

The
I N F L U E N C E
of
L O W T E M P E R A T U R E S
on
N E R V E .

(An Experimental Research).

Thesis submitted for the Degree of M.D. by

JOHN TAIT, M.B., Ch.B., B.Sc.,
(Crichton Research Scholar).

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Part I. INTRODUCTORY.

A highly interesting chapter in a history of Physiology would be the evolution of our knowledge of nerve and nerve conduction. Each important advance in experimental fact was usually the source of a fresh theory or new modification of old theory; and with these special theories are associated the names of veteran physiologists.

No one theory has ever been accepted as final. Our experimental knowledge has hitherto remained insufficient to decide between modern rival views.

In recent times there have been two distinct tendencies of speculation. On the one hand stress has been laid on the electrical phenomena, and an attempt made to explain nerve conduction on a simple physico-chemical basis. On the other hand conductivity has been classed as an elementary property of living substance in general, like "irritability" or "contractility", and referred for explanation to "chemical processes" too complex for us as yet to understand. Of late years the tendency on the whole has been to favour the latter view.

One of the chief obstacles in the way of

adopting it has been the failure to demonstrate fatigue in nerve. In the end of last century however, positive doubt was for the first time cast on the non-fatigability of non-medullated nerve.⁽¹⁾ Medullated nerve remained refractory until two years ago, when it was proved to show definite fatigue under the influence of asphyxia and of anaesthesia.⁽²⁾ About the same time fatigue of medullated nerve was got by cooling.

The present thesis, while touching incidentally on some subsidiary questions, contains the first systematic account of cooling experiments done on nerve in relation to its fatigability. Besides proving the fact of fatigue by means of low temperature it throws light on the question of temperature generally as affecting the properties of nerve.

The research of which it forms the subject was undertaken at the suggestion of Professor Verworn in the winter of 1903-4 in the Physiological Laboratory of Göttingen University, and was continued during the winter of 1905-6 in the Physiological Laboratory of Edinburgh University under the direction of Professor Schäfer.

I
Part ~~IX.~~ ~~CHIEFLY~~ HISTORICAL.

Section A :- Grünhagen's Method:

Conductivity v. Excitability.

In the year 1872 Grünhagen⁽¹⁾ by the introduction of a new method into nerve physiology, gave a start to a most important series of investigations. Already in 1858 Schiff⁽²⁾, from the local variations in excitability along a nerve, and from the behaviour of a nerve when a portion of it was subjected to the action of a constant galvanic current, had argued that nerve possesses two distinct properties, excitability, or the faculty of being excited at any point by an external stimulus, and conductivity, or the faculty of transmitting the excitation so caused from one part of the nerve to another.

These observations were soon backed up from the clinical side by Duchenne de Boulogne, Ziemssen and Weiss, Erb, Eulenberg⁽³⁾ and others. They showed that in certain peripheral paralyses a nerve supplying definite muscles might be incapable of responding to electrical stimulation, while the patient might still be able to move these same muscles at will. In other words nerves might retain the property of conducting impulses from

centre to periphery and yet be incapable of excitation by external stimuli. Ziemssen - Weiss, and Erb⁽¹⁾⁽²⁾ went further. They carried out experiments on frogs by nipping across certain nerves with forceps and then studying the reactions during the process of repair. Conductivity returned before excitability.

Munk⁽¹⁾⁽²⁾ carried out a very careful series of investigations on the dying sciatic nerve of the frog, and found that while excitability might be greatly diminished or even lost in peripheral portions, a relatively slight stimulus applied to a more central portion might still cause a full contraction of the muscle.

All these experiments, however lacked definiteness. It is to the credit of Grünhagen⁽¹⁾⁽²⁾ (see Fig 1)

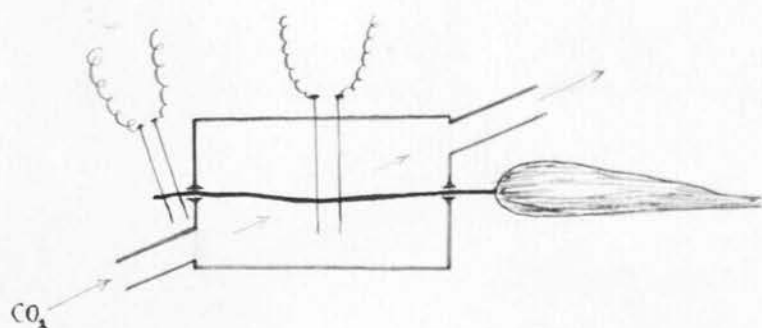


FIG. 1.

that he instituted a method by which the existence of these two separate properties was clearly established, and by which the extent of their independence might be with some exactness determined. He

enclosed the middle portion of a frog's sciatic nerve in a chamber through which a constant stream of CO_2 gas could be passed. The middle portion was thus subjected to the influence of the gas while the portions outside the chamber remained unaffected. On stimulating after the lapse of some time by means of electrodes fixed inside the chamber he found that the excitability had undergone a marked diminution, "while on the other hand," to quote his own words, "the excitability of the central portion of the nerve, which was not subjected to this narcotic influence remained constantly at the same height.... although the excitation started in it had to traverse the peripheral narcotised part. From this, one is justified in concluding that nerve excitation and nerve conduction should be separated as different processes".

With the pertinence of Grünhagen's deduction from his experiment we are however not immediately concerned; it is the method that claims our attention. It presents these special advantages:- (1) a localised piece of the nerve can be homogeneously acted upon by some external condition (presence of

gas) to the exclusion of the rest of the nerve; (2) the influencing agent, being electrically non-conducting, does not prevent the use of electrical stimulation, thus still affording quantitative measurement of the stimulus; (3) the length of the portion influenced by the gas can be varied at will by varying the size of the chamber. Finally its result shews that we have in it a most valuable means of determining the extent to which conductivity and excitability are independent of each other.

Since these experiments a hot dispute has raged round the question of the relative independence of conductivity and excitability, and the multiplication of observations on the subject made the question for a time very complicated.

Szpilman and Luchsinger⁽⁵⁾ using the vapours of ether, chloroform, alcohol and ammonia, distinguished two stages in the process of narcosis. In the first stage the excitability is lowered while the conductivity remains unchanged; in the subsequent stage the excitability is not yet absent, though greatly lowered, while the conductivity is totally gone. They pointed out that Grünhagen by using the weaker agent CO_2 had got only the first stage. The two stages of narcosis which these two observers thus established are of fundamental importance and will

often be referred to. These results correspond entirely with those of Efron⁽⁶⁾, who extended his observations to include the influence of various alcohols, different pressures, and different temperatures. Hirschberg⁽⁷⁾ repeated Grünhagen's CO₂ experiments while also using different temperatures. He did not get by this means the second stage of Szpilman and Luchsinger.

Gad, Sawyer and Piotrowski⁽⁸⁾ using various methods were able to repeat all the work of Szpilman and Luchsinger, besides directing attention to a new fact, viz. an initial rise in excitability and to a less extent in conductivity on narcosis. They also introduced an important modification into the method,

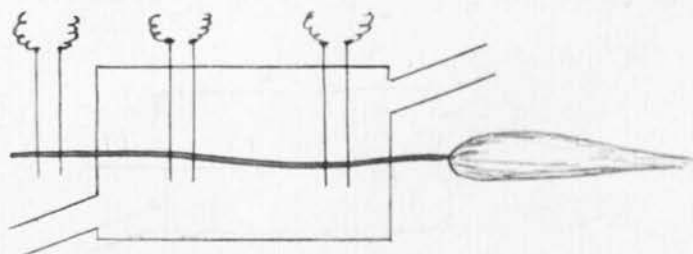


Fig. 2.

viz. they put two pairs of electrodes inside the chamber used for anaesthesia, one peripheral and one more central, although the observations they carried out in this way have since been shewn to be erroneous (see pp. 15-16.)

Gotch⁽⁹⁾ carried out a series of observations with ether and chloroform, in which special attention

was directed to the recovery of the nerve after anaesthesia. He got the two stages recognised by Szpilman & Luchsinger, while during recovery he found that conductivity returned somewhat rapidly, excitability on the other hand returned more gradually. Some experiments carried out under Gotch's direction (~~se~~)⁽⁹⁾ shewed that the second stage of nerve anaesthesia could be reached by the use of CO₂ gas, while recovery occurred in the same order as after chloroform or ether.

Waller (~~se~~)⁽⁹⁾ used isolated nerve and took the electrical response as an indication of the condition of the nerve. His observations, however, rather emphasised individual differences in the narcotic agents than helped forward generalisation on the question of the fundamental properties of nerve.

The question of the relation of conductivity to excitability had now become thoroughly complicated. As an indication of the difficulty of the subject at this stage one need only refer to the account given of it in some of the modern text-books of physiology. The careful and impartial summary of the evidence by Biedermann in his "Elektrophysiologie", 1895, p.p. 493, f.f., shews better than anything the need for some general principle to explain the facts. It tends to leave the mind of the reader rather in bewilderment than in enlightenment. Again the

section relating to " the influence of anaesthetics " on nerve in Schäfer's "Textbook of Physiology", Vol. II, 1900, p.p. 491-493 is more a ~~conflicting~~ series of observations on individual anaesthetic agents than a generalisation. The fact is, it was not possible to bring order out of the experimental facts at the time when these text-books were written.

As an interesting commentary on the difficulty of coming to definite conclusions on the subject, one may take the following two sentences, which Biedermann in his text-book, as if glad of an opportunity to generalise for once, prints in italics:- "From these (the experiments of Grünhagen, Luchsinger, Gad, Piotrowski, etc.) the very significant fact appeared that with local application of alcohol vapour, ether or chloroform, conductivity was as a rule first and most fundamentally affected before excitability underwent any perceptible diminution. With CO₂ on the contrary, as well as CO, conductivity is quite unaffected, while local excitability is quickly abolished". Not only is the statement in the first sentence a very doubtful deduction from the experiments quoted, but the separation of the action of CO₂ from that of other anaesthetics was soon proved wrong by the experiments just referred to as being carried out under the direction of Gotch. (p. 1)

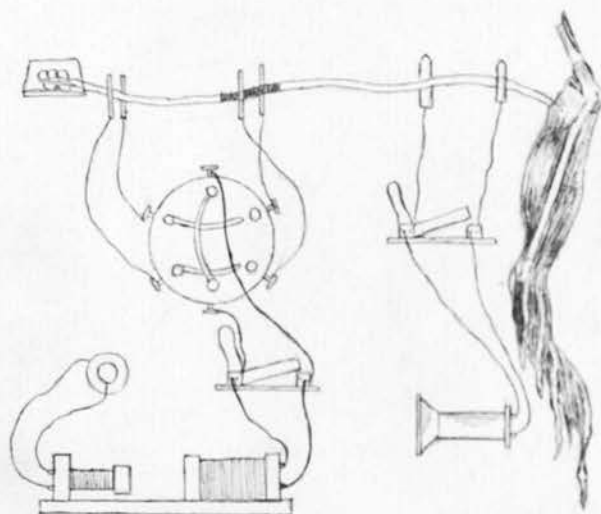
The subject was soon to be cleared up in

considerable measure. To understand how this was done we must go on to other experiments carried out by slightly different methods.

Section B. Wedensky's "Paradoxical Conduction".

We have with
It falls now to consider an investigation which Wedensky⁽¹⁰⁾ carried out on the fundamental properties of nerve. This observer anaesthetised the nerve by painting a portion of it with cocaine, chloral hydrate, or phenol.

Fig.3.



As indicators of the nerve condition he used simultaneously the telephone and the muscle response. The use of the telephone involved that the stimulation of the nerve should be rhythmic and rapid, — a tetanus of 100 per second he used as a rule. The responses of the muscle were accordingly tetani.

By means of the muscle record it was easy to confirm the existence of the two stages of Szpilman

and Luchsinger. During the first stage the excitability gradually fell away, as shown by the shorter and shorter distance of secondary from primary coil required to elicit minimal tetani in the muscle. The conductivity meantime remained constant. The second stage set in suddenly. All at once the conductivity began to fall rapidly and in a few seconds entirely disappeared. The excitability by this time was low, and it continued thus during further anaesthesia.

On using the telephone note however as indicator a remarkable phenomenon occurred. So long as he tested the excitability alone, the telephone record and the muscle record ran exactly parallel to each other, i.e. to keep the intensity of the telephone note constant during the progress of the anaesthesia he had to shift the secondary coil nearer to the primary, and at the same rate as he found necessary in order to elicit minimal tetani of the muscle.

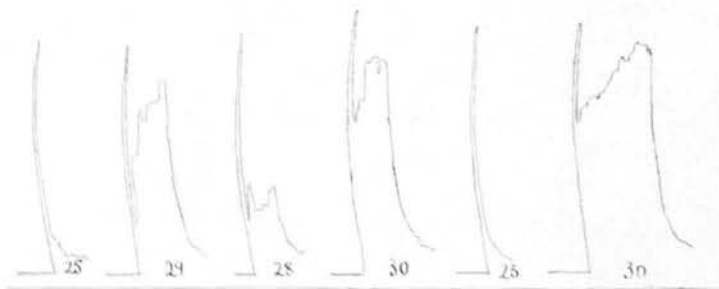
When he tested conductivity on the other hand he noticed that during the first stage, - the stage of apparently "undiminished" conductivity as shewn by the muscle record, - the tone underwent a considerable change. The note became weak, dull and mixed up with extraneous noises, and this was the case for all intensities of stimulation during the first stage. This change was a progressive one,

Fig. 4



(Curves reproduced from Wedensky's paper)

Fig. 5.



Later Anaesthesia

(From Wedensky's paper)

becoming more and more pronounced as anaesthesia went on.

His next observation was that in a certain stage of anaesthesia the tetanic contractions of the muscle (as elicited by stimulating centrally) were not as full and complete as before anaesthesia, and this was the case whether he used strong or moderate tetanising currents. Again, the tetani elicited by strong were weaker than those elicited by moderate stimulation, and more resembled those resulting from very weak tetanisation. The effect was in no way due to diminished conductivity of the peripheral end-apparatus, for peripheral stimulation with a third pair of electrodes caused full and energetic contractions. (See Figs. 4 and 5)

On further progress of the anaesthesia he found that strong tetanisation produced only an initial twitch of the muscle, (with often a final twitch in addition), while stimulation at moderate intensity caused tetanus. The telephonic record corresponded exactly; strong tetanisation caused either no sound at all or an initial "Knack" followed by silence, moderate tetanisation caused a weak and not quite pure tone, but one that corresponded unmistakably to the rate of stimulation used.

This remarkable and perfect parallelism between the records of the muscle and the telephone naturally surprised Wedensky. To make sure that the telephone sound was no backwardly propagated effect from the muscle itself, he put a drop of creosote on the nerve

close up to the muscle. Thereupon the contractions of the muscle ceased, while the nerve continued to give the same sounds as before. When he put creosote between the "conductivity" and the "excitability" electrodes the telephone tone disappeared.

He was consequently led to conclude that a stretch of narcotised nerve begins to alter as regards its conductivity much sooner than was formerly supposed. A "paradoxical modification" of the conductivity is established, in that rhythmical tetanisations are not transmitted regularly: further, strong impulses are transmitted with greater and greater difficulty, while weak excitations are handed on without prominent change.

It was interesting to see if the galvanometer would bear out the results of the telephone and of the muscle. The use of the galvanometer involves the interruption of the vital continuity of the nerve. Wedensky consequently brought the anaesthesia to a certain stage, keeping both nerve and muscle intact. After recording the muscle response he quickly killed a portion of the nerve with creosote and led off from the nerve the galvanometer current.

The results confirmed in the most striking fashion his previous observations. During the first stage of anaesthesia, provided strong shocks were used, the galvanometer records of stimulations starting at

both central ("conductivity") and peripheral ("excitability") electrodes ran parallel to each other from the start i.e. they shewed a regularly progressing diminution of both conductivity and excitability. When the conductivity had disappeared, as shewn by muscle and telephone, i.e. after the second stage of anaesthesia was established, the galvanometer no longer indicated the presence of any negative variation. When he stimulated peripherally, (excitability) the stronger induction currents still remained for a considerable time effective, but gradually fell off in amount as anaesthesia further progressed.

These results of Wedensky are of high importance. As we see, they were reached by employing rapid tetanisations of the nerve instead of single excitations, and by directing attention not to responses of the muscle elicited merely by minimal stimuli but to the result of stimuli of all degrees of strength; while the muscle records were controlled at every point by means of the telephone and of the galvanometer.

