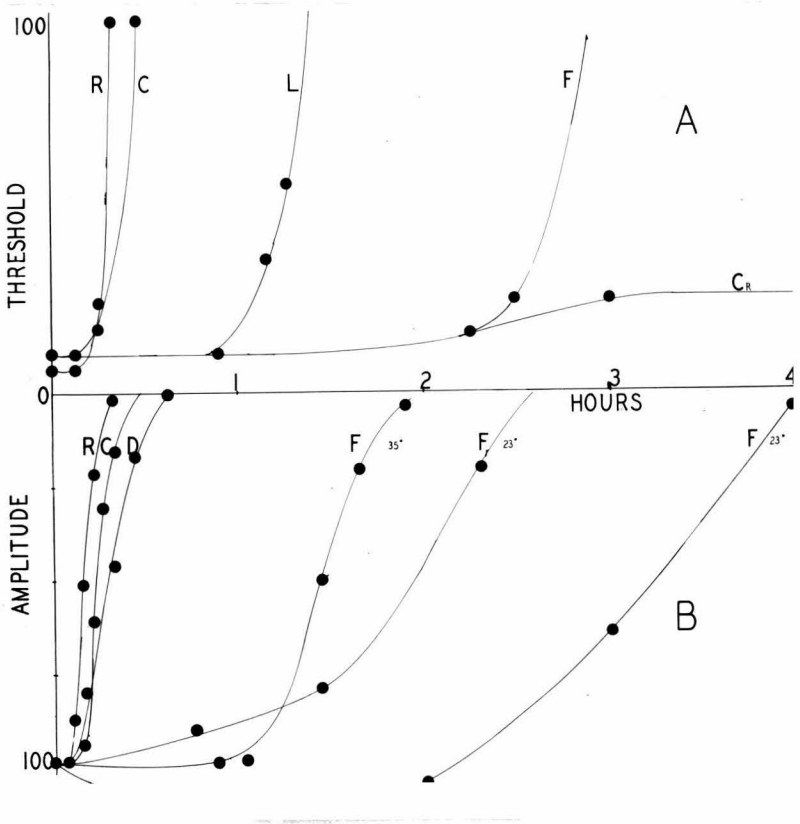


Fig 10.A. Curves showing threshold change in 0.02 volt units with time, hours, in nitrogen for rabbit nerve(R), cat nerve(C), lobster single fiber(L), bullfrog(F), and crayfish(Cr).

B. Curves showing amplitude change in percent maximum with time, hours, in nitrogen. A dog(D) nerve is included, and three bullfrog nerves. The right hand curve represents results from a frog nerve aged 3 hours in saline before anoxia. The other two curves are from fresh frog nerves one at 35 degrees, the other at 23 degrees centigrade.

The amplitude of the nerve action potential reduced during anoxia. The curve of the reduction was similar to the threshold rise curve for a nerve of the same animal. The action potentials of rabbit, cat, and dog nerves began to decrease in size after 5-10 minutes in nitrogen. The rabbit nerve potential change begins sooner than the potential changes in cat and dog nerve. The potential change curves from 3 frog nerves are included in the figure B. These curves reading left to right, were made from data from a fresh nerve at 35 degrees, a fresh nerve at 23 degrees, and a nerve bathed 3 hours in saline at 23 degrees. The curve of the fresh nerve at 23 degrees corresponds closely to the threshold curve directly above it in the figure. The amplitude began to decrease after $1\frac{1}{2}$ hours in nitrogen.

It was concluded from the threshold and amplitude data that rabbit nerve retained its normal activity for 5-10 minutes when asphyxiated; cat nerve and dog nerve slightly longer, 10-15 minutes; lobster nerve for nearly an hour, and frog nerve more than an hour.

The 2 other frog nerve curves are included in the figure to show how the effect of oxygen lack on excitability is influenced by temperature and aging in solution. It is seen that at higher temperatures the initial normal period is shortened and the rate of change of activity increased. The solution effect causes a prolongation of the initial normal period and a reduction in the rate of change of excitability.

Reduced All

The decrease in action potential amplitude during anoxia could be caused in several ways. Gerard (see introduction) concluded that the "all" response of each fibre was reduced by anoxia. Two other possibilities are the blocking of conduction in fibres at various places along the nerve, and decremental conduction. In order to investigate Gerard's conclusion the following experiment was devised.

A long frog sciatic was placed in the chamber and the partition sealed with vaseline. Nitrogen was made to enter one section, oxygen the other. The part of the nerve in nitrogen contacted 2 pairs of electrodes. The distal pair was for stimulation, the proximal for leading off. There was contact by the part of the nerve in oxygen with another lead-off pair of electrodes. Thus the nerve was stimulated in nitrogen, and the action potential recorded first in nitrogen and then in oxygen. With all the fibres conducting past both lead off pairs of electrodes the ratio of the 2 potential records would be 1. If the all of each fibre was reduced during anoxia then the ratio of the potential size in nitrogen to the size in oxygen would become less than 1. If the fibres began to stop conducting in nitrogen then the ratio in nitrogen to oxygen would increase to greater than 1. Due to leading off difficulties the initial ratio probably would vary somewhat.

The results from 2 frog nerves are given in Table VIII. In both cases the ratio decreased for the first $2\frac{1}{2}$ hours of

TABLE VIII

Nerve	Time in N_2	Ratio ($\frac{\text{spike in } N_2}{\text{spike in air}}$
229 Rana catesbiana	0 minutes	1.000
	40 "	1.000
	75 "	.834
	100 "	.680
	130 "	.640
	150 "	.550
	165 "	.562
	180 "	.635
230 Rana catesbiana	0 "	.800
	60 "	.783
	90 "	.705
	120 "	.700
	150 "	.685
	180 "	.705
	210 "	.750

anoxia. Then a slight increase was recorded. Thus the response in nitrogen in each fibre decreases until conduction blocking commences in the fibre.

To observe the reduced all response more clearly fine nerve bundles prepared from lobster nerves were asphyxiated in a double chamber. Stimulation was applied to the nerve in the aerated section. There were 4 lead off electrodes in the asphyxiation section which were numbered 1 to 4 progressing distally along the nerve. Lead 4 was common. Photographs of the action potential of a single fibre recorded with lead combinations 1-4 then 3-4, after 20 minutes and 115 minutes of anoxia are reproduced in Fig. 9. The 1-4 combination, top of figure, includes the artefact spikes from leads 2 and 3 as would be expected. (Marmont 1940). Thus from this one picture it can be concluded that after 115 minutes of anoxia this particular fibre conducted a normal sized spike past electrodes 1 and 2, but a greatly reduced potential past electrode 3. The contrast in size of the spike at lead 3 after 20 and 115 minutes of anoxia is clearly seen with leads 3-4, center pictures.

The lower pictures show similar results from another fibre in a larger bundle. The large bundle eliminated the artefacts. The 1-4 lead potential was much larger than the 3-4 spike after 70 minutes in nitrogen. After 90 minutes the 1-4 potential was the size of the 3-4 potential in the figure.

Thus the response of the single fibre definitely diminishes during anoxia but not uniformly along the fibre. Whether

the conduction is considered decremental, or whether it is considered irregularly reduced by the end-in effect becomes a question of definition.

Referring back to the frog sciatic experiment, a careful measurement of the 2 phases of the diphasic spike led off from the asphyxiated part of the nerve showed no change in the ratio of these potentials during anoxia. Thus the spike size changed an equal amount at each electrode, which suggests that decremental conduction is not likely to occur in regions of the nerve far from the cut end.

Refractory Period

To measure the refractory period variations during anoxia, 2 stimuli were necessary instead of 1. It was possible to change the single rectangular stimulus to 2 shocks of short duration by connecting the output of the square wave generator to a "separator" consisting of an audio transformer and 2 output diode tubes. The sudden potential rise at the start of the square wave placed an induced voltage of short duration on the plate of one of the tubes so that it conducted momentarily. The sudden potential drop at the end of the square wave caused the other tube to conduct momentarily. These brief passages of current were the 2 stimulating impulses the separation of which was controled by the duration of the square wave.

For a general study of the nerve refractory period during anoxia the second stimulus and the nerve impulse excited by it are made to occur in the relative refractory state from

the first nerve impulse. Thus the second nerve impulse could be reduced to only $\frac{1}{2}$ or 2/3 the magnitude of the first. It was assumed that the refractory period would vary directly with nerve excitability and become shorter than normal if the nerve were in a hyperexcitable state and longer if the nerve were hypoexcitable. This could be easily investigated by comparing the ratio between the amplitudes of the 2 nerve potentials at various times during anoxia. An increasing ratio value would indicate a decreasing refractory period and vice versa because an increasing ratio would mean the second nerve impulse was becoming larger which could only happen if the refractory period shortened so that the impulse occured during a more excitable state. Such ratios have been gathered in Table IX. Nerves 89, 87, 85, and 83 show a steadily decreasing ratio so there was no early shortening of the refractory period in these cases, but in all the other nerves there was an increase of the ratio value. This increase occured usually during the first 6 minutes of anoxia.

Let the difference between the first ratio at 0 minutes and the other ratio values for each nerve be defined as negative if the other values are smaller than the first one and positive if they are larger than the first one. By dividing this positive or negative difference by the first ratio value it is converted to a percentage difference plus or minus. When these percentage differences are plotted, a curve as seen in Figure 11 is obtained. Thus for nerve 89 after 2 minutes in nitrogen the expression is $\frac{750-572}{750} = 0.237$, and

TABLE IX

Ratios of the second impulse to the first when the nerves were stimulated with two stimuli so close together that the second fell in the relative refractory period of the first impulse.