They were confirmed by Boruttau^{//} (~~14~~) who likewise investigated the condition by means of the capillary electrometer. He writes: "I have been able to confirm out and out the discoveries in a series of analogous experiments, and have also found by registration of the movements in the capillary

electrometer that at the commencement of ether as well as of cocaine narcosis of a complete nerve, the steepness of the curve and the backward drop ("definitive Ablenkung") of the meniscus is considerably diminished i.e. when the nerve is strongly stimulated with very frequent currents (acoustic interruptor); while the curve of the ^{current} of action with its single peaks shews scarcely any change on stimulation with less frequent and weak induction shocks". Boruttau likewise reports that he cannot appraise too highly the telephone as a means of investigating changes in nerve conduction.

Wedensky further found that when he tested the excitability of a narcotised piece of nerve by means of electrodes applied to the anaesthetised portion, the phenomena altered with alteration in the direction of the exciting current. When the effective current had an ascending direction, so that the point from which the stimulus started lay more centralwards, the phenomena more resembled those got from the "conductivity" electrodes; when on the other hand he used descending currents to excite the nerve he got a quite regular and gradual fall in excitability. He concluded that the latter method of stimulation gives a record of pure excitability, the former method gives a mixture of excitability and of conductivity. Gad and Sawyer⁽⁵⁾, by using two

pairs of electrodes within the anaesthetising chamber had found no difference in effect according as they stimulated more centrally or more peripherally.

Werigo and Rajmist⁽¹²⁾ in every exact piece of work had previously reached the same conclusions in this regard as Wedensky, and had pointed out the mistake in Gad and Sawyer's experiments, while Tiberg⁽¹³⁾, working under Wedensky's directions, had in 1895 shewn that in the later stages of narcosis each 1 mm. added to the narcotised nerve causes an increased hindrance to the progress of the excitation. Curiously enough Gotch and Macdonald⁽¹⁴⁾ apparently from purely a priori considerations, had pointed out the fallacy that might arise from testing for excitability at any arbitrary point along the influenced portion of a nerve subjected to local depressing influence. The importance of this precaution has since been confirmed by many investigators.

[The present occasion may be used to discuss a question which, though not directly bearing on the subject in hand, is yet of considerable theoretical importance, ⁽¹⁵⁾ ~~It~~ is the relation which the electrical change bears to the actual excitatory process in a stimulated nerve. The intimate association of the one with the other has again and again been clearly proved — the experiments of Wedensky on "paradoxical conduction" are only a further illustration of

the fact. While there is distinct evidence that the electrical may exist without the excitatory process, e.g. the dissected out nerves of warm-blooded animals may be incapable of causing contraction of the attached muscle when stimulated, and yet give the electrical response (Hermann⁽¹⁵⁾), Fredericq⁽¹⁶⁾, Gotch and Horsley⁽¹⁷⁾; still, until quite recently (Gotch and Burch⁽¹⁸⁾), there has been no evidence of the existence of the excitatory alteration without the electrical; ~~(see however, [p. 18])~~

These experiments of Wedensky suggest an interesting question. A portion at least of the electrical energy can be tapped on the progress of the excitatory wave along the nerve; it is this energy that causes the vibrations of the plate inside the telephone. Does this draining away of electrical energy detract from the ability of the excitatory process to stimulate the muscle? In other words is the electrical change causally related to the contractions of the muscle, or is it a mere bye-product in the generation of the excitation along the nerve? *follows with written text*
~~A series of experiments on minimal stimulations of muscle, (1) without, and (2) with, an electric circuit applied to the side of the nerve would be interesting.]~~

See Gotch's paper on length of action

Section C. Question of Oxygen Supply for Nerve
Cells and Nerve Fibres.

Meantime a new series of investigations had been going on, which though at first directed towards the elucidation of processes within nerve cells, ultimately came to bear on the fundamental properties of nerve fibres. These investigations were started by Professor Verworn, and continued under his direction by his pupils in Göttingen. From our present point of view their first important result was the discovery by von Baeyer that in the presence of an oxygen-free neutral gas a nerve after some time loses both its excitability and its conductivity; on restoration of oxygen these properties return.

¹⁹ (1) Nerve Cells.

Verworn (²⁰), in an investigation into the meta- Strychnine
bolic processes in the nerve cells of the frog's
cord, had used strychnine to increase the dissimila-
tion relatively to the assimilation. The ultimate
paralysis which follows upon greatly increased activ-
ity of the cells after injection of strychnine he
found to depend in high degree on the fact that the
cells had used up their available supply of oxygen.
When exhaustion had set in he perfused the aorta
with normal saline, (the only possible food materials

being here H_2O and $NaCl$). No recovery occurred. When O_2 was present in solution in the normal saline the tetanic convulsions began again and within limits the more O_2 that was supplied the longer did the renewed convulsions last. The capacity of the nerve centres to undergo excitation he thus directly proved to be a function of the amount of available oxygen.

Winterstein⁽²⁰⁾ then studied the extent to which the two phases of metabolism, assimilation and dissimilation are affected by narcosis (ether, chloroform, alcohol, CO_2). It had long been known that narcosis interferes with dissimilation. By using Verworn's method of perfusion with solutions of O_2 he shewed that narcosis hinders assimilation to exactly the same extent as it hinders dissimilation.

Narcosis

Archangelsky⁽²¹⁾ in 1873 had shewn that when frogs are warmed up to a temperature of $29^{\circ} - 34^{\circ} C$, clonic and tetanic convulsions appear, which are soon succeeded by complete paralysis. Examination of the peripheral organs shewed that the cause of this paralysis lies in the centres themselves. Winterstein⁽²²⁾ examined this condition and found that, as in strychnine paralysis so in the paralysis of overheating, the condition of the nerve cells as regards oxygen is a factor of the first importance. With increase of temperature the amount of oxygen used up by the nerve cells increases rapidly in proportion to the amount taken in, so that after a period of greatly

Heat.

heightened activity the cells become exhausted of oxygen. Recovery does not occur with mere cooling; a fresh supply of oxygen must be available.

Next, von Baeyer⁽²⁶⁾₂₃ investigated the effect of low temperature on the assimilation of oxygen in the ganglion cells of the frog. He shewed that in the cold the assimilation of oxygen outweighs its dissimilation, so that in the cold an actual storing up of oxygen takes place.

Cold.

Curiously enough Regnault and Reiset⁽²⁷⁾₂₄ in 1849 had directed attention to the same fact. They were investigating the condition of hibernation and found that in one case a marmot actually gained 5-9 grms. in weight in five days during profound torpidity. This increase in weight could only be derived from some constituent of the atmosphere. During the hibernation of marmots generally they found that only about two-fifths of the O_2 taken in in respiration appeared in the CO_2 discharged.

The results of Winterstein and v. Baeyer were all confirmed and extended by Bondy⁽²⁸⁾₂₅ who further investigated the effect of narcosis, heat and cold on the metabolism of the cells of the frog's cord

(II) Nerve Fibres. ₂₆

It next occurred to v. Baeyer⁽²⁹⁾₂₇ to try if a nerve could possibly be paralysed by want of oxygen.

He adopted Grunhagen's method, and passed neutral gases (N_2 and H_2), carefully freed from the last trace of oxygen, over the nerve. After a period varying from 3-5 hours both the conductivity and the excitability became abolished. By once more supplying pure oxygen gas to the nerve recovery occurred in 3-10 minutes. These experiments were done at room temperature. With increase of temperature the process of asphyxiation occurred more rapidly. Not satisfied with using indifferent gases alone, v. Baeyer used chemical reducing means. He packed the nerve with iron filings which had been made red hot in an atmosphere of hydrogen, and found that by this means also the conductivity became abolished, to reappear when oxygen gas was once more supplied to the nerve.

The importance of these experiments need hardly be insisted upon. Their interest lies in their suggestiveness. Although they were taken to shew the existence of metabolism inside the nerve yet in the absence of proof of the existence of end-products, such as CO_2 , this deduction is not ^{absolutely} justified.

In his experiments v. Baeyer⁽²⁴⁾ had observed phenomena that bore a strong likeness to fatigue. When he stimulated by means of repeated short tetanisations a nerve that had been asphyxiated and again exposed for a minute or two to the air he obtained "first of all only a single response.

Thirty seconds later ~~recovery~~ (the stimulation being meantime suspended) ^{recovery} ^ was so far advanced that the muscle responded to three stimulations, and a short time later the nerve had again laid up such a quantity of O_2 that even on continuous stimulation no fatigue was to be seen".

v. Baeyer next endeavoured to determine if a nerve becomes asphyxiated more rapidly when it is kept in constant activity. He placed two nerves from the same frog inside the same chamber and exposed them together to the influence of N_2 gas. The one he stimulated continuously at the rate of 800 per second, interposing a block by means of ether narcosis between the asphyxiated portion and the muscle; the other nerve he allowed to remain unexcited. Numerous experiments shewed that in one case the stimulated, in another the non-stimulated nerve became first asphyxiated. Hence this method does not adapt itself readily to shew fatigue in nerve.

To sum up, these experiments lend countenance to the idea, tenable on general biological grounds, that a nerve while it is alive, "respires" in the wide sense in which we talk of respiration in a unicellular organism. That this probable respiration is increased by activity there is a slight amount of evidence to shew.

Now that it was known that a nerve could be

asphyxiated Fröhlich²⁸~~(28)~~ examined the effect of narcosis (ether) on asphyxiated nerve, and found that it prevented the assimilation of O_2 — just as had been found by Winterstein to hold for nerve cells. He further found that the condition of narcosis is no bar to the passing of O_2 out from a nerve when surrounded by an oxygen-free atmosphere; whence he concluded that the O_2 stored in the nerve is dependent on the partial pressure of O_2 gas in the vicinity. His experiments, too, shewed in the most marked manner that nerve, once exhausted of O_2 , is in a condition of extreme oxygen-hunger, and takes up with the greatest eagerness O_2 offered to it.

This observer next endeavoured to determine if the presence of a reserve store of O_2 in a nerve can be definitely proved. He asphyxiated nerves⁽²⁹⁾ and offered different amounts of O_2 to them, and found that a certain definite amount is necessary to restore completely the excitability and the conductivity. What falls under this amount causes an incomplete recovery. What is over this amount, while causing no further rise in excitability, yet serves to protect the nerve for a longer period against a second asphyxiation. This goes far to prove the existence of a reserve store of O_2 .

The time that is required to asphyxiate different nerves at room temperature varies within wide

*This was by
minimal
stimulus
method however*

limits. v. Baeyer had given a period of 3-5 hours. Fröhlich found a difference of 1-15 hours in different frogs and established the fact⁽³²⁾ that the nerves of animals kept long in the cold take a much greater time to asphyxiate than the nerves of animals that have been kept continuously exposed to a higher temperature. Further, he determined that the faculty of recovery from asphyxia depends on the state of nutrition of the animals. The nerves of badly fed frogs do not shew complete recovery from asphyxia; if the nutrition of the frogs is good the nerves recover perfectly.

I then endeavoured to carry out asphyxiation experiments with warm-blooded nerve. It is well known that the nerve of a living warm blooded animal can be freed for a considerable distance from the surrounding tissues and yet retain its properties for hours together. On the cessation of the blood supply to the muscle of a warm blooded animal inability to contract very soon sets in. For my experiments the peroneal nerve-muscle group of a live rabbit was used. The spinal cord of the animal was divided in the lower dorsal region. The peroneal nerve was separated out from the point where it enters the muscles in the upper part of the leg, right up to its exit from the pelvis, and in this latter situation it was cut across. The

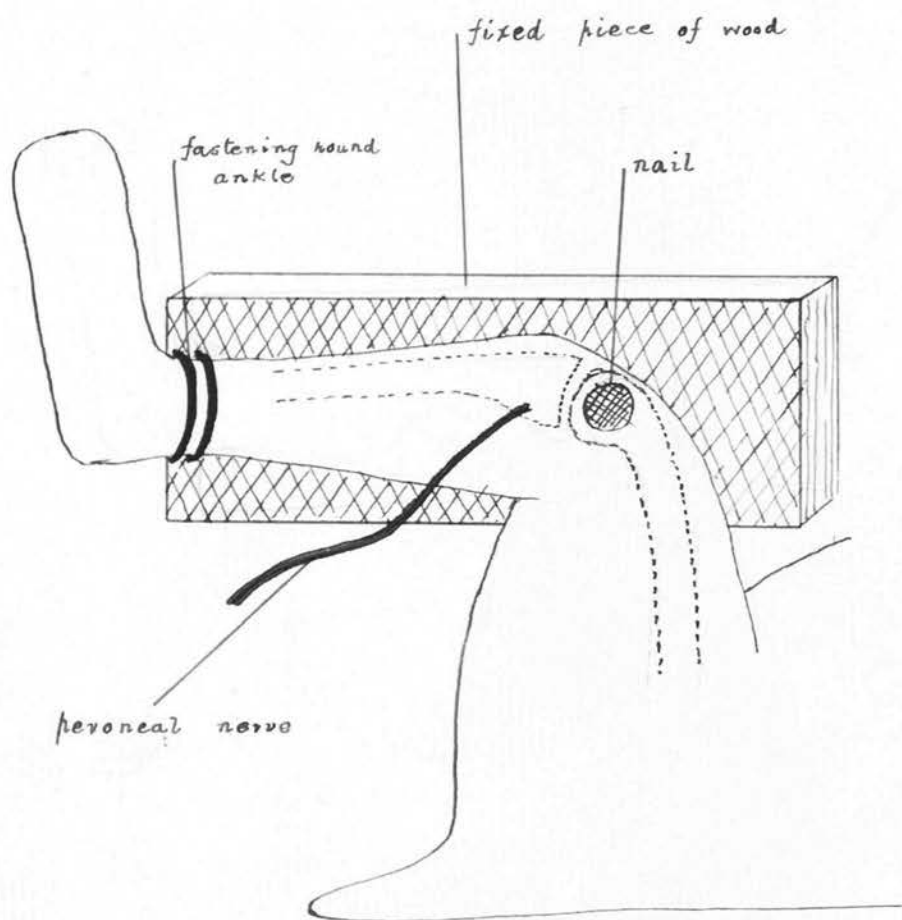


Fig. 6.

separation was easy because the peroneal and tibial nerves in the rabbit are not bound together in one common sheath as is the case in man.

The animal was laid on its back and the lower end of the femur fixed to a rigid piece of wood by means of a flat-headed nail passing through the bone just at the condyles, while one or two turns of tape round the leg just at the ankle served to keep the leg steady. Thus one got a rigidly fixed nerve-muscle preparation, stimulation of which caused flexion of the foot at the ankle. To prevent the possibility of any reflex interference with the responses the tibial nerve going to the calf muscles was likewise divided. The peroneal nerve itself was supported horizontally on electrodes, and lay in a direction perpendicular to the plane of the flexed leg and thigh of the animal. (See Fig. 7.)

On now surrounding the nerve by a chamber through which N_2 gas was passed, no diminution in either excitability or conductivity was found to occur even though the stream of N_2 was kept going for 15 hours. It then struck me that the persistence of the blood supply might prevent asphyxia. If O_2 were being continuously supplied by means of the blood-vessels the effort to exhaust the nerve from the outside would naturally be fruitless. Control experiments shewed that this conjecture was right, and

further I found that a continuous blood-supply is a sine qua non for the functioning of warm-blooded nerve.

Contrary to accepted ideas the nerves of warm-blooded animals possess a richly anastomosing blood supply. The combined peroneal and tibial nerves of the rabbit are in the thigh supplied by at least three visible arteries that enter at different points of their course. By much the largest of these is an artery that enters about the middle of the thigh and sends branches both upwards and downwards along the nerves. On the division of this vessel the central half of the peroneal nerve usually underwent a sudden drop in excitability, often the excitability became quite lost. The half which lay peripheral to the point of entrance of this large vessel remained as a rule fully excitable all along its length. The reason for this was found to be that recurrent vessels from the muscles enter the nerve below, the blood is pumped along these as far as the cut in the aforementioned large artery, and there it escapes, (it used to form as an ever-increasing drop which hung ^ependant on the middle of the nerve). From loss of pressure the blood fails to be pumped further backwards, whence the loss of excitability in the upper half of the nerve. This was beautifully shewn by cutting the nerve across with sharp scissors at different parts of its length. In the upper half —

the half which was unexcitable — no blood oozed from the fresh cut section. In the lower excitable half a section at any place shewed bright red blood oozing out from the lower end. Once or twice, too, after the drop of blood forming on the middle of the nerve had clotted, the upper half of the nerve became once more excitable. A cut with the scissors then shewed that blood was again flowing along this part. Obviously by the clotting of the blood in the mouth of the large artery of supply the pressure had become sufficiently raised to drive the blood into the central part once more. It was then found that if this vessel was ligatured before division the excitability of the central half of the nerve remained as a rule quite good.

In the course of a long continued experiment I sometimes omitted to keep the nerve moistened with Ringer's solution. In these cases the central part would become brown, dry and shrivelled, while the more peripheral portion would remain moist and white, and perfectly capable of functioning all the time. The latter condition was obviously due to persistent blood supply. Again, all efforts by means of very free dissection to prevent the blood from flowing back into the nerve from the side of the muscles proved fruitless. The blood supply of the muscle and the attached nerve are indissolubly bound up with

each other. An injection with methylene blue shewed the presence of fine vessels visible to the naked eye running longitudinally along the nerve into the muscles; and generally speaking it is the easiest thing in a youngish animal during life to demonstrate the presence of relatively large vessels running just inside the sheath of every moderate sized nerve in the body. A low power of the microscope shews them up very plainly.

These experiments throw into strong light not only the absolute dependence of the nerve on a blood-supply, but also the very complete nature of the anastomosis in mammalian nerve; further, that a warm blooded nerve may become temporarily inexcitable and after the lapse of some considerable time regain its excitability on the restoration of the circulation. They clearly suggest that mammalian nerve has a metabolism of a pronounced kind.

An attempt was then made by perfusion to replace the blood in the combined tibial and peroneal nerves by means of Ringer's solution, with the ultimate object of determining if a supply of O_2 is as necessary for warm-blooded as it is for cold-blooded nerve. All efforts however to perfuse the fine vessels of supply failed owing to the smallness of the calibre. Such experiments might succeed in a larger animal than the rabbit.

As was just stated mammalian nerve may undergo

temporary suspension of function owing to deficiency of circulation. The nerve is not dead, it resumes its capacity to function normally when blood comes back to it. This might possibly be correlated with the fact, previously mentioned (see p.17.) that the electrical reactions may be elicited from mammalian nerve at a time when the muscle response cannot be got.

Section D. The Relationship between Excitability and Conductivity further discussed.

In the course of his investigations Fröhlich had soon convinced himself that the interference with conductivity and excitability caused by asphyxia corresponds exactly with the interference with these properties by narcosis. The only difference is a difference in the time required to produce the various stages, asphyxia being a much slower process than narcosis, in the relation roughly of 10:1. By using the more deliberate asphyxiation method he was enabled ³⁰(~~86~~) to study the two "stages" of Szpilman and Luchsinger much more carefully than had before been possible, and could easily trace the passage of the one stage into the other. The method he used was the single twitch method with minimal induction shocks, the muscle being employed as the indicator.

On commencing the asphyxiation the excitability

Fig. 7.



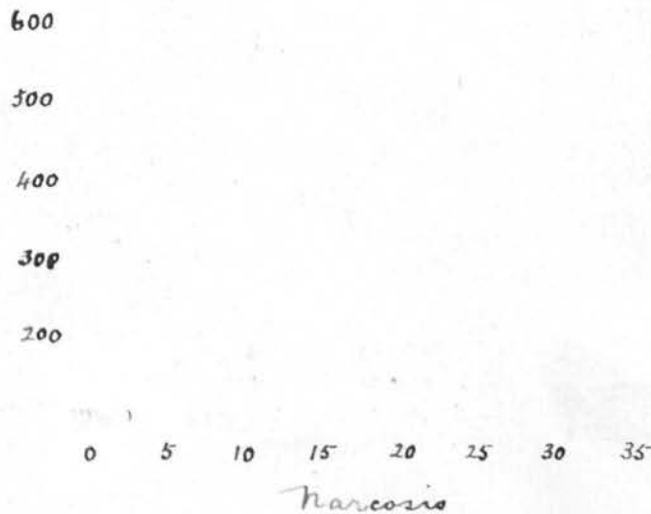
Abcissa gives Time in Minutes

Ordinate gives Distance of Secondary from Primary Coil in mm.

C = Conductivity

P = Excitability

Fig. 8.



Abcissa and Ordinate as above

Upper Horizontal line shows Conductivity

Lower Descending line shows Excitability

was first influenced, while the conductivity shewed no change. When the excitability had fallen to a definite point the conductivity began to fall very quickly, soon to disappear entirely. Meantime stimulation applied inside the chamber not far from the point of exit of the nerve towards the muscle, remained still effective. If the asphyxiation was further pushed the excitability sank more and more until the stimulation began to pass by "Stromschleifen" to portions of the nerve lying outside the chamber. Asphyxiation was never much pushed beyond this stage, for the nerve was apt to die.

If O_2 was supplied to the asphyxiated nerve the first signs of recovery occurred after a period of $\frac{1}{2}$ - 2 minutes, and at the peripheral point of excitation situated inside the chamber. At this spot a rise in the excitability took place. Only when the excitability inside the chamber had risen to a definite point did stimuli coming from the central electrodes become effective. The re-installation of the conductivity went on then so quickly that the steps could scarcely be followed. In a few seconds the original conductivity was back again while the excitability inside the chamber continued gradually coming back to its primitive height. Exactly the same results were got with narcosis by means of ether.

By using chambers of varying diameter so as to asphyxiate different lengths of nerve he was able to

Repeat my
Prolongation of
effect "experiments"
went of O_2 .
This may be
another method
of asphyxiating B.T.G.
muscle p. 49.

I wonder how I
got this result.
If Deaton's
experiments p. 32.

shew that the conductivity more quickly disappeared when a long stretch of nerve was acted upon than when a short piece was asphyxiated. As stated before (p. 16) Werigo and Rajmist⁽¹²⁾ had previously got the same result, and, even before Wedensky made his direct observations on a diminution in the power of conductivity in the early stages of anaesthesia, they had reasoned from the time required to abolish the conductivity when different lengths of nerve were exposed to the anaesthetic that the conductivity undergoes a gradual change from the first moment of anaesthesia.

Fröhlich, however made a further observation³⁰
(33)
He found on asphyxiating different lengths of nerve that there was not merely a difference in the time but also a difference in the degree to which the excitability must sink before conductivity is abolished. The longer the stretch of nerve affected by narcosis or asphyxia the less a drop in the excitability suffices to abolish the conductivity; the shorter on the other hand is the anaesthetised portion the more must the excitability be lowered before the conductivity disappears.

These results clearly prove that instead of being two distinct properties, as Grünhagen had imagined, the conductivity is a function of the excitability.

It is unfortunate that owing to the use of

induction coils the graduations of which were arbitrary the exact relation that holds between length of nerve anaesthetised and degree of depreciation of excitability necessary to cause disappearance of conductivity, were not in this minimal twitch method determined. The presumption is that if the anaesthetised portion were homogeneously acted upon all along its length, the nature of the relationship would turn out to be very simple. A series of quantitative investigations with a standard Kronecker coil, care being taken to make as precise delimitations of the anaesthetised portion as possible, would be ~~very~~ interesting.

Dendrinos⁽³¹⁾, working in Hering's laboratory with ether narcosis, had also emphasised the importance of the length of nerve anaesthetised on the result of narcosis. This observer, acting on the hint given by Wedensky, had not confined his observations to minimal twitches but had used single over-maximal stimulations as well. Direct observation by this method shewed that conductivity undergoes a decrease at a time, when, as tested by the minimal twitch method, it is apparently unchanged.

Noll⁽³²⁾, who worked in Biedermann's laboratory, repeated Dendrinos work with single over-maximal stimuli and confirmed the results. He also directed his attention to the question whether different

narcotic agents have specific effects on nerve, and concluded from experiments on ether, chloroform, acetone, ethyl-bromide, ammonia, acetic acid and carbon dioxide, that they all act in the same way exactly, the only difference being in the rapidity with which they produce their effect.

In this regard it must be pointed out that the idea, once entertained, that some substances exalt the conductivity of a nerve, relatively to the excitability, and vice versa, is now being abandoned. The tendency of recent work has almost invariably been to establish the fact that so-called anaesthetics have nothing peculiar in their action. A whole group of substances varying enormously in chemical constitution all have the same action on nerve. They are depressants and simply abolish the function of the nerve with greater or less rapidity. Fröhlich shewed that asphyxia acts in the same way. Any difference in the action of these things is not manifested by a difference in the behaviour of conductivity to excitability, but rather in the relative toxicity of the chemical substances, recovery from their action being taken as the test. To this point Waller first directed his attention. After him Wedensky emphasised the question. The only serious attempt in quite recent times to establish a specific action for any so-called nerve anaesthetic was that of Herzen⁽³³⁾ and Radzikowski⁽³⁴⁾ who claim-

From this point
of view the peculiar
initial action of
CO₂ (water) is
all the more
striking

ed that chloralose (or glyco-chloral) could entirely abolish the excitability without affecting the conductivity of a nerve. Their experiments were ~~very~~ severely criticised by Wedensky⁽¹⁰⁾~~(15)~~:

In this connection it is interesting to know if CO gas acts in the same way as N₂ or H₂. As we know, CO forms a firmer compound with haemoglobin than O₂ does. It is just possible that after CO asphyxia some compound may be formed in a nerve which might prevent recovery on the admission of O₂. I performed some experiments in Göttingen to settle this point. The CO gas, prepared by the action of concentrated H₂SO₄ on oxalic acid, and freed from CO₂ by washing with KOH, was allowed to run into the asphyxiating chamber. For some hours the excitability gradually decreased, the conductivity as tested by minimal stimuli, remaining constant. Finally the conductivity disappeared. On readmission of O₂ both conductivity and excitability soon came back again to the full extent, and the nerve showed a complete recovery. CO acts on nerve like any other neutral gas.

To fix our ideas we shall sum up what we know at this juncture of the relation of conductivity to excitability. The two stages of anaesthesia (Szpilman and Luchsinger) got by the minimal twitch method is only a first approximation to the truth. Just as Kepler's laws, not absolutely true themselves

formed the basis of Newton's generalisation, so the recognition of these two apparent stages was the starting point for more exact knowledge. There are in reality no two distinct stages of anaesthesia. Conductivity begins to be influenced from the start. By the minimal twitch method it is not possible to verify this by direct observation, one can only by reasoning on the results of anaesthetising different lengths of nerve reach this conclusion. By the use of the method of over-maximal stimulation one can directly shew that the conductivity begins to disappear at a time when it is still present for minimal stimuli. These facts are sufficient to prove that conductivity and excitability are not two distinct properties as Grünhagen held, but that conductivity is a function of excitability. When excitability is depressed conductivity tends likewise to be depressed.

Wedensky's method of using rapidly recurring stimuli with the "paradoxical conduction" that it disclosed, seems to indicate that the question is a much more complex one than had been supposed, but as we shall see even this paradoxical conduction is capable of analysis and of explanation.

Section E. Fatigue of Nerve.

Ever since Bernstein's ³⁵(~~58~~) classical experiments on "the fatigue and recovery of nerve" the question of the fatigueability of nerve has been a much discussed subject. The majority of the experiments that have been carried out on the subject have been done on motor medullated nerve. The method used has generally been to stimulate the nerve for a number of hours, and meantime exclude fatigue of the end-apparatus by preventing the impulses from reaching the peripheral organs. On removal of the block by which this was attained the activity of the peripheral organ was seen to be present in undiminished strength. Various kinds of blocks have been used. Bernstein ³⁶(~~59~~) and Wedensky ³⁷(~~40~~) used the constant galvanic current, Bowditch ³⁸(~~41~~) and ³⁹(~~42~~) used curara, Maschek ⁴⁰(~~43~~) employed ether, and in the case of secretory fibres Lambert ⁴¹(~~44~~) made use of atropin.

Waller ⁴²(~~45~~) took the electrical changes in medullated nerve as an index of the activity and photographed the galvanometric variation. The nerve was submitted to a short series of rapid excitations of similar intensity, which recurred at regular intervals of one minute, and the resulting effects shewed no evidence of diminution though fatigue.

Some few observers have used non-medullated nerve. In experiments on the cervical sympathetic fibres Eve ⁴³(~~46~~) found the vaso-constrictor apparatus

of the vessels of the ear still active after 12 hours stimulation. Again Howell, Budgett and Leonard⁽⁴³⁾ state that vaso-constrictor and cardio-inhibitory fibres show no functional fatigue after an hour's stimulation.

In Waller's experiments on isolated nerve he used various anaesthetic agents, including CO₂ gas. Large doses of CO₂ temporarily abolish the electrical response. Very small doses on the contrary increase its activity, and the swing of the galvanometer needle is increased when the nerve is thrown into action. Again, in a non-narcotised nerve mere excitation causes the electrical responses to improve at first, and in a manner exactly similar to the improvement from small doses of CO₂ gas; whence Waller concluded that activity is associated with the discharge of carbon dioxide.

Now to account for the fact that fatigue does not still occur in nerve Waller put forward the following tentative explanation. He says⁽⁴⁴⁾; "I wonder does this carbonic acid become altogether dissipated; may it not perhaps be reinvolved in some storage combination, as the nerve-fat, perhaps, that is so prominent a constituent of fully evolved nerve? Such nerve consists of proteid axis and fatty sheath; the axis — which is the off-shoot of a nerve cell — is the specially conductile part, the sheath is a developmental appendix, not directly connected

with any nerve cell. Yet, cut the nerve, and sheath as well as axis undergo Wallerian degeneration, which is evident proof of a functional commerce between sheath and axis. All these things to my mind reconcile themselves with the notion that the active grey axis both lays down and uses up its own fatty sheath, and that it is inexhaustible not because there is little or no expenditure but because there is an ample re-supply".

⁴⁵ In order to test this hypothesis, Miss Sowton (~~4~~) undertook at Waller's suggestion an investigation on the non-medullated olfactory nerve of the pike, and found that the galvanometric responses of the nerve became feebler on repeated stimulation.

Brodie and Halliburton, (⁴⁶~~47~~) not quite satisfied with the proof of fatigue in these experiments, sought to settle the question in another way. To avoid the use of the electrical response as the test of activity, they selected the non-medullated nerves that go to the spleen of a mammal. They blocked the impulses by means of low temperature ($0^{\circ} - 2^{\circ}\text{C}$), and found that even after 6 hours continuous stimulation the nerve conducted impulses just as well as at the start. They likewise made corresponding observations on ^{the} vaso-motor nerves contained in the sciatic nerve of the dog, the volume of the leg being recorded with a plethysmograph, and repeated Eve's experiments on the cervical sympathetic run-

ing to the ear of the rabbit. In no case were they able to demonstrate any functional fatigue.

These experiments of Brodie and Halliburton however, labour under one ~~very~~ serious objection, and the same objection applies to Bowditch's, to Eve's, to Lambert's and to Howell, Budgett and Leonard's experiments. They were done on warm-blooded animals in which, as was pointed out on page ~~86~~⁸⁵, the nerves while capable of functioning retain their blood supply more or less intact. So far as we yet know, it is quite possible that the nerves of warm-blooded animals may be easily made to shew fatigue.

But to return to Waller's hypothesis, apart from the function ascribed to the medullary sheath, the suggestion that fatigue is difficult to prove because the restitution processes are so perfect has much to recommend it. Thus Fröhlich's experiments brought into prominence the great rapidity with which O_2 is taken up by an asphyxiated nerve. While it took 3 - 4 hours to remove the conductivity of a nerve in an atmosphere of N_2 exposure to O_2 for $\frac{1}{2}$ -1 minute was sufficient to restore conductivity again for a long time. The nerve took up O_2 at a much faster rate than this element could be withdrawn from it.

Garten⁴⁷ (~~48~~) took up Miss Sowton's work on the non-medullated olfactory nerve of the pike, using the capillary electrometer instead of the galvanometer,

*Reasoning
not
conclusive.*

and got the same results as she did. On rhythmical stimulation with induction shocks in a constant direction he found that the excursions of the electrometer soon declined in amount. After a rest they returned in full amount again. In order to meet the objection that the result was merely an effect of injury at the point of stimulation, he stimulated at two places; the one point of stimulation he used for long continued tetanisation, the other (nearer to the capillary electrometer electrodes) he kept for short control stimulations. Thus, assuming that the electrical change gives a measure of the amount of the excitation, the fact seems to be proved that non-medullated nerve is capable of being fatigued.

In 1900 Carvallo⁴⁸ (55) reported that he had been able to shew fatigue of frog's nerve by cooling. He kept the muscle at room temperature and cooled the nerve to 0°C. By stimulating with single shocks every five seconds he found that after a period of half an hour the excitability had disappeared; and proved to his own satisfaction that both low temperature and stimulation of the nerve were necessary to cause this fatigue. I repeated Carvallo's experiments while in Göttingen, and found that what he took to be fatigue was merely a lowering of the excitability of the nerve by low temperature. He had fixed upon a stimulus that was just maximal at 20°C, and had used it all along. Naturally, when the tempera-

ture of the nerve fell this stimulus became no longer effective. In his proof that both low temperature and stimulation were necessary to cause fatigue he had not accurately measured his temperature; and generally speaking his whole conclusions were inaccurate. For one thing the conductivity of the "fatigued" nerve remained unchanged all along!