Nerve	Time in Nitrogen in Minutes										
	0	2	4	6	8	10	12	14	16	18	20
89	.750	.572	.460	.375	.222						
88	.733	.733	.625	.535	.694	.704	.714	.722	.751	.467	.310
87	.466	.306	.317	.286	.256	.220	.148	.167			
86	.670	.780	.780	.787	.803	.827	.713	.483	.386	.364	
86			.727	.754	.770	.400	.334				
85	.830	.842	.483		.400						
84	.740	.790	.800	.718	.645	.650	.545	.615	.600		
84	.880	.920	.906	.785	.825	.625					
83	.700	.700	.678	.545	.368	.355					
83	.555	.389	.305	.305	.305	.294	.250	.214	.136		

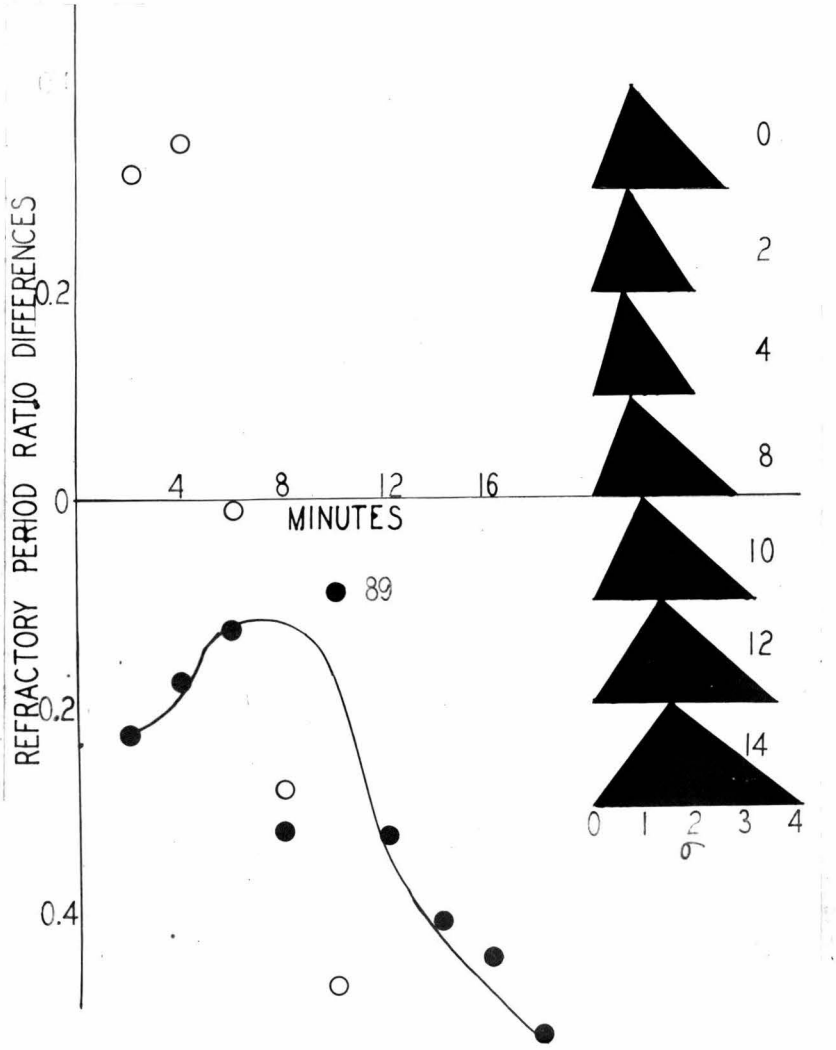


Fig 11. Graph showing the differences in size ratios of two nerve impulses at various times during asphyxiation.The second impulse is excited during the relative refractory state caused by the first.Results from rabbit nerve in solid circles,from cat nerve in open circles.Black triangles show refractory period after time(minutes)in nitrogen indicated by the number adjacent to the triangle.The positive slope represents the absolute period, the negative slope represents the relative period.

since the 2 minute ratio value is smaller than the ratio value at 0 minutes the final figure is -0.237. The average of all the rabbit nerve refractory periods has been plotted with solid dots, 1 cat nerve refractory period has been included, open circles.

By reducing the time between maximal stimuli to the value at which the second shock can no longer excite an action potential, a measure of the change during anoxia of the absolute refractory period for maximal stimulation was taken and by increasing the time between stimuli until the second nerve impulse was as big as the first, the total refractory period variation was measured. The results are pictured graphically as triangles in Figure 11. Each triangle was made from data taken at the time of asphyxiation indicated in minutes by the number adjacent to that triangle. The average of results from 2 rabbit nerves was used, and it will be noted that both the absolute and the relative refractory periods first decreased and then increased.

Chronaxie

The rheobases of 2 rabbit nerves were established with shocks of one second duration. The strength was then doubled and by varying the duration beginning at 0.01 sigma and increasing until the potential appeared, the chronaxies were determined. The nerves were then placed in nitrogen. The chronaxie of the first nerve was 0.12 sigma at the start of anoxia, dropping to 0.09 sigma after 9 minutes in nitrogen and rising abruptly to infinity after 22 minutes asphyxiation.

The second nerve had an initial chronaxie of ~~0.095~~ 0.095 sigma, ~~1.0~~ 0.1 sigma after 5 minutes anoxia, ~~1.3~~ 0.13 sigma after 10 minutes, and ∞ after 13 minutes. This nerve was one with a very short survival time. The chronaxie evidently changes very abruptly when the nerve loses its excitability from oxygen lack.

Membrane Depolarization During Anoxia

The cause of the reduced action potential in asphyxiated areas of a nerve could be membrane depolarization (Furasawa, Gerard, Koch, see introduction). This possibility was not difficult to test. A nerve was placed in the double chamber, the partition of which was then sealed. Oxygen or air was allowed to enter one section and nitrogen the other. 1 electrode in each section led to the D.C. amplifier previously described. The swing of a slow moving galvanometer was observed. If depolarization did take place during anoxia, as suspected, the electrode in the nitrogen section would become negative relative to the electrode in the air section of the chamber. The results of several such experiments with mammalian nerves are presented graphically in Figure 12.

The slow rise during the first 10 minutes in several experiments was due to drift in the apparatus which could not be completely eliminated. 6 rabbit nerves were asphyxiated, 1 of them twice. These results are plotted with solid lines and small dots. The figures in brackets mark the first and second asphyxiations of the one nerve doubly treated. The results from 3 cat nerves are plotted with dashed lines and

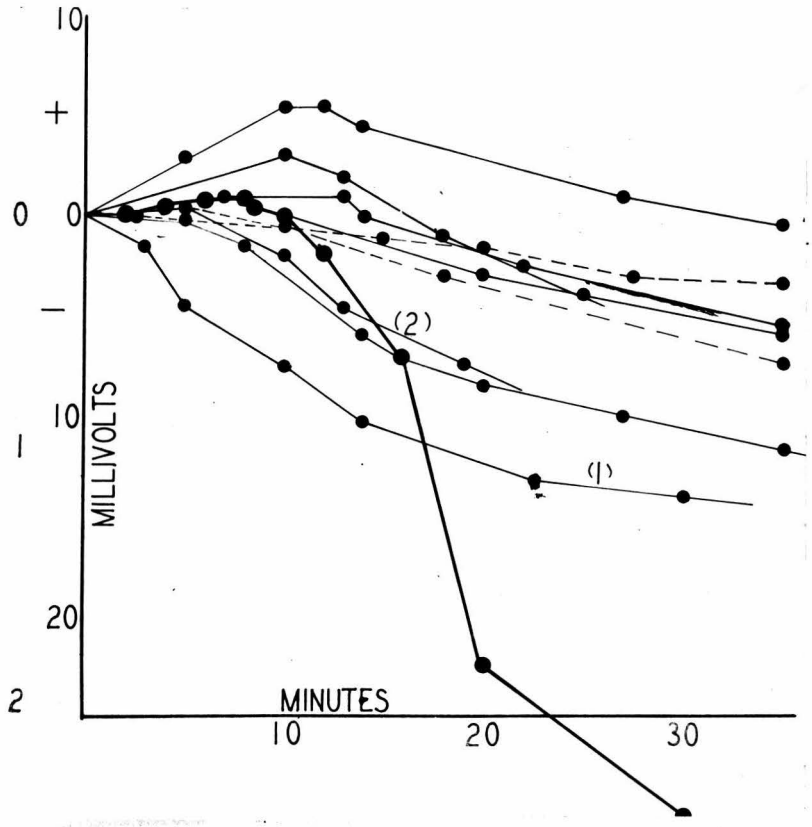


Fig. 12. Resting potential change with time in nitrogen as registered with one lead on section of nerve being asphyxiated, other lead on section of same nerve in air. Curve (1) oxygen was used instead of air. Curve (2) same nerve repeated with air. Curve with large dots plotted with scale furthest left. Others with scale nearest ordinate axis.

heavy line with large dots. The latter was graphed using the magnified millivolt scale on the very left of the figure.

The nitrogen electrode did become relatively negative, the change beginning after 5-10 minutes of anoxia, which is about the average time when the potential amplitude starts to decrease. The difference in potential between the asphyxiated and nonasphyxiated areas of the nerve was more pronounced when the nonasphyxiated area was in oxygen than when it was in air. This is recorded by curve (1) as compared to curve (2) both from the same nerve. For curve (1) part of the nerve was in oxygen, for curve (2) the same part was in air. The potential difference when oxygen was used was about 15 millivolts, and with air varied from 5 to 10 millivolts. Theoretically the membrane potential is about 60-70 mv so that the change observed was smaller than was originally expected. This smallness could be due to the fluid and connective tissue shortcircuiting the potential.

It is important to mention here, though it will be discussed in detail below, that this potential change of mammalian nerves was reversible with the admission of oxygen or air into the nitrogen section of the chamber.

2 frog nerves were asphyxiated at room temperature using the same recording apparatus. A potential became evident after about 30 minutes and increased to 30 mv in one case and 45 mv in the other after 150 minutes. This result was not reversible with the admission of oxygen or air into

the chamber. Again it was apparent that frog nerve differed from mammalian nerve.

It was concluded that the nerve membrane is depolarized during anoxia.

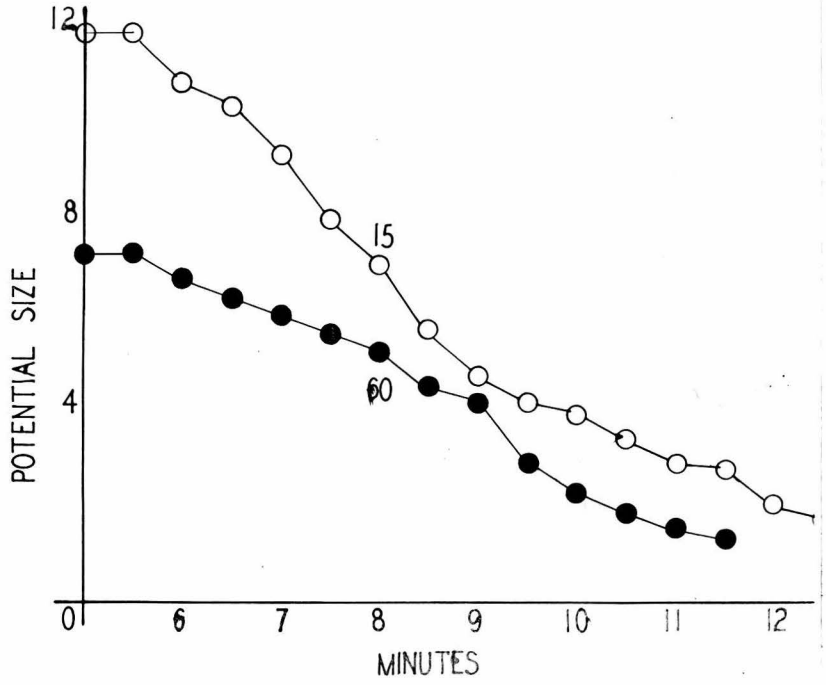


Fig. 13. Graph showing relative spike size with time in nitrogen for rabbit nerve with continuous stimulation at 60/sec. (solid circles), and at 15/sec. (open circles).