As we have seen (p. 21), v. Baeyer observed phenomena in a nerve recovering from asphyxia, which he took to imply fatigue.

While Fröhlich was examining the curves published in Wedensky's paper ⁽¹⁸⁾ it occurred to him that the phenomena might be due to fatigue of the nerve. At a certain stage of the anaesthesia shortly before the disappearance of the conductivity, strong and rapid tetanisation of the nerve calls forth only a single twitch of the muscle, weak and less rapid tetanisation causes tetanus. Fröhlich had found that the same thing occurs in asphyxia just before the disappearance of the conductivity. He consequently set himself to examine the condition more closely. ⁽¹⁹⁾

He kept the nerve narcotised with a dilute stream of ether, and examined it when it had reached the stage at which the conductivity is on the point of disappearing. By varying the rate of stimulation (from 150 to 3 or 4 per second), meantime keeping the intensity constant, he found that high frequency

caused only an initial twitch, a slightly less frequency caused also an initial twitch which was slightly higher than the first. Stimulation with still lower frequency caused tetanus, which increased in height with further decrease in the frequency — Optimum of Frequency — and finally decreased again in height with continued decreasing frequency of stimulation. Eventually he got incomplete tetanus. A frequency of 3 - 4 per second produced twitches quite separate from each other, but of the same height at first as the initial twitches which were got with the high rates.

The same results were got by altering the intensity, when the frequency was kept constant. Tetanisation with strong intensity caused a single twitch, a slightly less intensity caused a single twitch just higher than the first. Weaker intensities caused tetani, which at first increased with decrease ⁱⁿ intensity — Optimum of Intensity — and then fell off in height to finally disappear. Single shocks here, too, caused twitches of the same height as those got by tetanisation with strong shocks.

These results corresponded exactly with effects that Wedensky ⁽⁵⁰⁾~~(49)~~ already in 1886 had found in the nerve-muscle preparation of a narcotised frog and which Hoffmann ⁽⁵¹⁾~~(50)~~ who repeated the experiments using

had ascribed to an action on the end-plates of the nerve in the muscle.

That these effects in Fröhlich's experiments were not due to an altered condition of the end-plates or of the muscle was shewn by control experiments with a pair of electrodes applied quite peripherally on the nerve, i.e. near the muscle. Full tetani were then always produced. Besides, the telephone and galvanometer controls of Wedensky's later experiments, ¹⁰(~~10~~) and the capillary electrometer results of Boruttau (see p. ~~10~~) entirely negative any such objection.

The fact that in a certain stage of anaesthesia strong tetanising stimuli call forth only one twitch which in height corresponds to the effect of one single ^{stimulus}~~twitch~~, indicates that in this condition of the nerve strong and rapid stimuli are not summated, which must be due to a prolongation of the refractory period of the nerve. Experiments with two stimuli separated by a very short interval of time, shewed that in reality the refractory period of the nerve was greatly prolonged by anaesthesia. This had already been pointed out by Boruttau ⁵²(~~51~~).

Nay more, there was a definite relation between the refractory period as determined by two successive twitches, and the frequency of stimulation that in any given stage of the anaesthesia just caused an initial twitch. Supposing he found that with a de-

:finite intensity of stimulation tetanising shocks of 100 per second just caused an initial twitch, while similar shocks at the rate of 80 per second caused muscle tetanus, then two stimuli of the same intensity as had been used for tetanisation following each other at an interval of $1/100$ th. of a second caused no summation, while two similar stimuli, but separated by an interval of $1/80$ th. of a second caused summation.

This prolongation of the refractory period by means of anaesthesia may reach a considerable extent. Fröhlich found a refractory period in one case of as much as $1/10$ th. of a second.

The refractory period is a function not merely of the degree of the anaesthesia but also of the strength of the shock used to stimulate the nerve. Strong shocks shew a longer refractory period than weak shocks.

All this suggests that the reason why no one has succeeded in fatiguing nerve is that the refractory period under normal conditions is so short that in the intervals between the individual stimuli the nerve has sufficient time for complete recovery, and fatigue cannot set in. In the condition of narcosis and of asphyxia on the other hand, the refractory period is so much prolonged that on stimulation with repeated shocks that follow each other in an interval of time less than the refractory period, only the first stimulus is effective, while the second stimulus

Fig. 9.

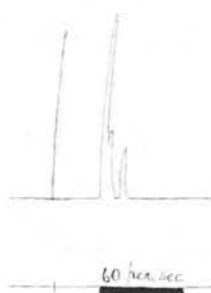
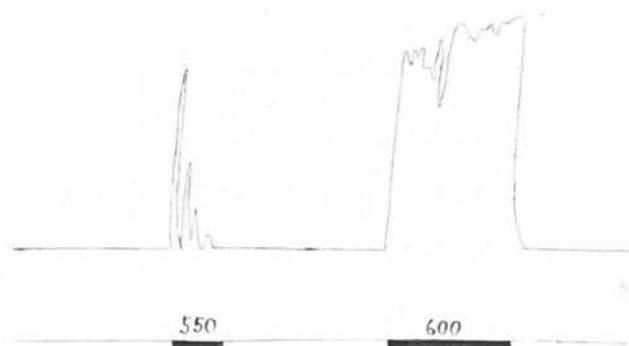


Fig. 10.

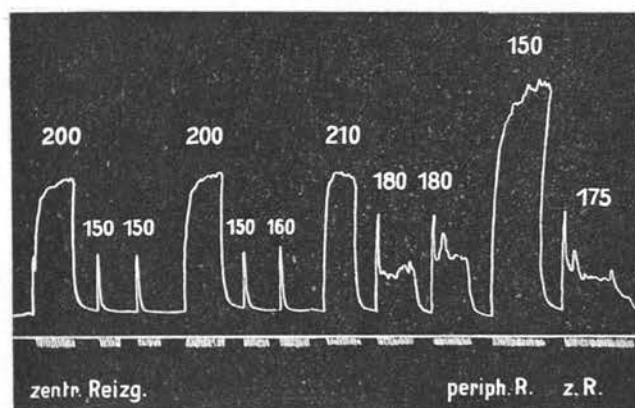


occurs in the refractory period of the first and is therefore ineffective.

Now, if instead of considering simply the single twitches that are got with rapid tetanisation and the complete tetani that follow much slower stimulation, we direct our attention to the muscle contraction that follows tetanic stimulation of the nerve at an intermediate rate, we find that it consists of an initial twitch followed by a series of twitches of decreasing height and finally by complete rest of the muscle. In figure⁹_^ we see two responses: the first is a single twitch recorded with stationary drum, the second is the result of tetanising at 60 per second at the same intensity. Had the tetanising shocks been more rapid we should have got a single twitch, had they been slower we should have got full tetanus. As it is we see that the twitches fall away and are succeeded by complete rest. This is a curve shewing fatigue of the nerve.

This proof of fatigue is ingenious and beautiful. It just misses being absolutely conclusive, because Fröhlich did not prove that the refractory period is prolonged by previous activity of the nerve. A possible objection might be that his anaesthesia was becoming progressively deeper, and that consequently the refractory period was with each small interval of time becoming longer. Until it is proved that the refractory period is actually pro-

Fig. II.



:longed by activity one cannot take this as unconditional fatigue. Nevertheless it is by far the best proof of fatigue of nerve that has ever yet been given.

As in my attempts to asphyxiate the nerves of warm-blooded animals I had worked out the operative technique for warm-blooded nerve it occurred to Fröhlich ~~(and me)~~ that by an adaption of the method we might be able to narcotise warm-blooded nerve sufficiently to show Wedensky's "paradoxical conduction". This we found ⁽⁵³⁾₍₅₅₎ quite possible in the peroneal nerve of youngish rabbits by means of ether and chloroform, and we obtained typical "fatigue" curves. For some reason we were unable to sufficiently narcotise the corresponding nerves of full-grown animals; whether it was that the connective tissue was more dense or the blood-supply better ^{or for what other reason} it is difficult to say.

Section F. Are Conductivity and Excitability really separate?

Before concluding this ^{part of our} historic sketch it will be of advantage to once more return to these two properties of nerve, conductivity and excitability so as to finally adjust our ideas on the subject.

Boruttau had found that the rate of transmission of the electrical change in nerve is delayed by narcosis. Fröhlich investigated this discovery by

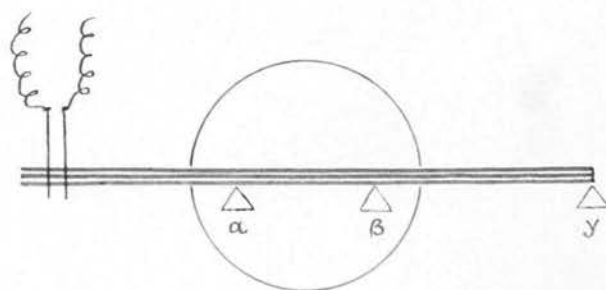
means of the muscle, and established the fact (54) that both asphyxia and narcosis produce a distinct delay in the transmission of the excitation. This delay is greater the greater the length of nerve anaesthetised or asphyxiated, as the case may be, and is most marked just at the stage when the conductivity (as tested by minimal induction shocks) is about to disappear. The slowing ^{of} ~~at~~ the impulse is confined to the affected portion; on passing through it the impulse once more resumes its normal speed.

As mentioned in last section (p.43), Fröhlich determined that the refractory period of the nerve is prolonged by narcosis and asphyxia, a fact to which Boruttau had already drawn attention (⁵²~~51~~).

Hand in hand with the prolongation of the refractory period there goes an increase in the actual duration of the electrical wave (⁵²~~51~~). This takes the form of a great prolongation of the descending portion of the curve of negative variation, as shewn by the capillary electrometer. The prolongation of the wave is obviously most intimately bound up with the increased duration of the refractory period. It is limited to the narcotised portion of the nerve, while peripheral to this the normal wave duration reasserts itself.

Thus we have three definite changes occurring in a narcotised stretch of nerve, ^{two at least} ~~three~~ limited to the

Fig 12.



affected portion:-

- (1) delay in rate of transmission,
- (2) prolongation of refractory period,
- (3) prolongation of electrical wave.

There is still a fourth change, this time ~~now~~
~~was~~ not localised to the narcotised part. This takes the form of a diminution in the extent or amplitude of the excitation as measured by electrical means. Boruttau first established this change in nerve which had been locally cooled⁽⁵⁵⁾. A similar decrement in the excitatory wave occurs in a narcotised or asphyxiated portion⁽⁵⁶⁾. On leaving the affected stretch of nerve the wave does not return to its original height, it remains permanently diminished, notwithstanding that its original short duration and its original rate are recovered.

This decrement is a function of the intensity of the stimulation. We see this from the experiments of Wedensky and of Noll. Boruttau and Fröhlich⁽⁵⁶⁾ set themselves to determine the exact relation between the two. They used two electrodes for registering electrical change on the longitudinal part of the nerve, the one, α , nearer to, the other, β , more distant from, the point of stimulation. (See figure 12.). By means of a third electrode, γ , placed in contact with the cross section of the nerve, they could lead off alternately from α and γ , or β and γ as the case might be.

In any given stage of narcosis then, although the demarcation current was exactly the same in each case, the negative variation with one and the same intensity of stimulation was less in β than in α . When they varied the intensity, the decrease in the negative variation from α to β with strong stimulation was much more considerable than with weak stimulation. The further the narcosis had progressed the more did this hold true. Thus, by tetanising at stated intervals, first with secondary coil set at a distance of 450 m.m. and then at a distance of 200 m.m., the negative variation in the first case decreased in the space of 20 minutes from 4 to 1 scale-divisions, in the second case from 75 to 3.

The decrement in the strong stimulation is great, that in the weak is proportionately slight, and in fact "as one can easily convince oneself, the size of the decrement is directly proportional to the size of the wave of excitation".

This explains how it is that the conductivity disappears at the same moment for both weak and strong stimuli, for the negative variation in each case becomes vanishingly small at the same point. It likewise explains how, at the commencement of narcosis or asphyxia, the conductivity appears unchanged for both strong and weak stimulations. For weak stimulations the decrement is extremely small, and

"As one can easily convince oneself" !!
Mathematically constituted Brontes.

This not true.
See p. 112.

the conductivity disappears only when the negative variation has sunk below the limit necessary to induce a minimal twitch of the muscle. For strong stimulations the decrement is greater, but the maximal twitch only decreased when the negative variation sinks under the limit necessary to call forth a maximal twitch. Once this occurs, the height of the muscle twitch decreases with decreasing extent of the negative variation, to disappear simultaneously with the effectiveness of weak stimulation.

The conductivity for weak and strong stimulations during narcosis is therefore only apparently unchanged, because the various alterations that the excitation undergoes, prolongation of refractory period, increased duration of the electrical wave, and delay in rate of transmission, do not have an opportunity of evincing themselves in the commonly used method of observation of the muscle twitches following single stimuli.

The only other question that it remains to answer is this. Why does the apparently unchanged conductivity disappear before the excitability?

As we saw on page 31, the time at which the conductivity disappears depends on the length of nerve narcotised. The shorter the affected portion of nerve, the longer the time and the greater the drop in excitability required to abolish the conductivity. Pursue this to the limit, i.e. narcotise

a vanishingly small portion of nerve, and the excitability and conductivity will disappear together. The "excitability" merely expresses the change at one given point owing to progressive alteration in the nerve through narcosis, the "conductivity" is an expression for the change in a whole stretch of nerve, for the wave of excitation undergoes steady diminution all along its course from one portion of the narcotised nerve to another. In other words there is no separation^{possible} between excitability and conductivity. They are merely different expressions for the same thing.

PART III.The Present Research.Section C. Theoretical.*The Question of Cooling*

It had long been held that the excitability of nerve is lowered by cooling, and raised by warming. Efron⁽⁶⁾ and Hirschberg⁽⁷⁾, who conducted experiments to determine the relation between ~~the~~ conductivity and excitability, found that the excitability apparently fell with fall of temperature. Howell, Budgett and Leonard⁽⁴³⁾ investigated the influence of temperature on nerve in a special research, and came to the same conclusion. Not till Gotch and Macdonald⁽¹²⁾ carried out their experiments, however, was the question settled beyond cavil; they found, taking special precautions against manifold sources of error, that the excitability of nerve to break induction shocks is definitely lowered with decrease in the temperature.

The rate of transmission of the nervous impulse is likewise lowered by cooling of the nerve. This was determined by Helmholtz⁽⁵⁷⁾ for frog's nerve, by Helmholtz and Baxt⁽⁵⁸⁾ and by Oehl⁽⁵⁹⁾ for the sensory nerves of man, and by Boruttau⁽⁵⁵⁾ for the motor nerves of numerous warm-blooded animals.

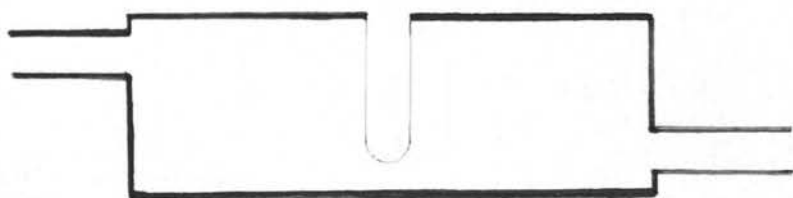
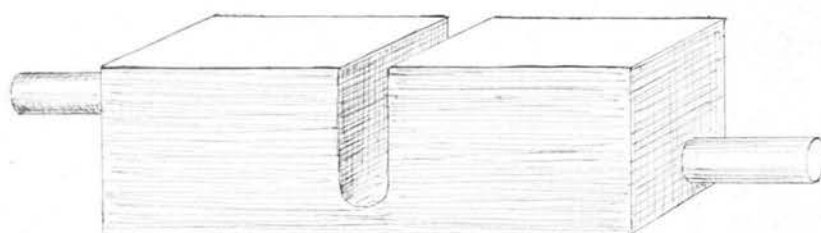
Again, Borruttau had found⁽⁵²⁾ by electrical observation that the amplitude of the wave of excitation under-goes a diminution with cold. The fact that the muscular response is altered in extent by cooling of the nerve is thus referred to by Gotch⁽⁵¹⁾: "On cooling a portion of a nerve, not only is the rate lessened, but the excitatory state diminishes in amount as it traverses the cooled region, and on emerging from this it continues with the same diminished intensity, so that it evokes a smaller muscular response than it would otherwise do".