RESULTS B

Recovery

Mammalian Nerve

When an asphyxiated mammalian nerve is placed in a fluid or gaseous medium containing oxygen, the excitability of the nerve recovers rapidly. The recovery times of 16 rabbit nerves, 15 cat nerves, 3 dog nerves, and 6 lobster nerves are presented in Table X. The left hand column of figures gives the time of the first signs of electrical activity after the beginning of oxygen treatment. This is expressed in seconds for mammalian nerves, minutes for lobster nerves. The right hand column gives the time (in minutes) which is required for the activity to reach its original value. All the mammalian nerves recovered in a like manner, the first signs of recovery appearing after 30-120 seconds of oxygen, complete recovery being attained after 5-20 minutes.

Lobster Nerve

Lobster nerves recovered more slowly, the first signs not being apparent for 5-20 minutes in oxygen. The original state of irritability was reached in 10 minutes by 1 nerve bundle, but the other 5 required 30 minutes or more. The recovery of a lobster nerve bundle is shown in detail in Figure 14. In this case a small sensory bundle was asphyxiated until only 1 fibre was still conducting. Then oxygen was turned on. After 5 minutes 1 fibre has recovered and its action potential has summated with the 1 present at the start of recovery. After 13 minutes the action

TABLE X

Recovery times of irritability in mammalian and lobster nerves. Figures in left column indicate time after oxygen starts that recovery first begins. Figures in right column indicate time required for complete recovery.

Rabbit Nerves

Nerve	Time start recovery	Time complete recovery
Medial	90 secs	8 mins
"	60	8
"	50	8
"	60	10
"	60	5
"	60	10
"	90	10
Peroneal	90	10
"	60	--
"	60	--
"	60	20
"	60 120	12 15
Tibial	60	10
"	180	10
"	90	10
Saphenous	90	15

TABLE X CONT.

Cat Nerves

Nerve	Time start recovery	Time complete recovery
Peroneal	60 secs	10 mins
"	60	10
"	90	10
"	60	13
"	60	10
Phrenic	60	5
"	60	5
Saphenous	50	10
"	50	15
Radial	45	--
Tibial	60	5
Medial Cutaneous	60	5

Dog Nerves

Peroneal	120	8
"	60	15
Tibial	60	10

Lobster Nerves

Whole Nerve	5 mins	--
"	5	1½ hours
Coser Bundle	6	--
	10	--
	10	--
	2	10 mins.

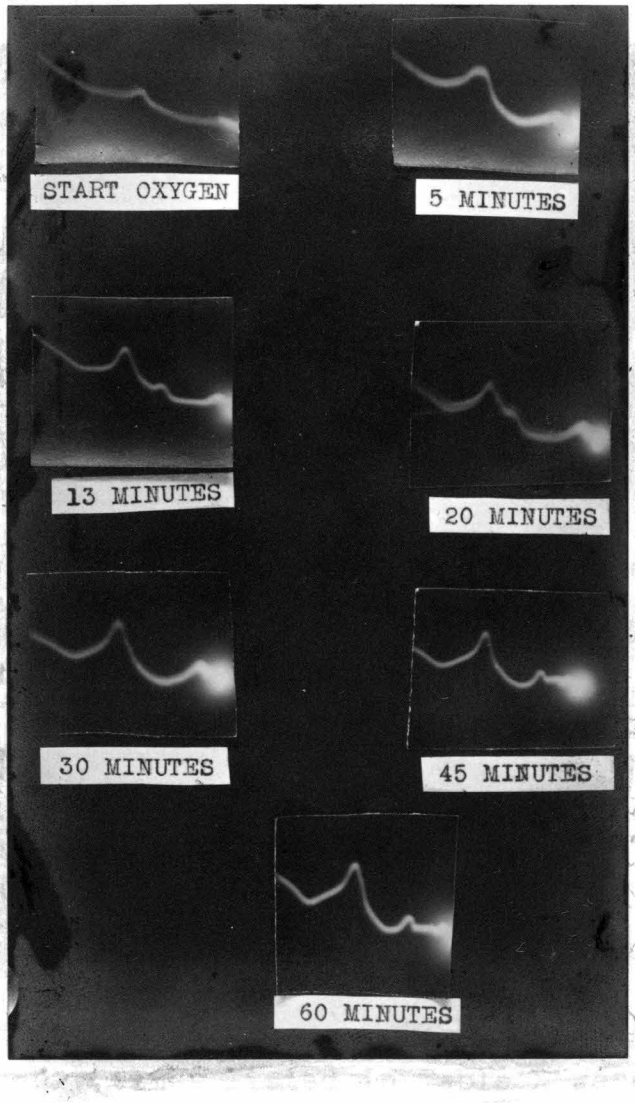


Fig. 14. Recovery of lobster nerve in oxygen. At start only one fibre conducting, two in 5 minutes, 3 in 13 minutes, 4 in 45 minutes, and 5 in 60 minutes. This is an excellent example of the conduction velocity change, reduced by the nitrogen, increasing in oxygen.

potential of a third fibre has appeared, but the conduction velocity is less than normal. (see below). After 20 minutes the conduction velocity has increased and summation with the other 2 spikes has begun. Summation is completed in 45 minutes, and the impulse from a fourth fibre has appeared again with a smaller conduction velocity. This one summates in 60 minutes and a fifth fibre has started to function.

Frog

Frog nerves behaved differently in oxygen after asphyxiation. Of the 11 nerves listed in Tables III and II nerve 121 recovered completely and 186 about 30% of normal at room temperature. The other 9 nerves never regained any of the activity they lost in nitrogen. Heinbecker (1929) and Gottschalk (1914) have reported many poor recoveries after frog nerve asphyxiation. To account for the poor recoveries the latter suggested the poisoning by high concentrations of metabolites collecting during anoxia. On the other hand Gerard and others obtained complete recoveries using frog nerves.

Effect of Asphyxiation Time on Recovery

The recovery times listed in Table X were from nerves given oxygen immediately upon the extinction of all nerve activity. Prolonging the anoxia beyond this period had a pronounced effect on the time required for the first signs of recovery to appear when placed in oxygen. Once recovery had started it progressed at about the same rate as already described. Below are listed a few recovery results after prolonging the asphyxiation with rabbit nerves.

Prolongation period (minutes)	Time in oxygen to first sign of recovery (minutes)
0	½
5	2
10	2½
15	3
15	5
25	7
40	5
60	no recovery
70	"
55	"

It was concluded that a) more than 40 minutes in nitrogen after complete asphyxiation destroyed some process necessary for recovery; b) if no signs of recovery were observed after 8 minutes in oxygen there would be no recovery.

Effect of Immersion in Solution on Recovery

An entirely different phenomenon was investigated during recovery with immersion, namely, the possible washing away of poisonous metabolites as suggested by Gottschalk (1914).

4 frog nerves were asphyxiated for 3½, 5½, 5½, and 6½ hours in nitrogen. The first nerve showed no sign of recovery after 30 minutes in oxygen, but did begin to recover after 30 minutes more, this time in aerated ringers. The recovery was complete after 10 hours. The second also failed to

recover in 30 minutes of oxygen alone, but began to recover after only 15 minutes in solution. Nerve 3 failed to show any response after 1½ hours in oxygen alone, but was 50% recovered after overnight immersion in the saline. The fourth nerve only recovered about 15% after overnight washing. These experiments confirmed Gottshcalk's and Frohlich's claims.

The effect of immersion was tried on mammalian nerves unable to recover after prolonged asphyxiation periods. A rabbit peroneal was asphyxiated in a nitrogen atmosphere for 1 hour. Signs of recovery began to appear after 5 minutes in oxygen so the nitrogen was immediately resumed for another hour. The survival time originally was 20 minutes, but was only 7 minutes the second time the nerve was asphyxiated. After the second hour in nitrogen oxygen failed to revive activity in the nerve for 30 minutes. This experiment was repeated with another rabbit peroneal from the same animal. The only difference was that the second nerve was immersed during the anoxia in saline. The survival times were about the same as of the first nerve. This immersed nerve began to recover from the second hour in nitrogen after only 2 minutes in oxygen. Each of 2 rabbit tibials were subjected to 100 minutes in nitrogen, one submerged in saline, the other in a gas atmosphere. Both nerves showed survival times of 20 minutes. There was no response from the nerve in gas after 20 minutes in oxygen, whereas the submerged nerve was more that 25% normal after only 4 minutes in oxygen. A cat

peroneal was submerged in saline and given 75 minutes nitrogen treatment. This nerve recovered completely after only a few minutes in oxygen. Previously it was found that no cat nerve recovered from more than 55 minutes in nitrogen.

It was concluded that whatever the recovery inhibitor was, it could not be removed by oxygen alone, but could be removed by oxygen plus physiological solution. Cowan has found that Maia nerve recovers somewhat in oxygenless sea water when no recovery was possible in oxygen alone.

Effect of Temperature on Recovery Time

3 rabbit peroneals were asphyxiated at different temperatures. Nerve 52 was asphyxiated first at 23 degrees. It began to recover 60 seconds after oxygen was applied and was completely recovered after 30 minutes in oxygen. The same nerve was asphyxiated again this time at 11 degrees C. Recovery did not begin for almost 8 minutes and was only 50% complete after 30 minutes in oxygen. Nerve 54 was asphyxiated the first time at 23 degrees. Recovery, begun in 2 minutes of oxygen, was complete in 20 minutes. The second anoxia was at 35 degrees and recovery began in 60 seconds and was completed in 15 minutes. The nerve was kept in oxygen for 1 hour more, a total of 75 minutes. Then it was asphyxiated a third time at 24 degrees and did not begin recovery for 4 minutes in oxygen, requiring 32 minutes to complete the recovery. Nerve 142 was asphyxiated at 21 degrees and recovery did not start for $3\frac{1}{2}$ minutes in oxygen. A second anoxia at 38 degrees was carried out on this nerve and recov-

ery began in 30 seconds of oxygen, being completed in 8 minutes. Although the survival times were increased by the lower temperatures which would affect the starting time oxygen application, the increase was not enough alone to account for the above results. Therefore it was concluded that the recovery time of mammalian nerves was markedly increased by cooling.

It was not practical to raise the temperature of mammalian nerves. However, this was tried with frog nerves in the hope of reducing the survival time and thus making recovery possible. The idea was based on the fact that mammalian nerves recovered poorly or not at all if subjected to long asphyxiation periods such as necessary to asphyxiate the frog nerve. 7 frog nerves were asphyxiated at 32.5 to 35 degrees C. The 2 nerves with the longest survival times at this temperature range, 130 and 210 minutes, failed to recover any activity. The 5 other nerves reacted as follows:

Nerve 135, survival time 128 minutes, recovered 65% normal in 5 minutes.

Nerve 120, survival time 120 minutes, recovered 80% normal in 15 minutes.

Nerve 153, survival time 110 minutes, recovered completely in 20 minutes.

Nerve 117, survival time 80 minutes, recovered completely in 30 minutes.

Nerve 156, survival time 70 minutes, recovered 80% in 5 minutes.

Nerves 117 and 120 were sciatics from R. pipiens which on the average have shorter survival times than the nerves from R. catesbiana (135, 153, 156).

It was concluded that the lack of recovery or poor recovery in frog nerve at room temperature was due to the long asphyxiation time, during which time recovery inhibitors collected. Temperature increase decreased this time and recovery became possible.

Frequency of Stimulus Effect on Recovery

Frequency of stimulus had no effect on recovery times. An example is given in Figure 18. A rabbit peroneal was subjected to continuous 60 cycle faradic stimulation causing maximal response, and another peroneal was stimulated by 1 shock every few minutes. They recovered identically.

Recovery of Threshold of Excitation and Amplitude of Action Potential

The threshold recovery curves and action potential amplitude recovery curves are feproduced in Fig. 15. The threshold recovery curve is marked T and is drawn through the data from 10 rabbit nerves (open small circles), and 1 cat nerve (solid small circles). The action potential amplitude recovery curve was plotted from the data of 3 rabbit nerves (large open circles). The data from a cat nerve (large solid circles), and a dog nerve (half filled large circles) were also plotted. The threshold units are 1/200ths of a volt (left hand ordinate), and the action potential amplitude is in percent of original size (right hand ordinate).

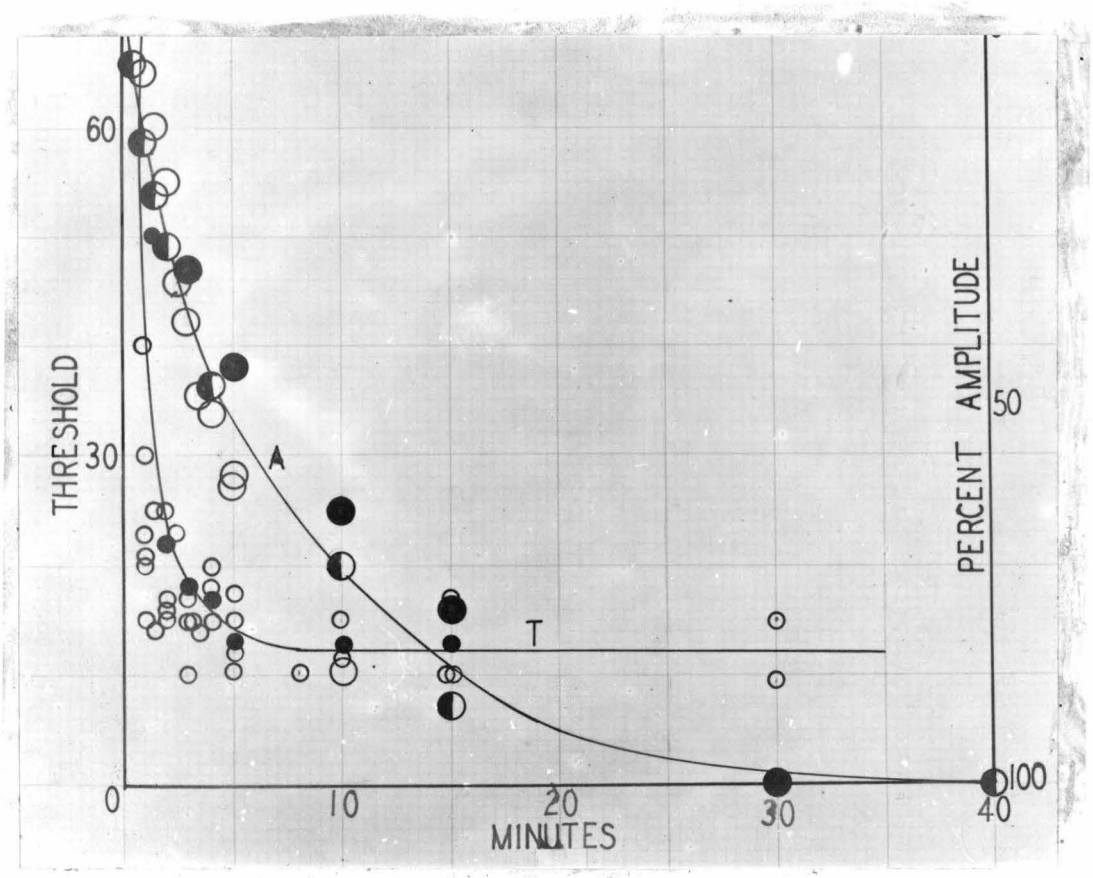


Fig. 15. Graph showing recovery in oxygen (minutes) of threshold (T) with small open circles rabbit, solid circles cat; and recovery of amplitude (A) large circles rabbit, solid circles cat, and half circles dog nerves.

The recovery of excitability progressed exponentially, approaching the original value. The threshold of mammalian nerves decreased abruptly toward the original value, attaining it in less than 10 minutes. The action potential amplitude was slower, requiring 20-30 minutes to reach the maximal size. It was 80-90% of this size at the time when the threshold was normal. No such threshold fluctuation as described by Lehmann (1937) were observed at any time during recovery.

The effect of prolonging asphyxiation upon the threshold recovery curves is plotted in Fig. 16. The number near each line designates the number of minutes after complete extinction of all nerve excitability that the nitrogen period was prolonged in that particular threshold recovery curve. There is a shift to the right as the time of prolongation increases, but the recovery curves maintain a consistently similar shape. Thus the chief effect of prolongation, as said before, is to retard the initial appearance of recovery. No effect upon the recovery rate once it is started is evident.

End-in Effect

The end-in effect caused by anoxia was observed to reverse during recovery, Fig. 17. The photographs are of the recovery of an action potential of a frog nerve at 36 degrees. The time intervals at which the pictures were recorded are indicated in the figure. The ratios of the positive to negative spikes were: at start--0.000, 5 mins--0.222, 10 mins--0.272, 20 mins--0.417, 30 mins--0.462. Thus at first there was only the negative spike, then a very small positive one

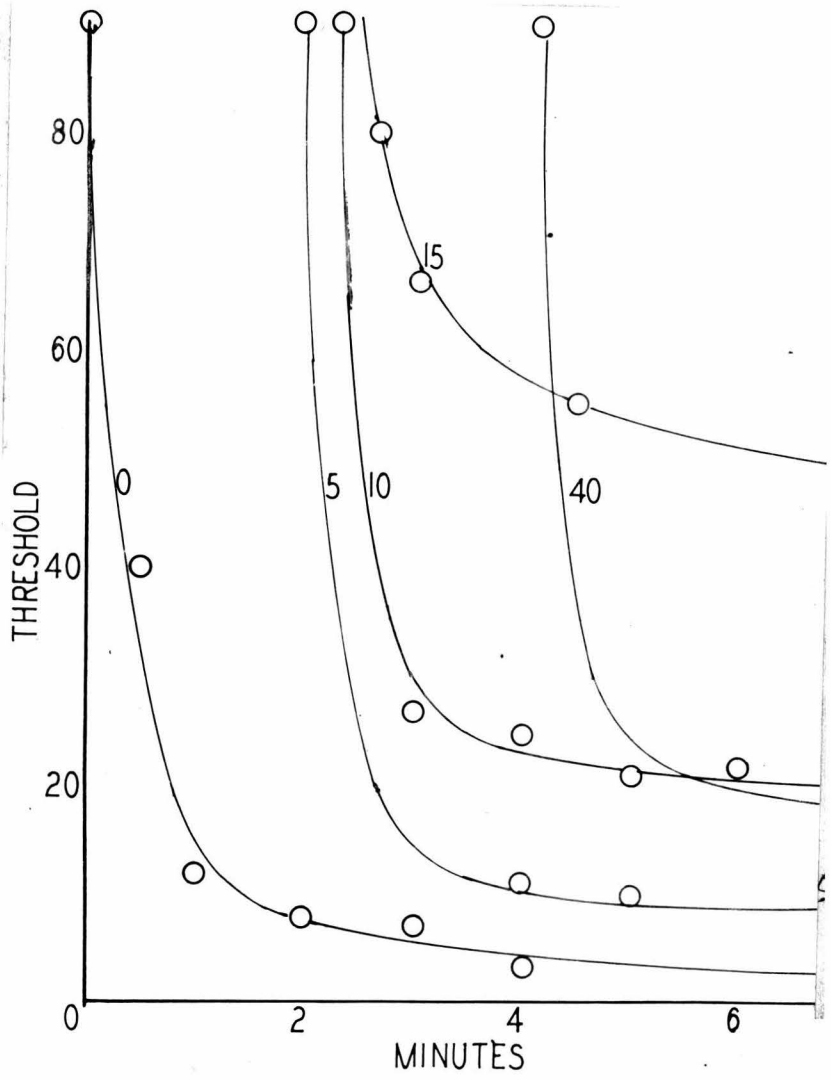


Fig. 16. Graph showing delay in recovery with increasing time in nitrogen. The figures indicate the time in minutes the nerve was held in nitrogen after disappearance of the action potential.