Boycott⁽⁶⁰⁾ working under Gotch's direction, found that the refractory period of nerve is prolonged by cooling. Boycott used the muscle response; Gotch and Burch⁽⁵⁸⁾ determined the same thing on isolated nerve by means of the capillary electrometer.

The descending part of the curve of negative variation is also prolonged by cold. This was first made out by Hermann⁽⁶¹⁾ and confirmed by Verweij⁽⁶²⁾ and by Borruttau⁽⁵²⁾.

Putting these fact together, one sees that cold has apparently much the same influence upon nerve as narcosis or asphyxia does. Seeing that the muscular responses associated with Wedensky's "paradoxical conduction of nerve" were got with both the latter influencing factors, it was interesting to see if they could be got by cooling. At the instigation of Professor Verworn I undertook this investigation.

Fig. 13.



Section A. Apparatus.(1) Cooling Apparatus.

In conducting local cooling experiments on the nerve of a nerve-muscle preparation, many precautions are necessary if one is to get reliable results. Care must be taken that neither the muscle nor the central end of the nerve is cooled. The cooled portion must be as nearly as possible at an equal temperature throughout its length. In working with very low temperatures, too, the nerve is apt to freeze if it touches any part of the cooling apparatus, hence it is necessary to cool the nerve by radiation rather than by actual conduction. Again, for experiments such as I had to carry out, it was found necessary not only to be able to alter the temperature at will, but to keep any one temperature constant for a more or less prolonged period.

The form of apparatus on which I fixed^{a form suggested to me by Prof. Brouha} was found well adapted to fulfil these requirements. It consisted of a small metal box (see figure ~~2~~) made out of $\frac{3}{4}$ inch square-sectioned brass tubing. A groove, $\frac{1}{4}$ inch wide by $\frac{1}{2}$ inch deep, was let into the upper side; this was lined with very thin copper foil and served to contain the nerve, which was stretched along it, near to, but not actually touching, the bottom. On each end of the box was a brass pipe which served to conduct cold fluid through it. As

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Fig. 14.

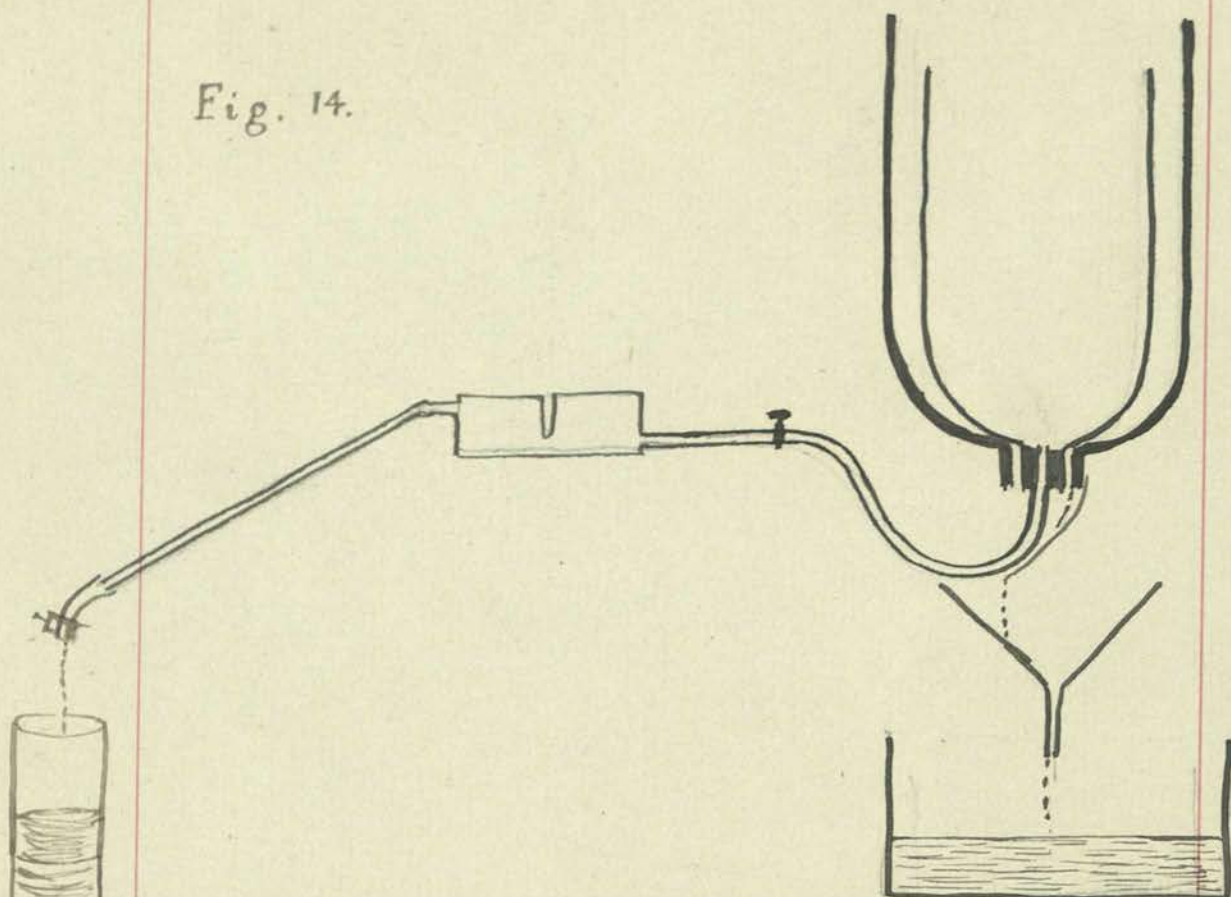
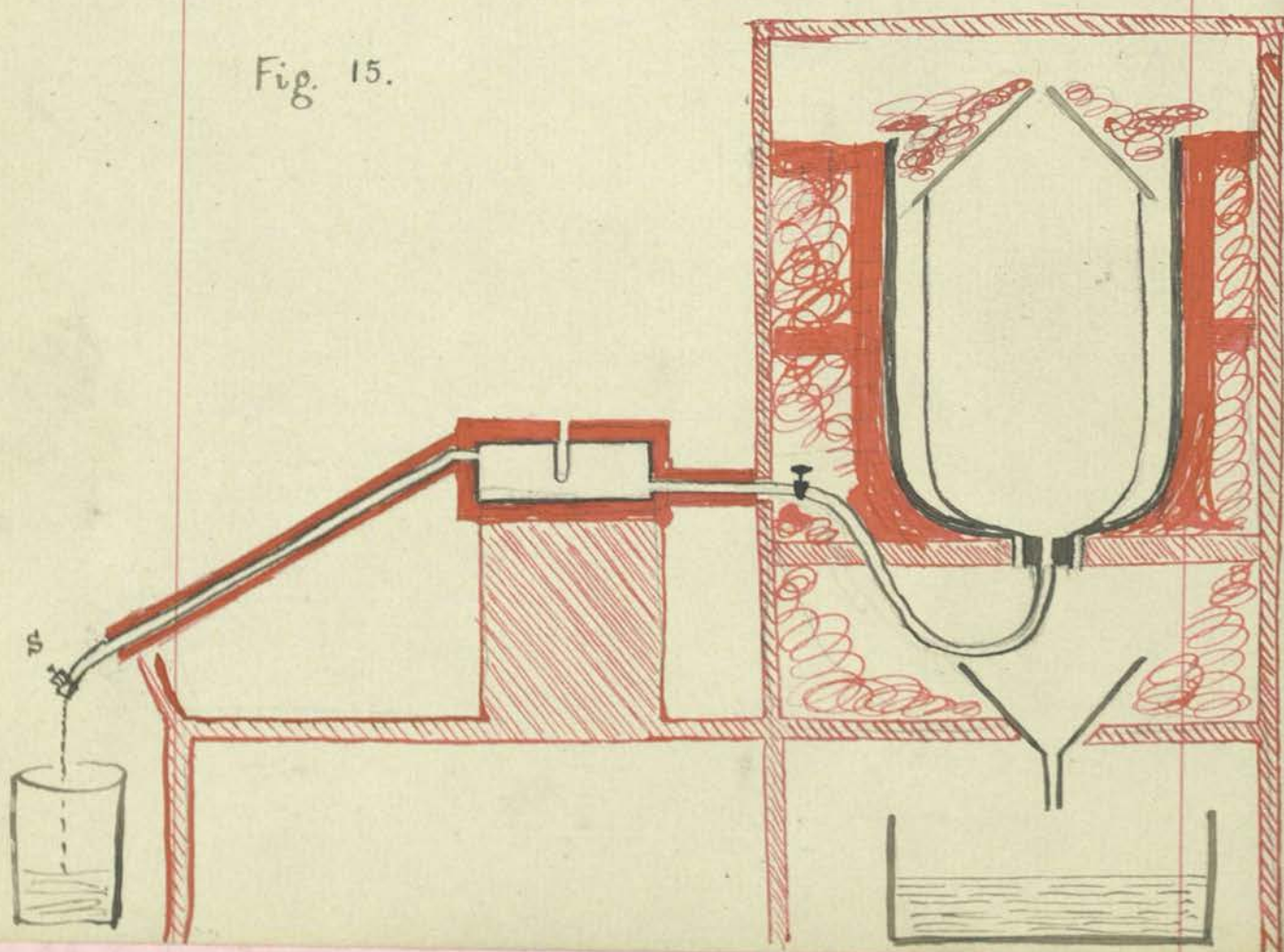


Fig. 15.



the fluid passed along and cooled the walls of the box, the radiation from the thin copper foil cooled the nerve.

The cold fluid (a mixture of alcohol and water to prevent freezing) was got from a special ice and salt cooling apparatus consisting of two glass vessels, one inside the other (see figure 14). The inner vessel (thin walled), containing about a litre and a half, was connected by a pipe running from the bottom directly to the brass box. Between the inner vessel and the outer one a mixture of ice and salt was put. The drippings from this escaped into a funnel placed underneath, and thence into a large glass container.

The diagram will explain better than any description how the apparatus was arranged. The upper figure shews the essential parts. The lower shews how insulation was affected, for all the parts designed to hold or convey cold fluid were thoroughly insulated with felt, while the brass box had an outside jacket of cork, to prevent radiation to the muscle. One thing is not shewn, viz., an additional vessel ^{holding} for fluid at room temperature. This was connected by means of a three-way cock, C, to the pipe leading to the brass box. Thus one could run either cold or warm fluid through the box. S is a screw clamp such as is used on rubber gaspipe for fine graduation of the flow. It was attached to a piece of rubber on the extreme end of the outlet pipe, and

served to accurately regulate the flow.

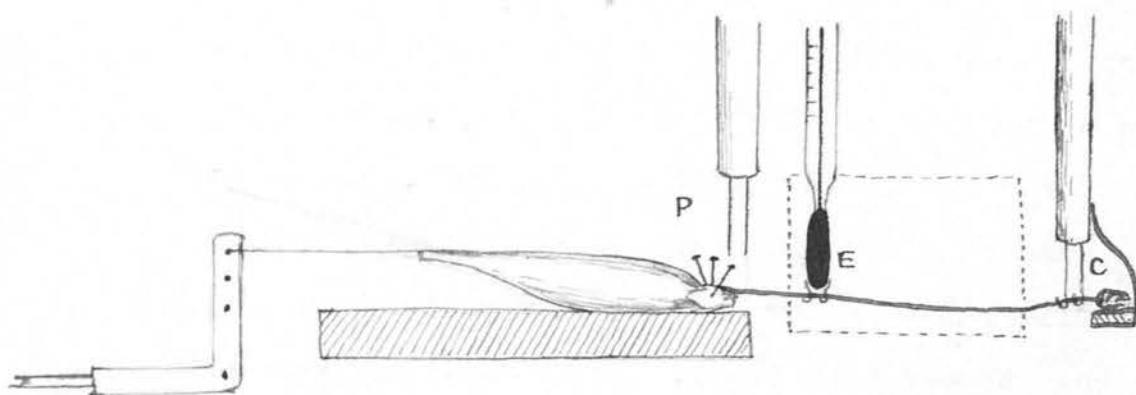
By means of this apparatus one could ^{with a little practice} keep the temperature of the brass box absolutely constant for from 20 minutes to half-an-hour, at any temperature varying from, say, $+3^{\circ}$ to -7° or -8° C. As far as constancy of temperature went, it functioned perfectly.

A preliminary series of experiments was done to determine the degree of the heat insulation near the brass box, and it was found that just outside the groove at a distance of $\frac{1}{4}$ " from the box, i.e. at the point where the muscle was fixed with pins, or where, in the case of a short nerve, the central electrodes were applied, the temperature was never more than 6° C below room temperature (12°), when the temperature in the groove was -5° C. The average temperature of the muscle, as very careful experiments shewed, was certainly never more than 4° below room temperature. When it was necessary that the muscle should be kept absolutely at room temperature, it was fixed in a small cork bath containing Ringer's solution, which was frequently renewed from a bottle kept standing in the room. As regards heat insulation the apparatus was probably ^{as} ~~more~~ efficient ^{as} ~~than~~ many similar pieces of apparatus that are assumed to be perfect.

(2). Nerve-Muscle Preparation.

The muscle was fixed on a small horizontal cork plate, the nerve and it lying in one straight line.

Fig 16.



To shew arrangement of electrodes in relation to nerve.

Three pins, one through the knee-joint, and one each through the connective-tissue attached to the femur and tibia, were used to fix the proximal end of the muscle. This insured that the nerve should not be pulled upon. The muscle lever gave a ten-fold magnification.

Two pairs of platinum electrodes, bent at the end in the form of a hook, served to support the nerve. The one pair (E electrodes) ran down alongside the bulb of a small vertical thermometer specially made for these experiments; by means of these the nerve was hung just underneath, and almost in contact with, the thermometer bulb. The other pair, fixed in a vertical vulcanite rod, supported the central end, so that the nerve hung like a telegraph wire between these two points of support. As the nerve was removed from the animal with a small piece of the spinal column attached, a support for this end was attached to the vulcanite carrying the C electrodes. Provision was made for applying a third pair of electrodes (P) to the nerve just as it entered the muscle. These were not always in use, however.

These electrodes, with thermometer etc., were all fixed relatively to the cork plate, and the whole system could be raised or lowered in one piece, so that once the muscle was fixed on the plate and the nerve laid in position, no alteration in the point of

stimulation could occur. This relative immobility of the system was found of the greatest importance in freezing experiments, where the slightest mechanical injury to the nerve irretrievably destroys it. The supporting apparatus was raised or lowered by means of a screw arrangement such as is used in the rough adjustment of a microscope. The position of the nerve in the bottom of the groove could therefore be accurately adjusted.

Special screws and sliding arrangements was also provided for altering at will the position of any member of the system relatively to any other, so as to make the apparatus adaptable to brass chambers of different thickness and to nerves of different length.

The thermometer carrying the E electrodes served to measure approximately the temperature of the nerve. It was always arranged at the peripheral edge of the cooled piece of nerve (conf. p. ~~12~~). A series of preliminary experiments ^{was} ~~were~~ carried out to determine how the indicated temperature varied with the position of the thermometer bulb in the groove. These shewed that if due care was taken to prevent the bulb from actually touching the sides of the groove, the variation, within the limits of position in the actual experiment was never 1°C . If the bulb touched the side the reading was ~~always~~ about 2° lower than it ^{otherwise was} ~~ought to be~~.

The greatest difficulty was to know ^{to what extent} ~~how~~ the indicated temperature corresponded to the actual temperature of the nerve, and this difficulty with this apparatus seems insurmountable. Experiments done by taking simultaneously the temperature of the fluid, first in the containing glass vessel, and then as it issued from the extreme outlet pipe, proved useless to decide the question. The indicated temperature of the nerve can only be taken as a rough approximation to the real temperature. The error, however, is probably a more or less constant one.

In cooling experiments, fortunately, no provision requires to be taken to keep the cooled portion of the nerve moistened with ~~the~~ saline solution. Moisture from the air condenses on the nerve, so that there is no fear of it drying. The projecting central part of the nerve was kept moist by means of a deep gutter lined with wet cotton wool, which was brought up under the nerve without touching it at any part, and served to keep the atmosphere in the immediate vicinity saturated with water vapour. The peripheral part of the nerve, as well as the muscle were carefully brushed from time to time with Ringer's solution.

(3) The electrical apparatus.

To stimulate the nerve a standard Kronecker coil was used. The current in the primary circuit was obtained from a large accumulator which was kept at the same potential by regular charging. The difference in potential was ^{2 1/4}~~two~~ volts. For rapid tetanisation a Bernstein's acoustic interruptor was interposed in the primary circuit, for slower rates the springs supplied with the Kronecker coil were used. In the one case the mercury contact surface was kept covered with alcohol, in the other it was continuously washed with a stream of tap water.