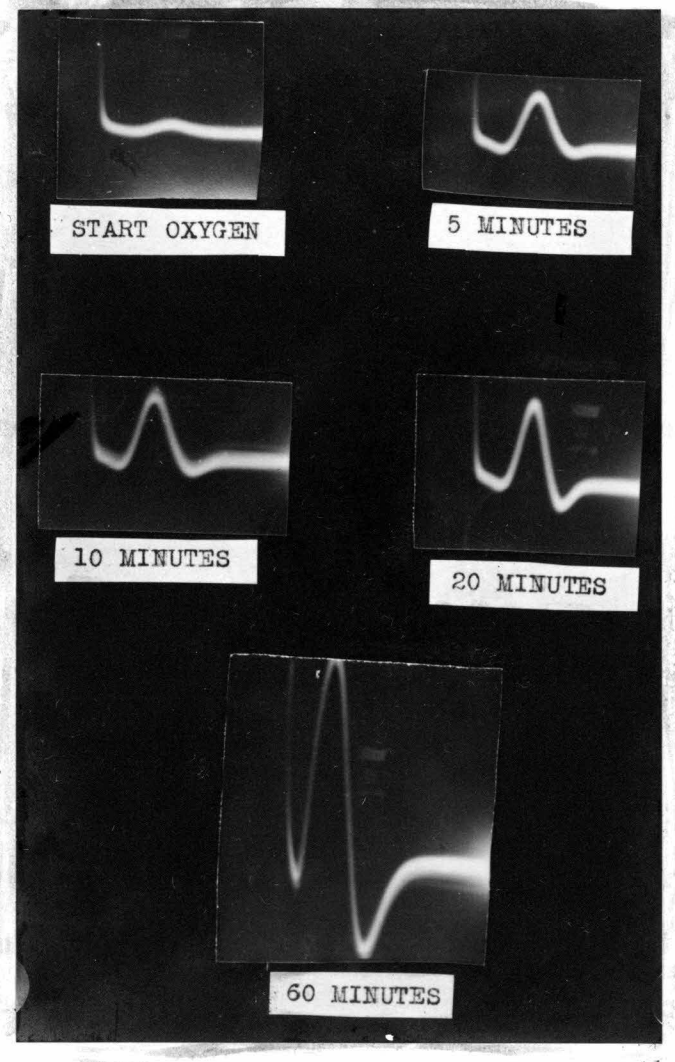


Fig. 17. Recovery curve of frog nerve at 36 degrees centigrade in oxygen showing mono-to diphasic change.

appeared at 5 minutes which increased in size rapidly in the 10-20 minute interval of oxygen. This effect was observed in the majority of experiments when the lead off electrodes were near the cut end of the nerve. It indicates that the tissue contacting the proximal lead became active before the tissue contacting the distal lead.

Refractory Period

The recovery of the refractory period of the nerve is tabulated in Table XI. The manner of calculating was the same as was used for the effect of anoxia on the refractory period, except here the last ratio of each nerve (10 minutes) was the value used for subtraction to obtain the ratio difference and for dividing to obtain ratio difference. Thus, taking nerve 87, for example, at $\frac{1}{2}$ minute the expression is $\frac{.000-.583}{.583} = -1.000$. The graph, Fig. 18, only shows the first 5 minutes of recovery. The action potentials were recorded and measured for every 30 seconds during recovery. The refractory period recovery was very rapid after the first minute until the third minute, following which the ratio decreased so that after five minutes the recovery was very slow.

The average of 2 experiments in which just the absolute and total refractory period recoveries were made, showed that in 1 to 2 minutes after oxygen was started the absolute period was 0.90 sigma and the total refractory period was 2.84 sigma. At the start of asphyxia these same 2 nerves averaged an absolute refractory period of 2.89 sigma, so within 2 minutes it

TABLE XI

Ratios of the second nerve impulse to the first when the nerves were stimulated with two shocks following so close in succession that the second fell in the relative refractory period from the impulse of the first. Shows the recovery in oxygen of the refractory state of the mammalian nerves, rabbit nerves.

Nerve	Time in Oxygen in Minutes									
	0	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	10.0
93	.000	0.143	.222	.308	.389	.435	.435	.488	.528	.560
88	.000	0.000	.000	.428	.572		.750			
87	.000	.000	.156	.167	.270	.286	.326	.367	.394	.583
86	.000	.000	.250	.214	.736					.893
86	.000	.000	.143	.375	.555		.600			
84	.000	.000	.250	.400	.430		.353	.364	.381	.740
83	.000	.000	.000	.100	.200	.315	.428		.609	.667

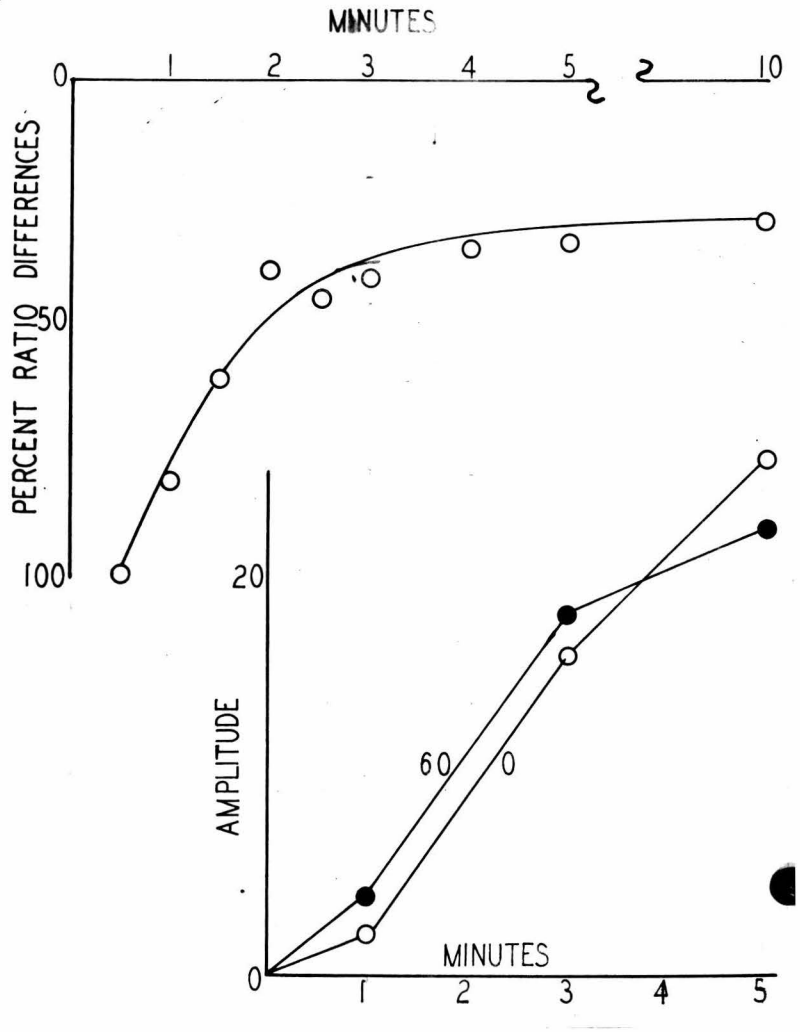


Fig. 18. Refractory period recovery curve in oxygen (upper).
Same recording system as used with asphyxiation, see text. Effect of no stimulation and 60 cycle continuous stimulation on recovery of the same nerve (lower).

was possible for the refractory period to recover completely. This suggests that the limits of the refractory states of the nerve recover their original values rapidly, but the variations of nerve irritability within these limits caused by asphyxiation recover more slowly. In other words, if the curve representing the recovery of excitability in a nerve fibre after the passage of a nerve impulse will attain its 2 original limiting values before it will attain its original shape. No attempt was made to investigate this possibility.

Oxygen "Rests" and Repetitive Anoxia

19 nerves (rabbit and cat) were asphyxiated 2 or more times with different oxygen "rests" separating the anoxia periods. The results were as follows. 9 nerves were given an oxygen period of 20 minutes or more and showed the slightest decrease in the second survival time compared to the first. 10 nerves were given oxygen for less than 20 minutes and showed a decrease in the second survival time compared to the first of 15-20%. Those given only a 5 minute oxygen rest had an average second survival time 22% shorter than the first. Those given 10 minutes had average survival times 12% less than the first. All these nerves were first "aged" an hour or more in saline to eliminate the initial effect of "aging" described in the asphyxiation section. Thus an oxygen period of 20 minutes or longer is necessary for complete recovery. These results conform with Gottschalk's reports for frog nerve.

Depolarization Recovery

The results of depolarization recovery experiments are

shown graphically in figure 19. The curves with the small circles represent the data from rabbit nerves, the large circles represent data from a cat nerve at higher amplification to be read with the millivolt scale to the right of the ordinate line. The open circles represent data from nerves asphyxiated 220 minutes and 90 minutes. The cat nerve was asphyxiated 90 minutes. The rabbit nerves for 30 minutes.

Repolarization of the nerves began after about 2 minutes in oxygen. All preparations did repolarize to the normal value, the large part of the process occurring during the first 2 and 5 minutes in oxygen. The belated start of the repolarization was probably due to the prolonging of anoxia 30 minutes. Rabbit nerves are usually asphyxiated in 20 minutes.

The apparatus was not designed or built so that action potential studies could be made simultaneously with polarization studies, but it was only a matter of a few minutes to transport the chamber from this apparatus to the oscillograph. This was done in the cases of those preparations asphyxiated for long periods of time (220, 90 minutes) which would not be expected to recover unless immersed in saline. None of these nerves showed any action potential response although the membrane had been repolarized at least 5 mv during the 15 minute period in oxygen before the action potential measurements were attempted (small open circles, large solid circles). Thus the action potentials were still absent after some 20 minutes of aeration, though the asphyxiated portion of the nerve was well repolarized by then. The 2 rabbit

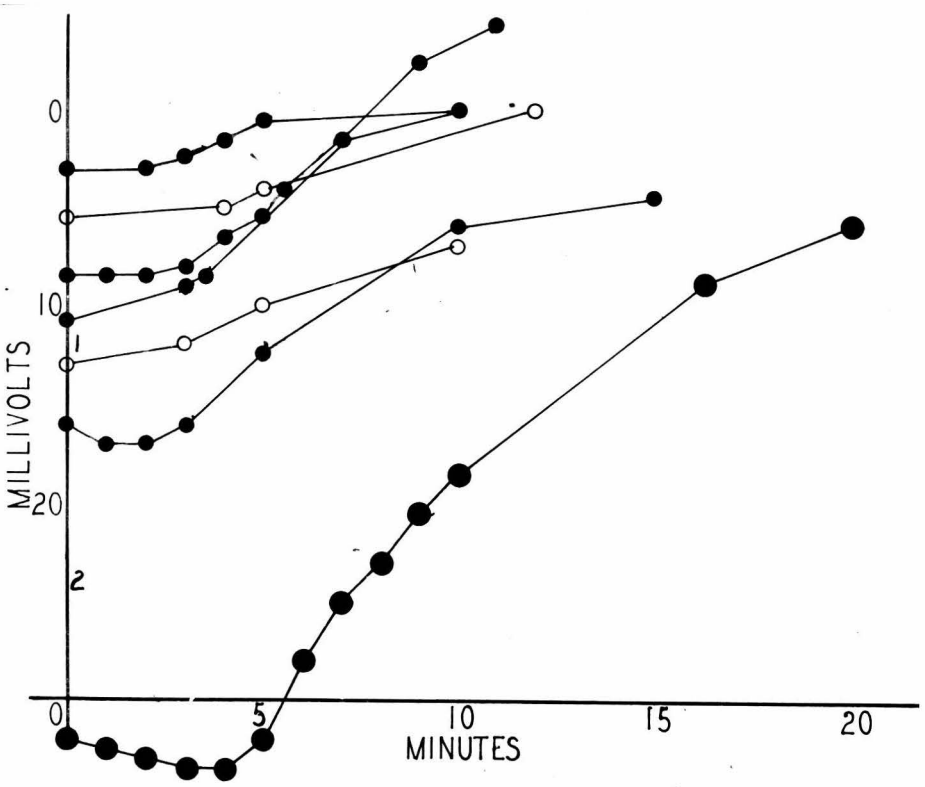


Fig. 19. Curve showing reduction with time (minutes) in oxygen of the resting potential between a lead on the nerve in nitrogen and one in air on the same nerve. Small circles are results from rabbit nerves, the open circles indicating nerves asphyxiated 220 minutes and 90 minutes. Large circle from cat nerve data using the figures to right of ordinate.

nerves were capable of producing a 1/10 normal spike after 2 hours washing in saline, and the other nerve, cat, recovered its spike completely after 3 hours washing.

The possibility that the moisture entering the chamber with the gas caused fluctuations in the apparatus which might be interpreted erroneously as nerve polarity changes was dispelled by an experiment using frog nerves. 2 frog nerves were depolarized with nitrogen as described in a previous section. Oxygen was then admitted to the chamber. No visible change occurred for 15 minutes in 1 case and only a 5 mv restoration after 105 minutes in the second case. This relatively very small recovery in the case of the frog nerve where a 65 mv depolarization was obtained with nitrogen, could easily have been due to slight depolarization of the section of the nerve held in air from aging. No action potential could be obtained from either preparation.

The conclusion is that some change takes place in the membrane during oxygen lack, besides the depolarization, which must also recover before the nerve can make use of the repolarized membrane to conduct an impulse.

Toxic Substance

Experiments by Heinbecker (1929) have proven that neither lactic acid nor CO_2 is the metabolite causing the poor recoveries in nerves after long anoxia periods. Since then Cowan has suggested the collection of high concentrations of potassium ions outside the nerve, leaking out from the inside, as being the cause of the poisoning of the nerve. Several ex-

periments were devised to test this supposition.

First it was observed that 20 times normal potassium ion concentration in the physiological solution extinguished rabbit nerve activity in about 1 hour confirming the findings of Graham (1933). Higher concentrations were poisonous in a shorter time, lower concentrations took longer to take effect.

A rabbit peroneal was first placed in Tyrodes solution with 10 times the normal potassium ion concentration and kept there for $1\frac{1}{2}$ hours. It was then asphyxiated while submerged in the same solution. The anoxia was maintained for 1 hour and 40 minutes. All activity in the nerve ceased after 20 minutes in nitrogen. The nerve began to recover, still submerged in the same solution, after 4 minutes of oxygenation.

2 rabbit tibials were asphyxiated simultaneously in a nitrogen atmosphere for $1\frac{1}{2}$ hours. One was then placed in aerated physiological solution and the other was placed in aerated physiological solution containing 20 times normal potassium ion concentration. Both recovered identically.

A rabbit peroneal was asphyxiated in the normal manner, becoming inactive in 15 minutes. It was given a 50 minute oxygen rest and then immersed in 20 times normal potassium ion concentration solution for 10 minutes. A second asphyxiation period was then begun with the nerve still immersed. The spike disappeared again in about 15 minutes and after 2 minutes of oxygenating the solution, the nerve began to recover. Although the action potential never quite regained

its original size, the threshold was normal after 15 minutes of oxygen.

Another rabbit peroneal was kept in the 20 times normal potassium ion concentration solution for 40 minutes. The action potential was about 30% its normal size after this period. The nerve was then asphyxiated and had a survival time of only 5 minutes. However, the spike reappeared again after only $2\frac{1}{2}$ minutes of oxygenation of the solution.

Thus it was concluded that a nerve could recover from asphyxiation even if prolonged, as well in a 20 times normal concentration of potassium ions in the surrounding medium as in normal physiological solution. The 20 times normal potassium ion concentration was chosen because this is the concentration found inside the normal nerve, and it would be impossible to obtain a higher amount of this ion from leakage out of the nerve during asphyxiation. Therefore the nerve can recover from asphyxiation in a concentration of potassium ion higher than that possible biologically, and hence potassium cannot be the toxic substance.

pH Change of Solution During Asphyxiation

A group of several nerves were asphyxiated immersed in $1\frac{1}{2}$ cc of solution for 2 hours. Others were simply placed in the same amount of fluid and allowed to remain there for 2 hours with oxygen bubbling through the medium instead of nitrogen. The pH decreased 0.15 units during anoxia compared to 0.10 units in oxygen. This difference of 0.05 units is not enough to have a poisoning effect.

Test of Solution Surrounding Nerves Asphyxiated Long Times

3 cat medial nerves immersed in 1½ cc Tyrode solution were asphyxiated for 2 hours. All activity ceased in 40 minutes. At the end of the 2 hour period these nerves were replaced in this solution by a cat radial nerve. The latter was subjected to nitrogen and had a survival time of 20 minutes. Recovery was complete in 5 minutes after oxygen was admitted to the chamber, the nerve still being immersed in the fluid. Another radial nerve was placed in the same medium and asphyxiated. Its survival time was 40 minutes and it was completely recovered also 5 minutes after oxygenation of the solution. Thus 5 nerves asphyxiated for a total of 3 hours did not produce enough "toxic" substance to be biologically detectable in 1½ cc of physiological solution.

DISCUSSION

When a nerve is placed in nitrogen it is able to maintain its original irritability for some time. This must be due to an energy reserve bound in the nerve. What the composition of this reserve is, is not known. However, it is known that its manufacture is dependent upon oxygen. This is indicated by the results of varying the length of the oxygen intervals between repeated asphyxiations. With only short oxygen "rests" the second survival time is shorter than the first. The cause of this reduction of survival time is the abbreviation of the initial normal period. The rate at which the irritability disappears once this change begins is always the same. Thus after the recovery of the nerve activity in oxygen, which is very rapid, the reserve is formed and at least 20 minutes are required to complete the process.

This reserve energy idea is not new. Gottschalk found that a 25 minute period in oxygen was necessary to "fill" the reserve in frog nerve. Lehmann noted the decrease in succeeding survival times with repeated anoxia in mammalian nerve, but makes no mention of the correlation of the oxygen interval between anoxia treatments and this change. When the reserve is exhausted, the threshold for electrical stimulation rised and the action potential spike diminishes in size. Each of these changes when plotted forms an exponential curve (see Fig./0). An attempt to explain these changes on the basis of the membrane theory of conduction

will be made. Let us consider this theory briefly.

Normally a nerve maintains on its membrane a charge which is an electrical double layer, negative on the inside and positive on the outside. This can be recorded as a demarcation potential between injured areas, contacting the inside, and non-injured areas of the nerve. Recently it has been measured directly as the potential across the membrane between a fine needle electrode inserted inside a single nerve fibre and an electrode contacting the outside of the fibre (Curtis and Cole 1940). This potential is due to the unique membrane property of selective semi-permeability whereby concentration gradients of different ions are set up across the membrane. The potassium ion concentration, for example, is 20 times greater inside a nerve fibre than in the normal surrounding medium (Schmitt 1940, Cowan 1939). Calcium ions, on the other hand, cannot penetrate the membrane at all and remain outside of the fibre. Thus the membrane potential is really a diffusion potential and its voltage is given by the expression

$$V = \frac{RT}{nF} \log \frac{C_i}{C_o}$$ derived by Nernst (1897), where R is the gas constant, T is the absolute temperature, n is the ion valence, F is the faraday equivalent, C_i is the concentration of the ions inside, and C_o the concentration of the outside of the membrane. If the potassium ion concentration ratio inside to outside is substituted for $\frac{C_i}{C_o}$ the expression becomes

$$V = 0.058 \log 20, \text{ or } V = 75 \text{ millivolts.}$$

Cole and Curtis measured a membrane potential of 40 mv, and action potentials from single fibres have been recorded as high as 60 mv (Marmont 1940). Allowing for some shunting due to the conductivity of the surrounding medium these results suggest that the potassium ion gradient alone could be the cause of the membrane potential, although it is possible that other factors enter in.

During anoxia, besides those changes already described, the demarcation potential declines (Koch, Furasawa, and Gerard) and if only part of the nerve is asphyxiated, this part becomes electronegative to the unasphyxiated part. Without oxygen, therefore, the nerve membrane becomes depolarized. This is probably due to the loss of the property of selective semi-permeability and hence the disappearance of the ion concentration gradients. Thus the membrane potential is extinguished and, as it is, the action potential declines. The exponential manner of this decline would be predictable from the diffusion potential equation.

The velocity of conduction is reduced by asphyxiation as seen in the single fibre action potentials (Fig. 19) and in a group of fibres from mammalian nerve (Fig. 20). This is probably due to 2 factors. Firstly, the reduction in size of the spike potential makes breakdown of neighboring areas more difficult and hence not as quick. The nerve is in a refractory state. Lillie's iron wire model provides an example of this point. When an iron wire is placed in 70% nitric acid it develops an oxide coating on its surface.