Considerable difficulty was found in determining whether both "break" and "make" shocks were to be considered effective in stimulating the nerve. The Helmholtz side-wire does not adapt itself to rapid contact interruptions.

Most investigators who have used rapid excitations skip this difficulty, and assume either that both "make" and "break" act as effective stimuli, or that only break shocks excite.

It seems to me impossible to say ^{it is hard to say} whether one alone or both stimuli are effective at any given stage of an experiment.

Experiments with single make and break shocks do not decide the question, for with increasing rapidity of stimulation the distance of secondary from primary coil requires to diminish in order to produce effective tetanisation of the muscle; so that the effects

Until quite lately I was ~~unaware~~ ^{not} ~~until~~ the application
unaware of the fact that by the application of a suitable
condenser in the primary circuit ~~best~~ ^{use} shocks may be
produced relatively submerge the make shocks.

of rapid and of more leisurely interruption are not comparable. X The figures that are here given represent the number of complete vibrations per second of the interrupting spring. [*Follow*]

The electrodes were so arranged that on the break of the primary coil a current in the descending direction went through the nerve. The electrode wires were kept as nearly as possible all parallel with each other, and were not twisted. This was found to be a vital precaution. By a combination of two Pohl commutators without the cross-wires, it was possible to send a stimulus through any one of the three pairs of electrodes at will. There was a simple key in the primary circuit, and a short circuiting key in the secondary circuit.

The above description applies to the apparatus as used by me in Edinburgh. The apparatus I had in Göttingen for example did not include a Kronecker coil; and many minor points tending to greater accuracy were not taken into consideration in Germany.

Section B. Wedensky's Effect by Cooling.

On now cooling a portion of the middle of the nerve by lowering it into the cold chamber it was found that when the temperature sank below 0°C tetanisation of the central end at a rate of about 100 per second gave the phenomena which Wedensky had described under the title of "paradoxical conduction of nerve", and which Fröhlich had ^{shown} ~~shown to be~~ ^{was} due to fatigue. When the temperature had become constant and stood at say, -2°C , the secondary coil was placed at just such a distance from the primary that no contraction of the muscle followed on tetanisation. On moving it 1 cm. nearer to the primary a weak tetanus followed stimulation, on moving it another 1 cm. nearer the tetanus was stronger; and thus it went on until the tetani attained a maximum. They then began to fall off in height as the secondary coil was brought still nearer, curves like Fröhlich's "fatigue" curves (see illustration facing p. 42) began to appear, and finally with strong tetanisation single twitches were got. (See ^{Fig.} ~~Chart~~ 1.)

That this condition was not due to fatigue of the muscle was shown by the simple device of reversing the direction in which the secondary coil was moved. Instead of bringing it nearer to, it was now moved farther away from the primary coil, and the same muscular effects were repeated in the reverse direction. The tetani began gradually to climb up as the

intensity of the stimulation decreased, until a maximum was reached; they then fell away in height and ultimately with weak tetanisation disappeared. (See ^{Fig.} ~~Chart~~ 2.) At the same time as this phenomenon was present peripheral stimulation of the nerve with a third pair of electrodes quite close to the muscle showed full tetani with all moderate and strong intensities.

The correspondence with the condition described by Wedensky as resulting from anaesthesia, and the similar condition described by Fröhlich as resulting from asphyxia, was seen to be more complete when one varied the rate of stimulation keeping the temperature still constant. On stimulating at a slow rate full tetanus occurred even with the strongest intensity; stimulation with a very high rate produced the "paradoxical condition" even more distinctly in that single twitches were got with tetanisation at even moderate intensities. These single twitches, or as Hoffman has named them ⁽⁵¹⁾ ~~(20)~~ "initial tetani" (Anfangs-tetanus), corresponded in height to single maximal twitches of the muscle evoked by one break shock. Finally if the temperature were ^{raised very quickly} raised, say, to $+1^{\circ}\text{C}$, the condition passed away for the highest rate of stimulation at my disposal. If the temperature were still further lowered the "paradoxical condition" was more easily elicitable.

From all this one concluded that:-

(1). So far as the response to induced currents is concerned, cold has ^{apparently} ~~exactly~~ the same effect on nerve as asphyxia or narcosis, ~~does~~. A low temperature corresponds to a deep degree of narcosis or asphyxia as the case may be; a higher temperature corresponds to a lighter asphyxia or narcosis.

(2). For examining the condition that Wedensky called paradoxical conduction, cooling of the nerve is a more satisfactory method than either narcosis or asphyxia, ^{curves obtained are more regular. This is probably due to the fact that the} in that the physical condition of the nerve during an experiment can more readily be kept constant.

Variations in Results, Some Unknown Factor

Section D. The Previous History of the Nerve.

The results described in last section I got in Germany in February 1903. On repeating the experiments in Edinburgh in November, 1905, I failed entirely to shew the same effect. I had begun the work expecting to get the effect without any difficulty. It was found that no matter how low one reduced the temperature of the nerve, the tetani kept maximal for all strong stimulations.

Remembering that Fröhlich had got the effect in the most pronounced form when the conductivity as tested by single minimal twitches was just on the point of disappearing, I cooled the nerve gradually, testing excitability and conductivity at each success-

ive stage. Excitability gradually fell and conductivity kept constant, but before conductivity disappeared the nerve always froze and put an end to the experiment.

It occurred to me that the rate of stimulation I was using was perhaps too slow. The highest rate I could get with the Kronecker springs was 192 per second. A Bernstein's acoustic interruptor, giving a maximum rate of 256 vibrations per second was accordingly interposed in the primary circuit, and even with this rate the condition refused to appear. It was still possible that owing to the production of waves on the surface of the mercury contact in the Bernstein apparatus, the vibrating point did not make contact in a regular fashion. The interruptor was accordingly removed from the table on which the rest of the apparatus stood, and set on the stone ledge of a window where it would be less affected by accidental vibrations. This precaution proved as ineffectual as any other.

Still looking for an error in the physical apparatus, I removed the iron core from an inductorium so as to abolish magnetic hysteresis, and make the growth and decline of the current in both primary and secondary coils more abrupt, while to compensate for the loss of the core a current of greater intensity was used. I still failed to get the effect.

The greater the length of nerve affected by

narcosis or asphyxia the more readily is the conductivity brought to the point of disappearance. Imagining that the brass cooling chamber was too narrow, I had a larger one constructed, and used the biggest frogs I could get for the experiments: all of no avail.

Finally I took all the apparatus down and built it up afresh. The fatigue effect still remained absent.

It then seemed that the most probable cause of the anomaly must lie in the condition of the nerve itself. In Germany I had got the result with both rana temporaria and rana esculenta, so that it did not depend on a "specific" difference in the frogs.

// Seeing that I was looking for a fatigue effect, and knowing as I did that the nerves of summer frogs are less tolerant of exposure etc. than the nerves of winter frogs, (conf. too, p.p. 19 - 21), while Fröhlich had found (see page 24) that cooled nerve takes a much longer time to asphyxiate than summer nerve, I ~~argued~~^{imagined} that fatigue ~~must~~^{might} be more readily got in warmed than in cooled frogs. A batch of frogs was accordingly kept in the room for some days at a temperature of about 12°C, some of them were even warmed up till tetanic convulsions appeared, and even then their nerves gave the same negative result.

Professor Schäfer suggested that as I ~~had~~^{found by the effect of} got the result in Germany in February, during the cold

Seeing that the temp of the influenced part of the nerve can be kept very constant while the corresponding factor is varied & Frohlich's experiments on the degree of anoxaemia or of asphyxia is ^{known} ~~well~~ ^{from our experiments} ~~given with time~~ it was found to examine the local effect more accurately than had before been possible.

time of the year, I should try the effect of cooling the frogs on ice before using their nerves.// On adopting this plan to my ~~great~~ satisfaction I got the paradoxical effect at once.// Not only so, but it soon became plain that the one condition that requires to be satisfied in order that the Wedensky effect should be easily elicited by local cooling is that the nerve should be taken from an animal that has been well cooled beforehand. Thereafter I always kept the frogs for some time previous to an experiment on ice, and never failed to get the desired effect. The nerves of frogs taken from time to time directly from the ranarium, so as to serve as controls, never gave the effect unless the weather happened for some time previously to be unduly cold. Again, if a frog which had been cooled on ice for some time was exposed even for half-an-hour to a temperature of from 20° to 30°C just before dissection of the nerve the effect was found not to be present.

Section E. The Wedensky Effect more closely
Examined.

Now that one could reckon with certainty on obtaining the effect, it was possible to examine the condition more accurately than had before been possible; for, as said before, the temperature of the influenced piece of nerve can be kept rigidly constant,

while the corresponding factor in Wedensky's and Fröhlich's experiments, viz., the degree of anaesthesia or of asphyxia, constantly varied. For the sake of clearness we shall take up the various alterations in the muscle response in the order in which Wedensky described them.

(1). The Height of Muscle Contraction as Influenced by the Temperature of the Nerve.

As we saw (p. 13), the first observation of Wedensky on the reaction of the muscle to anaesthesia of the nerve, was that the tetanic contractions were not so full and complete as before anaesthesia. In his experiments he had to exclude the possibility of this being a mere fatigue effect in the muscle itself. In my experiments there was the additional possibility that the temperature of the muscle might vary along with the variation in the temperature of the nerve. To avoid this contingency the muscle was fixed in a small bath of Ringer's solution at room temperature, and the solution frequently replenished with fresh fluid at a constant temperature. A few observations with a thermometer placed in the bath sufficed to shew that with these precautions the muscle did not vary in temperature more than 1°C . Such a variation is negligible, and besides, the alterations in the temperature of the muscle did not correspond with those in the temperature of the nerve.

On now cooling a portion of the nerve it was

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written that
A*

Chart 3 photo

Chart 10 photo

found that the muscle responses to central tetanisation of the nerve were less pronounced than before, in that the tetanus curves were lower. At first this change was not so marked, but when the temperature of the cooled piece of nerve fell to a definite point which varied for different preparations each succeeding change of 1° in the temperature of the nerve was denoted by a distinct falling off in the maximum height of the muscle tetanus; and, generally speaking, the lower the temperature of the nerve the more marked was the drop in the height of the muscle contraction corresponding to a drop of 1°C. in the temperature of the nerve.

Figure
~~Charts 3~~ and ~~4~~ ^{representing curves from the same preparations} show this change, in its final stage at least, very plainly. In ~~Chart 3~~ ^{Fig.} we see, (i) the general height of the tetani got with varying intensities of stimulation at room temperature; (ii) the drop in the height of the tetani when the nerve was cooled to -1° ; (iii) the return to the primitive height when the nerve was quickly raised out of the cold chamber again, thus proving that the effect is not one of fatigue of the muscle.

Fig.
~~Chart 4~~ (tracings i and iii) shews the relatively large drop in height of tetanus corresponding to a drop of half a degree in the temperature of the nerve, when the latter is already much cooled. A drop of another half degree suffices to abolish the muscle response entirely (tracing iv), while the height returns

more or less to normal on simply raising the nerve out of the chamber and waiting for a minute or two till it shall have attained approximately the temperature of the room. (tracing ^Y~~X~~).

My previous experiments with preparations from non-cooled frogs had taught me not to look for such a marked diminution in the height of the muscle tetani on cooling of the nerve; and, as a matter of fact this marked response of the height of the muscle contractions to alterations in temperature of the nerve goes hand in hand with the readiness with which the Wedensky effect is got by cooling. In other words the excitatory process in the nerves of non-cooled frogs does not undergo such a diminution in magnitude by traversing a locally cooled portion, as it does in the nerves of frogs that have been previously cooled.

In Chart 5 we see tracings taken from a preparation that had not been so well prepared by previous cooling of the frog as the preparation that served to give the tracings of charts 3 & 4. Here, as we see, the Wedensky effect is just elicitable with the highest rate of stimulation used in any of my experiments (256 per second). The nerve was cooled to the point of freezing, and the contractions are nevertheless only slightly lower than those previously obtained at room temperature. In this case, it must be admitted, the warm bath

was not used, and further, the rate of stimulation does not correspond to that used in the experiments of which charts 3 & 4 are records, but nevertheless the general fact is plain; and the same kind of thing has occurred in my experiments too often, and under too many varying conditions to admit of any doubt as to its constancy.

(2). Incomplete Tetani with Strong Stimulation.

After Wedensky had noted the
demonstration inherent of the tetani his
next observation, if we remember, was that when the anaesthesia had advanced to a certain stage the muscle contractions got with strong tetanisation were less complete than those got with weaker stimulation and more resembled those obtained with submaximal tetanisation. A reference to ~~Fig 4~~ ^{Fig} will shew the justice of this remark. In the first series of tracings shewn there, the gradual rise of the tetani to a maximum and their subsequent falling off in height as the strength of stimulation is increased, is ~~beautifully~~ ^{well} shewn. ~~But~~ A further fact, not mentioned by Wedensky, is also indicated, viz, that the tendency of the tetani to climb is more or less abolished, and a change has rather set in in the opposite direction. The tendency of the curves is now to decline rather than to rise in height, and this decline may be pretty regular as is indicated in the first curve marked 19 in the Chart.

From such curves alone it might ~~be~~ ^{as it was argued by Forthick} with ~~some~~ ^{considerable} shew of reason ~~argued~~ ^{is} that the nerve ~~was~~ being

*follow with
written
page. B*

fatigued, for the outside conditions are practically unchanged. The muscle is presumably as able to respond to stimulation as ever; the temperature of the nerve is the same; the stimuli that come from the electrical apparatus do not vary. ^{Successive} ~~the~~ excitations in the nerve in traversing the cooled region ~~must consequently~~ ^{undergo} a change ~~either in kind or~~ ^{in such a way as to be less than effective}

~~in degree.~~ Adopting the latter, and more probable, hypothesis, this change in degree can be only due to the passage of similar excitations immediately before. Such a process of reasoning is only possible when one keeps the influenced portion of the nerve under constant conditions.

But it must be granted that all the incomplete tetani got with strong stimulation do not shew this regular decline, and it is interesting in this regard to refer to some of Wedensky's own curves which do not so plainly conform to the declining type.

Nevertheless the very irregularity of ~~the~~ ^{most} tetani got in this manner is in itself a ~~most~~ striking phenomenon, ^{and as we shall see it occurs again and again when nerve is cooled.} [To this point however we shall return, when by more refined methods we are able to show a perfectly regular fall in the exciting value of succeeding excitations. (See p.)]

(6). The "Initial Tetanus" (Hoffmann.)

These incomplete tetani from strong stimulation gave way, as Wedensky found, on further anaesthesia to single twitches. It was reserved for Fröhlich

to point out -- & I have confirmed the fact again and again -- that the height of these single twitches corresponds to the height of a single maximal twitch of the muscle; as if only the first member of a series of excitations had succeeded in emerging from the affected part of the nerve with a strength sufficient to be maximal, while the succeeding ones all drop below minimal strength or do not get through at all. (See *Chart 3, Initial twitches, showing that their height is proportional to the strength of the stimulus.*)

Such initial tetani are to be seen in any of the ~~Charts 1-4~~ ^{Tracings in figures - Fig. 2}. In ~~Chart 2~~ ^{Fig. 2}, their presence is complicated by the appearance of another phenomenon, the initial twitch, or "Anfangszuckung" of Bernstein (63). These initial twitches occasionally occur on weak tetanisation and the apparently arbitrary way in which they do occur led to a lively dispute between Kronecker and Stirling (64) on the one hand and Bernstein (65) on the other. The latter had found them present when the rate of stimulation began to be as high as 250 (~~really 125~~) per second, the former two physiologists had been unable to observe them and denied their existence apart from physical errors in the apparatus. Their presence is indicated in a number of the charts in my possession but in none so plainly as in ~~Chart 2~~ ^{Fig. 2}, where their unvarying height suggests some mechanical cause for their production in this special instance. Generally however, these initial twitches vary in height with

varying distance of secondary from primary coil, as shewn in figure, $\oplus$, on the opposite page, where for some reason or other they have suddenly begun to appear, whereas in $\oplus$ and $\oplus$, ^{Figs. representing} records taken a few minutes previously from the same preparation, they do not occur.

To return however to ^{the} initial tetani, their existence may be taken as an indication that the ~~under the given conditions~~ Wedensky effect ^{is} fully established, and, generally speaking, the better the Wedensky effect is established, the weaker the tetanisation, or, what amounts to the same thing, the lower the rate of stimulation required to produce an initial tetanus.