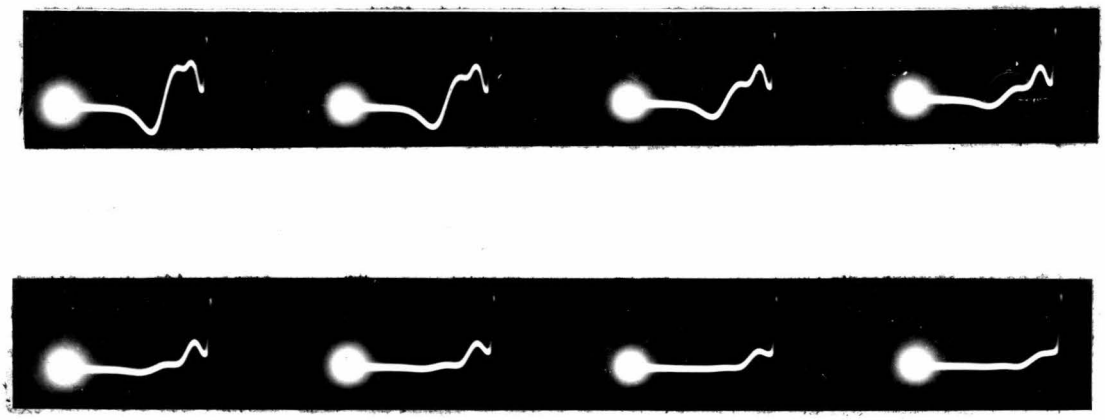


Fig 20. Potential of rabbit medial showing recovery of two groups of fibers, the velocity of one, greatly reduced by asphyxiation, increasing in oxygen

When this coating is completed the wire is in a "passive" state. If the surface of the coating is scratched off in a small area the whole coating disintegrates along the wire but at a very definite rate, not instantaneously. The disintegrating area moves along the wire in a manner analagous to the movement of the nerve impulse along the nerve. If during the formation of the coating, however, before it is completed and the wire is said to be "refractory", it is scratched, the disintegration occurs at a much slower rate. Whether it is a potential between the coated and non coated area or not which causes the breakdown of the oxide coating is not known. (Lillie 1919, 1921). Secondly, and more likely, the rise of the local response in the nerve fibre around the negative stimulating electrode is slower and reaches the threshold value for setting off the propagated spike potential at a later time (Hodgkin 1939). In other words, the time constant k of the local potential (Hill 1935) is increased.

The refractory period is lengthened by anoxia. This means that it takes longer for the nerve to restore its charge than originally. Without oxygen the power to move ions against a potential gradient becomes more difficult. Frohlich, Heinbecker, and Gerard have all reported elongation of refractory period, but Cooper argued against it. Since the recovery time remained constant and only the last period enlarged she claimed the refractory period remained constant and that there was decremental conduction. In other

words she observed that the muscle contractions did not summate unless the time between the 2 stimuli, the second 8 times the strength of the first, was increased. This meant, by the decrement idea, that the second impulse grew smaller so that it did not get through the chamber. Increasing the time interval allowed it to become longer at the start, and therefore proceed through the chamber. But, the very fact that the impulse decreased in size at the start means that the refractory state was becoming longer and smothering the response. Thus, Cooper's argument for decremental conduction demands a variable refractory period for its foundation.

The rise in threshold can be explained by assuming an increase in the time constant k of the change in local response. Thus stronger stimuli became necessary to force this response over the threshold of the propagated spike. The accomodation factor probably changes too which makes the cause of the threshold change a more complicated mechanism.

Graham (1933) found that the action potential was reduced, the threshold for excitation increased, the velocity of conduction reduced, and the refractory period increased by increasing the potassium ion concentration in the solution surrounding the nerve. This was also observed in the present work. Actually such a change in the outside medium simply reduces the potassium ion concentration gradient across the membrane and hence reduces the membrane potential. It is logical to suppose that a similar change in the potas-

sium ion gradient takes place due to the ion's leaking out of the nerve during anoxia when the property of selective semi-permeability is lost (Cowan 1934).

It is important to mention here the results of varying the calcium ion concentration around the nerve during anoxia. Excess of the ion caused the survival time to be prolonged, lack of it shortened the survival time. It is known that the permeability of a cell membrane varies inversely with the calcium ion concentration in the surrounding medium. Thus with excess calcium in the solution the nerve membrane is less permeable and it would require less energy to maintain the potassium ion concentration gradient. This is because the ions cannot easily leak out. With a low calcium concentration the reverse would be true. Therefore it would be expected that the reserve energy in the nerve would be used up quicker with the nerve in a solution void of calcium than it would in a solution with an over abundance of this ion, which is exactly what was observed.

The end-in effect can be explained on the depolarization theory. To begin with, the area immediately around the cut end of the nerve is partially depolarized by the injury. Therefore, without oxygen, the remaining depolarization is completed in a shorter time than it takes to depolarize an area completely polarized at the start.

The oxygen lack effect is different than that of narcosis. This was observed by Frohlich by adding ether to the nitrogen atmosphere surrounding a frog nerve. The irritabil-

ity of the nerve was rapidly extinguished, but recovered in the nitrogen when the ether was removed. It has been noted (Wiersma and Keighley, by communication) that a "threshold" concentration of KCN exists in crustacean nerves. If this concentration is increased by a very small amount the nerve activity disappears immediately. When the concentration is lowered again the activity reappears abruptly. Schmitt* narcotized a nerve with CO and obtained a sudden recovery of the action potential when a strong light was focused on the nerve. These very sudden changes could not occur if the membrane were depolarized, therefore the narcotic must attack some conduction process in the membrane without changing the electric double layer. Schmitt suggests that there is a photo sensitive hemin like enzyme which is poisoned by the CO. The toxicity from long anoxia periods may be due to a similar poisoning of a conduction process.

Wiersma (1933) reports decremental conduction caused by KCN. Cooper (1923) claims that decremental conduction results from asphyxiation (see note). Most of the results of the present work are against this possibility, except in the area about the cut end. The potential decline in this area is not a real conduction with a decrement, but is due to the smaller degree of polarization of the membrane as pointed out above. However, the lower pictures in Fig. 9 suggest that there is a slow decline in the potential of a lobster single fibre as the spike progresses in the asphyxiation chamber.

*1930

The pictures were interpreted, at first, as indicating that the end-in effect progressed along the whole asphyxiated length of a nerve. Later experiments (Frog nerve, Fig. ~~10~~ 7) showed that this was unlikely which leaves only decremental conduction to explain the phenomenon. Thus at present it is not possible to argue this point until further experiments have been carried out.

During recovery in oxygen, the changes which occured in nerve irritability with anoxia, are reversed. The nerve membrane regains its selective semi-permeability, ion concentration gradients are reformed and the membrane repolarizes. This occurs rapidly. The threshold drops back to its normal value even before the membrane potential has reached its normal value. This is because, as we have seen during anoxia, conduction can occur when the normally maximal action potential is not attainable. The velocity of conduction is slow and the refractory period long at this stage. They rapidly regain their normal values again.

As pointed out above, prolonging the anoxia period produces a toxic effect on the nerve irritability. When the asphyxiated nerve is placed in oxygen, the membrane can repolarize but cannot conduct an action potential until the nerve has been washed in saline. Even oxygen free saline can partially eliminate this blocking of conduction (Cowan 1934). CO_2, lactic acid, and potassium are apparently not the causes of this inhibition. Thus, acidity changes are ruled out as the possible instigators of the phenomenon.

A possibility not tested to date is NH_3 (Harvey 1933) which if given off by nerve.

The oxygen uptake rate of nerve has been found to have a pronounced effect upon the nerve behavior during anoxia. From data of other publications (Gerard 1932, Gerard and Chang 1936) together with data from the present work a very interesting relationship between oxygen uptake and survival time becomes evident. First, the oxygen uptake rate of a nerve increases with heating, decreases with cooling. The survival time of a nerve does just the opposite. Secondly, the oxygen uptake rate is reduced by high concentrations of calcium ion in the physiological solution, and is increased by the absence of the ion from the medium. Again, the survival time does just the reverse. Thirdly, the oxygen uptake rate at the cut end of a nerve is higher than in normal uninjured areas, and the survival time in the cut end region is shorter than in the normal areas. Thus, it appears that the survival time varies inversely as the oxygen uptake rate.

To investigate this problem, the oxygen uptake rates of rabbit, dog, lobster, and the two species of frog used were taken from the table in the review by Gerard. The average value from two oxygen uptake rate experiments on cat nerves using the Warburg technique was included. They are listed below.

Rabbit nerve	280	cc/gm/hr
Cat nerve	200	"
Dog nerve	190	"
Lobster nerve	80	"
R. Pipiens nerve	40	"
R. Cates nerve	30	"

If the above surmise is correct the ratio of the rabbit nerve oxygen uptake rate to the cat nerve oxygen uptake rate should be equal to the ratio of the cat survival time to the rabbit survival time, the inverse ratio. Since the 23 minute survival time of the rabbit nerve was taken from a mean of 28 nerves it is statistically more accurate than any other values and will be used as the basic survival time. Setting up the ratio gives $\frac{280}{200} = \frac{X}{23}$, X = 32.2 minutes. The mean survival time of the 10 cat nerves was about 33 minutes. (Lehman recorded a 30 minute survival for cat nerve.)

Dog nerve irritability should survive about the same length of time without oxygen as the cat nerve activity on the basis of their both having a similar oxygen consumption rate. This was true of the mean of 3 dog nerves. 3 nerves is not a large enough number for other than a very rough approximation. From the expression $\frac{280}{190} = \frac{X}{23}$, X = 33.9 minutes. This figure predicted by theory is not only in close agreement with the 33 minute survival time found experimentally with dog nerve, but bears out the postulation that the dog nerve should have a similar survival time in nitrogen as the cat nerve.

The lobster nerve should survive much longer on the ratio

basis. From the expression $\frac{280}{80} = \frac{X}{23}$, X = 80 minutes. Actually the mean survival time of 6 nerves was 77 minutes, the excitability of the 3 nerves left intact lasted 75 minutes each in nitrogen.

The same equation gives theoretical survival times of $2\frac{1}{2}$ hours for R. pipiens and $3\frac{1}{2}$ hours for R. catesbiana. Actually the nerves of R. pipiens had a survival time between $1\frac{1}{2}$ and 3 hours while the survival times of the bullfrog nerves ranged between $2\frac{1}{2}$ and 4 hours so again the order of magnitude of theoretical value and actual value are within comparable limits.

Therefore the survival time does vary inversly as the oxygen uptake rate. This means also that in all nerves the reserve supply is approximately the same, assuming that when anoxia is started the nerve draws on the reserve supply at the rate indicated by the oxygen uptake rate. Examination of the ratios of the initial normal periods strengthens this argument. The ratio of rabbit nerve initial period to lobster nerve initial period is 7/35 and the ratio of oxygen uptake is 10/35 for the same nerves. This is a good approximation. Similar ratios of 1/10 to 1/9 hold between rabbit and bullfrog initial periods and their respective oxygen uptake rates.