Thus in Charts 6 and 7 we find a series of curves taken from the same preparation at different temperatures; of these curves those to be presently enumerated have all been recorded with the same rate of stimulation (112 per second). No. 6 shews that with the nerve at a temperature of 0°C the initial tetanus has not yet appeared, though the strength of stimulation is 1000 units. In No. 7 taken at -1°C , the initial tetanus is present somewhere between 500 and 1000; in No. 8, taken at -1.5° , the initial tetanus is definitely present at 600, if not before; in No. 9, at -2°C , there is an initial tetanus at 500. In No. 11, we see the height of single maximal twitches from break shocks at varying intensities, and they correspond to the

Yellow with
Chart C
Chart 6 & 7

height of the initial tetani in No. 9. No. 10, taken with a much lower rate of stimulation shews that even at the low temperature of -2° , the Wedensky effect for that rate is not established and there are no initial tetani.

The presence of an initial tetanus, then, shews that for the given conditions the Wedensky effect is thoroughly established, and its position in a series of curves taken with regularly increasing intensities is an ~~almost reliable~~ index ~~to~~ to the degree in which the effect is present.

^{Now}
~~Seeing that~~ the position of the initial tetanus in such a series of curves varies with different nerves though the other conditions remain the same, and it might be expected that ^{under appropriate} ~~in extreme~~ conditions some nerve-muscle preparations might shew nothing but initial tetani for all intensities of stimulation; and this, indeed, is the case. In figure

we see a series which is just on the point of becoming entirely initial tetani. This record was taken with the highest rate of stimulation used by me, viz. 256 per second, and at a temperature of -2.6° . Again in figure ^{exp 9 at diff temp} we see in the lower line a series of initial tetani got with a rate of 214 per second and at a temperature of -2° ; while in the upper line, taken a few minutes later at a temperature of $+1^{\circ}$ to $+1.5^{\circ}$ the normal kind of tetanus has returned again. Thus under ^{special} ~~exceptional~~

✓ follow with written sheet D

conditions it is possible for the Wedensky effect to be represented purely by initial tetani. *See also Chart 7.*

This suggests a further question. What happens to such a series of initial tetani when, say, the temperature is still lowered, or the rate of stimulation increased, or, in short, the conditions further modified in such a direction as we know already to facilitate the appearance of the Wedensky effect? The answer to this question we shall most conveniently postpone until later (see p.

).

(4). Rate of Stimulation.

It will already have become abundantly clear that the rate of stimulation is ^{an} ~~almost~~ important factor in the establishment of the Wedensky effect. The effect is invariably better obtained when the rate is high than when it is low. We have already seen an instance of this in Chart 7, when the Wedensky effect, easily elicitable at a rate of 112 per second, disappears when the rate is changed to 30 per second. Chart 8 shews the same thing; in the upper line we see a series of complete tetani taken at a rate of 106 per second, in the lower line taken at 160 per second the Wedensky effect is fully present; the temperature all the time had remained at 1°C.

[In the upper tracing in chart 8 we see evidence of a phenomenon which was very often observed.

*Follow with
E*

When a preparation was just shewing signs of the proximity of the Wedensky effect, in that the tetani following stimulation at high intensities, (800 - 2000 units) began to fall off slightly from the height of the preceding tetani, stimulation at a still higher intensity brought the tetani back to their full height. In the present chart the tetanus at 4000 is higher than that at 2000. This is due to a physical cause, viz. that the electrical excitation skips over a portion of the cooled part of the nerve and directly excites it at a distance from the point of application of the electrodes. For this the physiological continuity of the nerve is not necessary, as may be shewn by nipping the nerve across with forceps close up to the central edge of the cooled part. Strong electrical excitation of the uninjured central end then still suffices to cause contractions of the muscle. This physical escape of the current, or "Stromschleifen" as the Germans call it, is a prominent source of error in working with high intensities of stimulation and for this reason in most of my experiments I did not use stimuli of more than 2000 units.]

In Chart 9, again, we see the effect of a more varied series of rates of stimulation. In the lower line, where the temperature is kept constant throughout, (the lowness of the indicated temperature, -4.3° C. is probably due to touching of the side of the

groove by the bulb of the thermometer), the series 1 and 2 were taken at a rate of 188 per second, 3 and 4 at a rate of 214 per second, 5 at 102, 6 at 188 again, and 7 once more at 102.

In experiments with an interruptor that consists of a vibrating steel spring, it is naturally difficult to obtain a graduated series of curves showing the effect of different rates of vibration, such as is possible in the case of different intensities of stimulation, the rate being meantime kept constant. The charts, shewn, however, are sufficient to indicate the general effect of varying the rate of stimulation, and the result is exactly the same as can be produced by varying the intensity. High rate corresponds to high intensity in its influence on the tetani of a Wedensky effect.

(5). The Length of Nerve Cooled.

A still greater difficulty is present when we try to vary the length of the cooled part of the nerve. In the absence of a special piece of apparatus with more than one compartment for fluid at different temperatures, the only method is to first of all observe the condition of the Wedensky effect in one definite preparation with given low temperature, and given rate of stimulation, and then at once repeat the observations when a chamber of different size has been adapted to the pipe through which the cold fluid comes. I had three chambers

of different width, 1 inch, $\frac{3}{4}$ inch and $\frac{1}{4}$ inch respectively. I often observed that when the Wedensky effect was only with difficulty elicitable by the use of the $\frac{3}{4}$ inch chamber, that on using the largest sized chamber it became fully present.

^{Fig 24}
~~Charts 10 and 11~~ represents an experiment carried out to shew the dependence of the Wedensky effect on the length of nerve cooled. In the lower line of ~~Chart 10~~ ^{the figure} we see that with one inch of the nerve cooled to -1.7° an initial tetanus is first got at an intensity of 600. For accuracy this observation was repeated twice. In the upper line a series of tetani at a constant intensity of 1000 are inscribed at varying temperatures. Although the thermometer in this case, from insufficient adaption to the coldest part of the groove, probably registered a higher temperature than the actual temperature of the coldest part of the nerve, yet we see that at a temperature of -3°C , almost complete tetanus follows stimulation at 1000. Only when the temperature falls to -3.2 are there indications that the Wedensky effect is on the point of appearing.

~~[This chart also shows well the rapid fall in the height of a complete tetanus from $+4^{\circ}\text{C}$ to -3°C when the nerve is in a suitable condition for showing the Wedensky phenomenon. See p. 60.]~~

^{possible}
 In order to guard against a fallacy which will afterwards (~~III, Section F.~~) become more plain to us

After the inscription of this tracing the nerve was once more put back in the larger chamber, and the Wedensky effect was found to return to about its primitive extent at a temperature of -2.4°C .

[Follow with F]

viz. that with rise in temperature of a cooled preparation ~~no matter for how short a time~~, the Wedensky condition ^{may} tends to pass away never to return again by subsequent cooling, the nerve was once more put into the large cooling chamber, and as we see (tracing No. 6) the Wedensky effect once more returns to about its primitive extent at a temperature of -2.7° .

(6). Spontaneous Disappearance of the Conductivity.

As we saw on page 41, Fröhlich found that in the case of anaesthesia and asphyxia that the Wedensky effect is best got when the conductivity, as tested by the method of minimal twitches, is just about to disappear. So much stress did he lay on the importance of the disappearance of the conductivity, not only as indicating a definite stage in the anaesthesia, but as an index to the near presence of the Wedensky effect, that when I first began to experiment I examined the cooled nerve as much for disappearance of the conductivity as for appearance of the Wedensky effect. So closely did I think the two things related to each other that when I failed to get the Wedensky effect, I was led off into a research extending over a month and a half, on the relation of conductivity and excitability to cooling of the nerve. I wished especially to determine if by any chance the conductivity does disappear simply by cooling, and before freezing of

the nerve sets in. I failed entirely to observe such a condition.

On discovering however, how to prepare the nerve so as to elicit the Wedensky effect, it was interesting to see if the conductivity could now be made to disappear at a temperature above the freezing point of the nerve. In a suitably cooled preparation this may be easily done as we see for example, in chart 4, tracing iv. Here at a temperature of -2°C the conductivity is abolished, although the nerve has not become frozen. When the nerve freezes the muscle is seized with convulsive twitchings, and it is quite easy by constant observation of the preparation to be sure that the nerve has not yet reached the freezing point. Similar instances of the spontaneous disappearance of the conductivity I have often observed, and always in those cases in which the Wedensky condition at a relatively high temperature, say -1°C is well established.

Section F:- Temperature Relations of the Nerve.

In all previous investigations on nerve at different temperatures the tacit assumption^{has been made} that any effect brought about by alteration of the temperature of a nerve is reversible. For instance, if a nerve be taken at, say, 10°C , then raised to 14° ,

and once more brought back to 10° , it is taken for granted that the nerve is once more in the same condition physiologically as it was at the start. As we shall see in this section, there is abundant reason to believe that such a process as the above is not reversible, and that the nerve in its final condition is different from what it was at the beginning.

(1). Different Degrees of the "Cooled" State.

From the results brought out in last section it must have become clearly evident that to obtain the Wedensky effect by means of low temperature, the cooling must be a kind of double process. First of all the animal must be cooled, and thereafter the dissected nerve partially cooled. It has also become apparent that even then, under exactly similar physical conditions, the extent to which the effect can be elicited varies with different nerves. Now, I have invariably found that in proportion as the previous cooling of the frogs was thorough and prolonged, so was the Wedensky effect pronounced.

A reference to Charts 3, 4 and 5 will shew what is meant. The animal from which the first two of these charts was got had been kept for three weeks at a temperature of 0°C ; the frog corresponding to chart 5 was kept only for three days at the same temperature. The Wedensky effect in the former case is well established, in the latter case

under even better physical conditions it is hardly present. The longest period that I have kept a frog on ice was 5 successive weeks; in no case did I get the Wedensky effect with such a low rate of stimulation as in the preparation from this frog. Not only so, but I have found that in a batch of frogs kept for the same considerable length of time at 0°C , gave effects of very nearly the same degree. During the process of cooling of the frogs slow changes are established in their nerves, whereby the Wedensky effect is more and more easily elicited by a subsequent partial cooling of their nerves.

Again if we compare Chart 4 with the upper line in Chart 10, or with tracing 4 in chart 11, we see that the records are practically similar. That is to say a preparation in which the conditions, apart from previous cooling, are as favourable for the production of the effect as one can make them, corresponds in its reactions with another in which the physical conditions are not nearly so favourable. This, taken with general known facts regarding nerve, leads us to conclude that previous cooling alters the properties of a nerve only in degree and not in kind. Were the physical conditions still more favourable, it is probable that the Wedensky effect might be elicited in any nerve by simple local cooling, without previous treatment of the frog.

The physical condition which, theoretically at

any rate, allows of the greatest range of modification is the rate of stimulation. The length of nerve that one can cool is limited, the lower limit of temperature to which one can bring the nerve is defined by the freezing point, while as we have seen there are experimental limits to the strength of stimulus that one may use. If the rate of stimulation could be raised ad libitum it is possible that the Wedensky effect might be obtained from any frog nerve-muscle preparation, simply by cooling the nerve.

(2). Change undergone by Nerve on Keeping.

I had not experimented long with cooled preparations until I began to notice that the preparation from one side of a cooled frog would give the Wedensky effect perfectly in the morning, while the sister-preparation, which had meantime been kept lying in Ringer's solution in a beaker in the room, would fail to give the effect in the afternoon. This I observed on quite a number of occasions. The fact was so marked that it was difficult to believe that in each case the leg used by me in the morning had happened to be better cooled during the life of the animal than the leg which I used in the afternoon.

If we compare Chart 5 with Chart 12 we shall see what is meant. In Chart 5, the record of the fresh preparation, the Wedensky effect is present at

-2.5°C. In Chart 12, the record of the opposite "kept" leg, there is no trace of the effect when the temperature is at least as low as -2.4°C.

Chart 13 is more convincing. It is a record from the opposite limb to that which gave the tracings in Charts 10 and 11. As we have seen in these latter two Charts, the Wedensky effect was fully present when one inch of the nerve was cooled. In Chart 13, where the preparation had been kept overnight, the effect, under similar conditions, has almost passed away.

A comparison of Charts 14 and 15 shews the same thing. The tracing in Chart 14 taken from a frog that had been steeped only half-an-hour, shews, with a rate of 192 per second, the Wedensky effect. The tracings in Chart 15, taken from the opposite leg after four hours steeping, shews almost no Wedensky effect, at a similar rate of stimulation, even though the temperature has been lowered to the freezing point of the nerve.

As in these instances, however, there must always be a suspicion that the "kept" preparation is not in the same condition to begin with, as the preparation which was used fresh, it was necessary to test the effect of "keeping" on one and the same preparation. Charts 16 and 17 shew the result of such an experiment. The same preparation that gave the Wedensky effect so well seen in Charts 3 and 4,

gives, after 24 hours steeping in Ringer, only an indication of the same effect, and that when the temperature is brought down to the verge of the freezing-point.

There is no need to multiply illustrations. I have found the same thing again and again, and have more than a dozen charts in my possession all of which prove it.

Here, then, is a change taking place in a dissected nerve in a direction exactly opposite to that which it undergoes inside the body during the process of cooling. Not only so, but it takes many days of cooling of the animal ^{to} ~~the~~ establish the Wedensky effect in high degree, the whole thing passes off after a few hours steeping in Ringer.

(3). Is the change undergone on Keeping a Reversible one?

The next question was: Does this change, undergone by the nerve on keeping, depend on the temperature at which the nerve is kept? Seeing that the one change — the change undergone on cooling of the animal — takes place with low temperature, might not the same effect be brought about by simply cooling a nerve outside the body?

In order to decide this point, a preparation was taken which, after lying in Ringer for some time at room temperature, just shewed the effect and no more. (See chart 18, lower line). After the record

was taken the preparation was at once removed and put into Ringer at 0°C , where it was kept for 24 hours. On now being tested it shewed that the Wedensky effect had, if anything still further passed away (same chart, upper line). Hence there is no reversibility at least within a short period of time.

A still better illustration is afforded by Charts 19, 20 and 21. The preparation of which these charts are records had been kept for 4-5 hours after dissection continuously at a temperature of 0°C . On examination (see the charts) the Wedensky effect was found not to be present even when the temperature was lowered actually to the freezing point. On the other hand the freshly used sister-preparation gave the Wedensky effect quite well at a higher temperature. (See Chart , tracing 5).

These instances are however not entirely convincing. The example that ~~had~~ best proves the point can be seen in charts and . In Chart initial tetani and nothing else are got at a rate of 30 stimulations per second — an extreme degree of the cooled condition. After the same preparation had been kept 92 hours in Ringer at 0°C full tetani are got at a rate of 128 per second (Chart). That is to say, the change is not merely non-reversible outside the body, but actually goes ahead in spite of the preparation being kept at 0°C . At the same time the frog from which this preparation had been taken

was never cooled below 0°C .

(4). Does Temperature affect the Rate of the Change?

Failing reversibility by low temperature, it now remains to enquire if the change which a cooled nerve undergoes extra corpus can be delayed by low temperature. To settle this question I took two preparations from the same cooled frog and kept them for four hours, one at 0°C ., the other at room temperature (11°). At the end of that time I tested first the preparation kept at room temperature, and found the Wedensky effect almost gone. In the other preparation tested immediately afterwards, the effect was still present in marked degree. This direct experiment confirmed what I had already strongly suspected from the different behaviour of preparations that had been kept at room temperature as compared with those kept at a low temperature. We consequently conclude that the change which a cooled nerve undergoes extra corpus, takes place at a more rapid rate with high than with low temperature.

To this process of return from the cooled condition there must however be a limit. We cannot suppose that in a nerve cut away from its supply of nutriment a change should continuously go on, whereby the tissue reacts in what would seem a more efficient fashion than before. This only emphasises the necessity of carrying out these experiments with much higher rates of vibration than I have been

able to do by means of the mercury-contact interruptor.

(5). The Far-Reaching Nature of the Wedensky Test
for Nerve.

We are now in a position to appreciate the importance of the Wedensky test in the physiology of nerve. Part of its value lies in the fact that it supplies a means — hitherto wanting — of measuring fine gradations of difference in the reactions of nerve.

By the application of this standard we have discovered that the influence of temperature on nerve is more fundamental and withal more complex than has hitherto been supposed. Not only do different nerves vary considerably according to the previous history as regards temperature, of the animal from which they are taken (a fact already well established); but, after dissection special changes slowly go on in any given nerve, over the rate of which temperature has a decided influence (a new fact); finally, the immediate reactions of the nerve depend on the temperature at which they are for the moment tested (again well known).

The first change takes place with the natural relations of the nerve to nerve cells, blood-supply, &c. intact; the second change is in the absence of these connections; in the third instance temperature operates as a mere condition, affecting the immediate reactions of a, for the moment, definitely determinate

structure.