It is known that the nerve metabolic rate falls off during the first hour or two in saline. In serum the fall is less rapid than it is when the nerve is in saline. It was found that the frog nerve followed the inverse law when

placed in physiological solution, but the mammalian nerves did not. The mammalian nerve survival time decreased with time in saline until an equilibrium was attained after ½-1 hour. It must be concluded that when a mammalian nerve is placed in a physiological solution some metabolic process is changed which either does not exist in the frog nerve or is not variable. Schmitt's* suggestion that the reduction in the metabolic rate is due to a healing over of the cut end does not solve this problem, for the healing over would probably occur in both types of nerve.

The protein content of the serum might be the cause of the difference between anoxic behavior (and metabolic behavior) of nerve in serum and in saline. However, the protein content of frog blood is about the same as that of mammalian blood, on the basis of simple acid and heating tests, so that the cause of the different reactions of these two types of nerves remains obscure.

In conclusion it should be repeated that at no time was the threshold recovery curve published by Lehmann observed. Nor were peroneal and saphenous nerves found to differ in their respective behaviors during anoxia consistently, as claimed by him. Lehmann has suggested that alteration of the calcium ion balance is the cause of the variations he observed in the irritability of mammalian nerves during anoxia. If this is true, then the change in the calcium ion concentrations would have to be exceedingly large, because in the present work nerves were immersed for 30 minutes in 250 times normal calcium ion concentration and solutions containing no

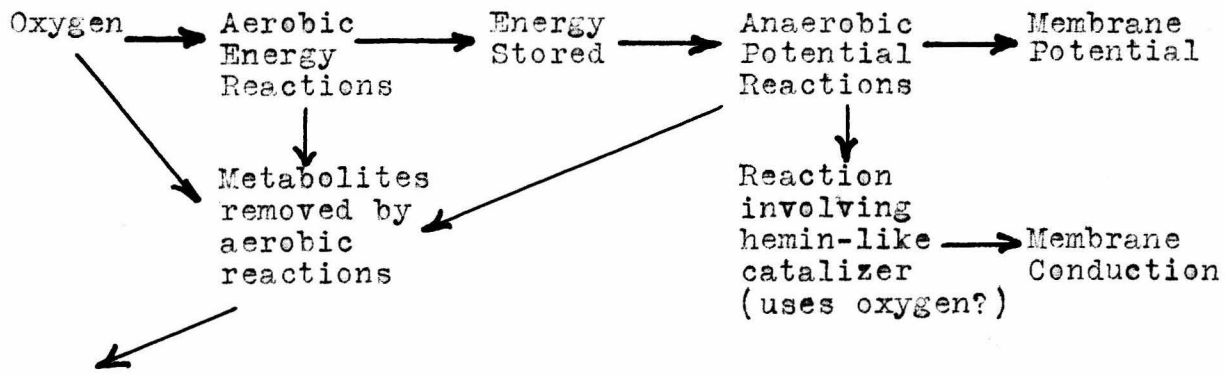
*/936

calcium whatsoever without initiating any tremendous variations of threshold or action potential as brought about by anoxia.

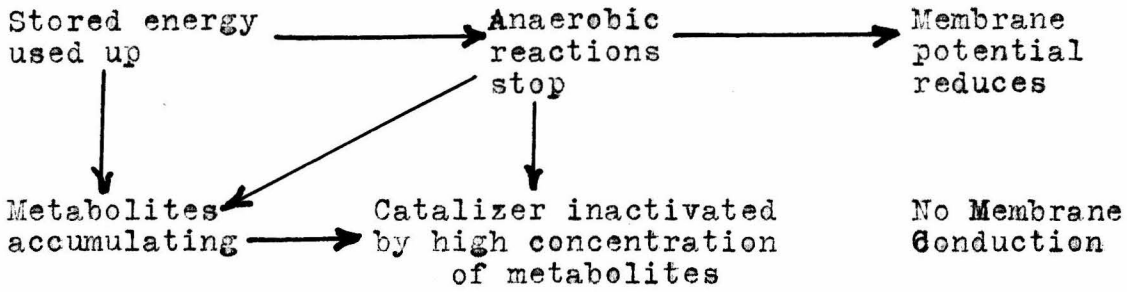
A scheme is presented, next page, which pictures how oxygen lack may affect the metabolism and irritability of the nerve.

The nerves of Cambarus clarkii do not fit into this picture at all. If there are any aerobic processes connected with excitability in crayfish nerves they are so small as to escape detection by the methods used here. It is of interest that the nerves of two related animals, lobster and crayfish, should react so differently to oxygen lack. It is worthy of more investigation to find just where the crayfish nerve respiration takes a turn strange to lobster, frog, and mammalian nerve. The crayfish is able to spend long times buried in mud where there is practically no oxygen at all. The lobster leads a more active life on the sea bottom. There are probably others in the animal kingdom which also do not fit the scheme. More experiments of a comparative nature are necessary to lead to an understanding of the reasons for these variations and to a more complete scheme to include all types of nervous tissue.

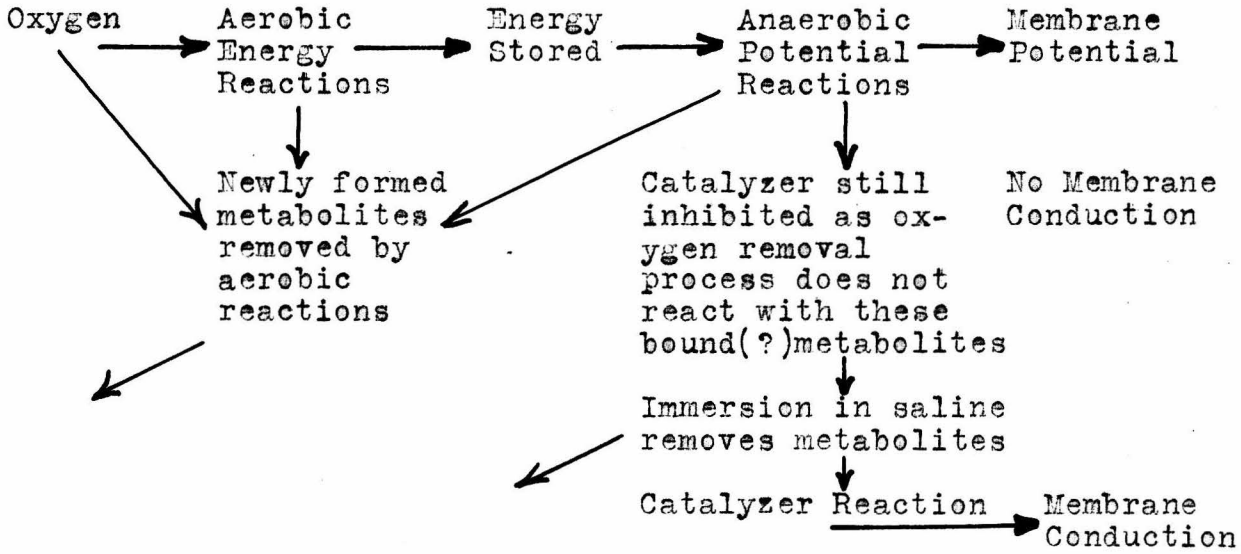
NORMAL



ASPHYXIATION



OXYGEN RECOVERY



SUMMARY

A comparative study was made of the effects of oxygen lack on the peripheral nerves of dog, cat, rabbit, frog (2 species, crayfish, and lobster. Dog and cat nerve activity survived about 30 minutes, rabbit nerve activity about 20 minutes, lobster nerve activity over an hour, Rana pipiens nerve activity 1½-2 hours, Rana catesbiana nerve activity more than 2½ hours, and crayfish nerve activity 9 hours without oxygen.

It was found that the nerve membrane becomes depolarized during anoxia.

The ratios of the survival times of nervous activity without oxygen were found to be inversely proportional to the ratios of the oxygen consumption rates of the same nerves (these rates taken from Gerard).

The refractory period, relative and absolute, was prolonged by asphyxiation.

The survival time of mammalian nerves varied inversely with temperature change and time washed in solution before asphyxiation.

The survival time varied directly with calcium ion concentration in the physiological solution.

The survival time of the mammalian nerve was not changed by immersion of the nerve in saline during asphyxiation, was held longer at the initial value by bathing the nerve in serum and was not changed by prolonged stimulation.

Recovery of activity was rapid and complete in mammalian

nerves with oxygen following asphyxiation of not too long duration was partial or not at all in frog nerves unless the temperature was raised to shorten survival time, was complete but slower with lobster nerves.

Prolonged asphyxiation of mammalian nerves in vapor up to 45 minutes shifts the starting point of recovery.

To recover from more than 45 minutes in nitrogen the mammalian nerve had to be immersed during the asphyxia or washed after it in physiological solution, indicating possible accumulation of toxic metabolites. Potassium is not the metabolite which acts poisonously.

No recovery of threshold pattern as Lehmann described was observed.

Oxygen "rests" of 20-30 minutes were required to duplicate the original survival time.

A possible scheme for the nerve metabolism is discussed. It is based on the assumption that there are two reaction groups, one aerobic, the other anaerobic.

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NOTE

Adrian (1922,1923)* has suggested that a narcotized nerve conducts with a decrement.This suggestion was based on the results of a series of experiments in which varying lengths of nerve were subjected to a narcotic.An impulse excited by a strong stimulus was observed to traverse a longer narcotized length of nerve than the impulse from a weaker stimulus.The impulse excited by the strong stimulus was larger at its origin than the one initiated by the weak stimulus.It was concluded that the impulse amplitude became gradually less as it traversed the narcotized region,and that due to its larger size at the start,the impulse from the strong stimulus was still existant though very small when it arrived at the normal region beyond the narcotized area.The weak impulse died out completely somewhere in the narcotized area.

Cooper based her claim for decremental conduction in asphyxiated nerve on this same principle.The original size of the impulse excited by a stimulus following the least interval became smaller as the interval increased with asphyxiation of the nerve.In traversing the asphyxiated region,this small impulse died out.On the other hand,the impulse stimulated after the recovery time remained large enough at the start to be able to travel through the asphyxiated region.However,since the least interval,and eventually the recovery time,did increase during anoxia,it must be concluded that the refractory period of the nerve increased during asphyxiation.This is contrary to the claim of Cooper.

* E.D.Adrian and A.Forbes, Journ Physiol, vol 56, 1922.

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