A physical analogy may make this more clear. Imagine that we wish to test the specific electrical resistance of a watery solution of a salt. The result will depend on the temperature at which the experiment is made. Temperature here acts as a mere condition, and the result will always be the same provided we test the same solution at the same temperature. But the strength of the solution may vary. Suppose we set the vessel containing the solution in the open air outside the laboratory, and let it stand for some days. Speaking in a very rough fashion, when the outside temperature is low the water element of the solution will be added to by rainfall and the amount of the increase will be in proportion to the lowness of the temperature. When the solution is now taken inside it is permanently removed from the influence of rainfall, and subjected, let us suppose, to the action of evaporation, which acts in a reverse direction to that of rainfall. This process again depends on temperature, in that the higher the temperature is, the faster will be the rate of evaporation.

Not to press the analogy too far, we may take it that something of a similar nature occurs with nerve. When the nerve is in relation to blood-supply etc., and is then cooled, some definite progressive structural alteration goes on in it. When the nerve is removed from its connections this structural change is brought

to a standstill, and an alteration in a reverse direction is established. This proceeds fast or slowly according as the temperature is high or low. Meantime, imagining the structure of the nerve to be fixed at any one moment, the reactions vary according as they are examined at a high or at a low temperature.

Once more, when we take into account the elementary processes that lie at the base of the Wedensky effect we are struck with the comprehensiveness of this test for nerve. For Fröhlich (p. 43) has shown that a knowledge of the exact stage at which an initial tetanus sets in is equivalent to a knowledge of the refractory period of the nerve for a definite intensity of stimulation. Again the height of the muscle tetanus gives an indication of the falling off in intensity of the excitatory process as it traverses an affected portion of nerve. Finally, as the results of Fröhlich and others (p. 46) have indicated the rate of passage of the stimulus may ultimately be found to stand in direct relation to the Wedensky phenomenon as well.

Section G. Review of Previous Work with
Reference to Temperature.

It is interesting from our present point of vantage to examine some of the work of previous

investigators on the "physiological constants" of nerve.

(1). The Refractory Period.

One "constant" that has attracted much attention is the refractory period. This has been investigated by three distinct methods.

The most frequently used of these methods is tetanisation of the nerve at a rapid rate with the object of determining the highest number of vibrations per second that can keep a muscle in continuous tetanus. Kronecker⁽⁵⁷⁾ Bernstein⁽⁶³⁾, Engelmann⁽⁶⁸⁾, Kronecker and Stirling⁽⁶⁶⁾, Roth⁽⁶⁹⁾ and Langdon and Schenck⁽⁷⁰⁾ have all used this method, and their results vary from 20,000 to 500 vibrations per second. Only three of the original papers have been accessible to me, and in two of these, Kronecker and Stirling's and Bernstein's, neither the temperature at which the experiments were carried out, nor the previous treatment of the frog are indicated. In the remaining one, that of Roth, who at a rate of 4,000 per second still got continuous tetanus, the statement is found "Vorliegende Arbeit wurde während der Sommerferien in Zürich ausgeführt". To other results which Kronecker and Engelmann obtained we shall have occasion to refer later.(p.).

The next method is that of recording the muscular response to two successive stimuli. Helmholtz⁽⁷¹⁾, Sewall⁽⁷²⁾ and Boycott⁽⁶⁰⁾ made use of

this method. Sewall puts down the limit as about .001 second; his work was carried out from October to January in Baltimore. Boycott, working in conjunction with Gotch and Burch, was the first to shew by this means the high importance of defining the temperature at which the experiments are carried out. The work of the Oxford investigators is a model of accuracy and carefulness. It partially forms the basis for the present thesis.

Boycott used winter frogs. The preparations were soaked in normal saline for $\frac{1}{2}$ - 4 hours before being tested. The refractory period was found to be at room temperature, (14°C.), about .001 second. This result corresponds with the value given by Sewall, who presumably also used cooled frogs. "A summated muscular effect was never noticed when the stimuli were less than .0005 second apart", while with a low temperature it might be diminished to even .02 second. "It is impossible to give exact values for the relationship which undoubtedly exists between the temperature of the nerve and the necessary interval which must elapse before a second induced current can be an effective stimulus".

Boycott seems to have been unaware of the influence that the winter condition of the nerve might have on his results, for, criticising the work of Kronecker and Stirling, who got tetanus at a rate of 20,000 per second, he says: "At 15°C it

seems impossible to place this interval [the refractory period] at less than .0005 second". For all we know the work of these two ^{ys} physiologists may have been carried out on summer frogs, in which there is considerable reason to believe the refractory period is much higher than in winter frogs.

The third method used to investigate the refractory period consists in recording by means of a very sensitive capillary electrometer the electrical response of a nerve to two stimuli. This method was used by Gotch and Burch⁽²¹⁾. The preparations were in most instances kept in physiological saline solution for 24 hours before being tested. They do not mention whether the frogs used were winter or summer animals. They found that "the electrical response is so much affected by temperatures varying between 0°C. and 10°C. that it was imperative to maintain uniform temperature conditions".

They carried out four separate sets of experiments, (1) with the nerve all at the same temperature, (2) with the excited portion at a temperature higher than that of the observed portion, (3) with the excited portion cooled, while the observed portion was warmed, (4) the electrical changes were observed at two different portions of the same nerve all at the same temperature.

In the first case they found that at a temper-

ature of 4°C the nerve gives no electrical excitatory response to a stimulus, if a preceding effective excitation has occurred at an interval of less than .008 second. "The duration of this critical interval is dependent upon the intensity of the stimulus, the condition of the nerve, and the distance between the electrometer contacts". "On lowering the temperature of the whole nerve below 4°C , the critical interval is increased". "On raising the temperature of the nerve the duration of the critical interval is decreased".

In the second instance, where the conditions corresponded to those in my experiments, they found that when the stimulus passes through a cooled portion the refractory period is prolonged. Boycott by locally cooling a portion of the nerve between the seat of excitation and the muscle had found the same thing.

But when they came to the third and fourth investigation they found "a number of anomalous instances", "which point unmistakeably to the conclusion that no second electrical response may be perceptible even when there is every reason to believe that a second excitatory disturbance is present".

First of all when the seat of excitation was cooled whilst the part connected with the electrometer was warmed they often found that when two stimuli were arranged to succeed one another at an interval less than the critical interval for the cooled nerve, so

that only one response occurred, two responses were recorded in the distal portion of the same nerve when this portion only had its temperature raised.

This certainly looks anomalous. Boycott, generally speaking, was unable to repeat the same kind of thing by means of the muscle record so long as he worked with the excited part at a low temperature and a peripheral part at room temperature. "On the other hand a result of a different character was sometimes obtained. If a preparation is taken which at $13^{\circ} - 15^{\circ}\text{C}.$, as is not infrequently the case, fails to shew any muscular summation when excited by two stimuli at .002 interval, such summation may frequently be observed when a peripheral area only is warmed to $20^{\circ} - 30^{\circ}\text{C}.$ ".

It must be remembered that Boycott worked with fresh winter nerve. He first of all tested the refractory period with the whole nerve at $13^{\circ} - 15^{\circ}\text{C}.$ All this time, as we have found reason to believe, internal changes were going on in the nerve in the direction of shortening the refractory period. By the time the peripheral part was heated to $20^{\circ} - 30^{\circ}\text{C}.$ the refractory period of the central part may have been already diminished (and he only got the result "sometimes") sufficiently to take up the two stimuli at .002" interval, while the peripheral part would naturally hand them on. To guard against this fallacy one would require to once more cool down the peripheral

portion to $13^{\circ} - 15^{\circ}\text{C}$, and test to see if the double excitation were not still present.

The same objection cannot be urged against the results of Gotch and Burch, however, for they cooled down the peripheral portion again and found that the refractory period was once more prolonged.

Although in Gotch and Burch's experiments the length of the cooled portion was generally less than that of the warmed portion, it is difficult on any physical basis to imagine that a refractory period undergoes a progressive increase on passing through an affected part of the nerve. Besides this is contrary to the known general facts regarding what occurs in a narcotised or cooled portion of nerve, where the depressing action is homogeneous all along (See Part II, Section F.). Hence any attempt to explain the anomaly on the ground of the relative lengths of the heated as compared with the cooled part is not happy.

Let us take it that the change in period takes place suddenly on passage from a heated to a cooled portion of the nerve, or what is equivalent to the same thing that a potential change in period occurs on passing from a cooled to a heated portion. Now there is no reason to believe that the capillary electrometer any more than the muscle is infinitely sensitive to minute alterations in the nerve. The intensity of the stimulus they used throughout their investigations "was fully" adequate to evoke a maximal

response in a muscle, it being in all cases a break induced current producing a marked sensation on the tongue", and even with this strength of stimulus the excursion of the capillary electrometer is not very large. Consequently when a wave of excitation — whether it be the essential nervous process or the electrical accompaniment — is not recorded by our apparatus we are not at liberty to assume that no excitation is there.

Suppose that in a cooled nerve two stimuli at a distance of .008 second are just recorded as one, it may quite well be that the actual wave of change is double, with the second portion present in much smaller degree than the first. On arriving at the warmed portion if we could assume that the second small wave is in some way reinforced, we could explain the anomaly.

Now, there are observations pointing to a property of what one might call "resonance" of excitation in certain tissues, i.e. a property whereby a preceding excitation paves the way for the effectiveness of another slight excitation provided certain time-limits are observed. For instance Richet⁽⁷³⁾ found in the case of the claw-muscle of the cray-fish that a succession of stimuli of sufficient frequency may suffice to excite a muscle contraction, although each stimulus when alone is so weak as to produce no response (For illustration see Schäfer's "Textbook" of

Physiology, Vol. II, p. 380). Engelmann⁽⁷⁴⁾ found in the case of the smooth muscle of the ureter that tetanisation with comparatively weak stimuli may produce a response when strong single induction shocks are inadequate to do so. This is quite a different phenomenon from the "stair-case" effect, which applies to discharges of the excited organ, not to the taking up of the actual excitations.

What happens in one irritable tissue may happen in another. Richet says that Fick⁽⁷⁵⁾ demonstrated the same thing for nerve. In any case, there is quite a possibility that a small excitation arriving at a portion of the nerve where the refractory period is again shortened, may, in virtue of the fact that an excitation has just preceded within a definite suitable space of time, be considerably more effective than it otherwise would have been. On this ground we might explain the anomaly of Gotch and Burch's result. Finally, there is always the simpler possibility that the excitation was not limited to the immediate neighbourhood of the exciting electrodes. (see p. 77).

In their fourth series of experiments Gotch and Burch applied two pairs of electrodes connected to the capillary electrometer at different portions of the same nerve, one pair nearer to, the other pair more distant from the exciting electrodes. Although the whole nerve was at the same temperature they found in

some rare cases that when the peripheral pair recorded two electrical responses the central pair recorded only one; in other cases they got the opposite effect.

From these experiments they concluded that an excitatory process might in a nerve without a corresponding electrical excitation, but in the absence of simultaneous records from the muscle the conclusion is not very convincing.

(2). Other "Constants".

These need not detain us so long. Experiment has invariably shewn that the nerves of cooled animals are modified in such a direction that the transmitted excitation takes longer to reach its height in any given portion of the nerve and longer to die down again, than is the case in the corresponding nerves of summer animals.

Thus if we take the actual duration of the electrical response to a single stimulus, the results of different observers are found to vary within considerably wide limits. Bernstein⁽⁶³⁾ gave .0007 second as the duration; Hermann⁽⁶¹⁾ .005 second, at 5°C; and Head⁽⁷⁶⁾, who worked with winter frogs, .02 second. Fuchs⁽⁷⁷⁾ determined the same constant for the non-medullated nerve of the cephalopod, Eledone moschata; he used for the most part cooled animals and got results varying from .008 to .02 second. Boruttau⁽⁷⁸⁾, who worked with summer animals found always a shorter duration.

Again, as to the rate of propagation, Boruttau (78) found in summer cephalopod nerve a speed of $3\frac{1}{2}$ - $5\frac{1}{2}$ metres per second, while in winter nerve the speed was 1 metre per second. Weiss⁽⁷⁹⁾ has recently stated that in the frog the rate is independent of temperature; at any rate within the range of 0° to 20° , which he used. I have not been able as yet to refer to the original communication but it would be interesting to know if he worked with summer or winter frogs, and with "Kept" or fresh preparations.

Section H. Fatigue of Nerve.

Apart from physiological alterations in a nerve, the presence of the Wedensky effect in any given degree depends on certain perfectly definite physical quantities; (1) the length of nerve cooled, (2) the degree to which the portion of the nerve is cooled, (3) the rate, and (4) the intensity of stimulation. Here for the first time in physiology it would seem that we could apply a definite quantitative standard to the condition of a nerve at any one moment and say, "This is a nerve of 200 units, while this is one of 250", much in the same way as one might classify dew-points in physics.

The possibility of such a mathematically definite statement in biology is striking, but in reality none more striking than the attempt of ^{the upholders of} a physical theory of nerve conduction to reduce the properties

of nerve to quantitative law. The fact is, nerve, almost more than any other tissue, lends itself to physical measurement. Nevertheless under the heading of "physiological alterations" there is included a great deal. In the first place there are nutritional and specific differences in nerve; secondly — and this is the most important subject of the present section — the nerve undergoes changes from use.

(1). Specific and Nutritional Differences.

If we take a number of frogs belonging to different batches and cool them for the same considerable length of time, there is a difference in the degree to which the Wedensky effect is present in them. This I observed very markedly on the only occasion on which I carried out such an experiment. I had received a number of large Esculentia frogs from Germany, and kept half a dozen of them for a space of $2\frac{1}{2}$ - 3 weeks among melting ice. Along with them four or five of the Temporaria variety, from the ranarium of the laboratory, were kept for the same period. The nerves of the German frogs all gave results of about the same degree, the nerves of the laboratory frogs, varied both from those of the German ones, and inter sese. I unfortunately did not pay special attention to the state of the nutrition in any of these animals. Fröhlich, if we remember (p. 24) observed different capacity for recovery from asphyxia in starved as compared with well-

fed frogs. Again, Head⁽⁷⁵⁾, experimenting on the duration and extent of the electrical change in nerve found great differences in frogs of different states of nutrition. The nerves of ill-fed frogs gave hardly any electrical response. Again, the nerves of Rana Temporaria always reacted better than those of Rana Esculenta. A series of nutrition experiments in relation to the Wedensky effect would be valuable.

(2). Physiological Alteration from Use.

In a consideration of fatigue of nerve we must clearly understand what is meant by the expression "fatigue". A mere absence of conductivity in a living nerve is not fatigue. Thus, the conductivity may be totally abolished by asphyxia, by narcosis, by freezing, or even as we have seen (p. 81) by simple low temperature. Before one can definitely say that a tissue is fatigued one must prove that it is either incapable of functioning or then shews a diminution in functioning capacity, because of previous activity.

The method by which Fröhlich proved fatigue (p. 45) is familiar to us. As there must always be some doubt as to the normal stability of the nerve in anaesthesia or in asphyxia, it was very interesting to get the same results as Fröhlich by simple alteration of temperature (p. 62). As however, during the research other kindred phenomena, which can only be referred to a Katabolic alteration in the nerve

through activity, have appeared, we shall devote some time to a discussion of them.

(a). Repetition of a Descending Tetanus.

If in a nerve shewing the Wedensky condition one keeps the temperature absolutely constant, and tetanises for a second or two at regular short intervals, one gets an effect as shewn in Charts 22, tracings 3, 4 and 6. The descending tetanus falls off in extent and becomes very soon reduced to a single twitch. A similar condition is seen in Chart 23, tracings 3 and 5, in Chart 24, tracings 2, 3 and 4, and in Chart 25, tracings 2 and 3. That ^{this} is not due to muscle fatigue is shewn by the simultaneous records of ascending tetani, whether elicited by stimulation from the central (Ch. 22, tr. 3; Ch. 23, tr. 2) or from the peripheral (Ch. 22, tr. 7; Ch. 23, tr. 4) electrodes. That it is not due to progressive slight fall of temperature is proved by the fact that in Chart 24, tracing 3, the temperature was so arranged as to gradually rise during the experiment.

In all these cases the nerve was tetanised for about three seconds at a time and a rest of about three seconds was given between tetanisations. In the interval between the tetanisations the nerve has time to recover to a certain extent but each time the recovery is less complete than before.

The only valid objection that might be brought against this method of demonstrating fatigue is that

the internal structure of the nerve ^{may be} ~~is~~ slowly altering through change in temperature quite apart from functional activity. This objection is negatived by an experiment that will immediately be described.

(b). Diminution in the Reverse or Second Half of a Wedensky effect.

If we turn over once more to Chart 2 we observe that the second or reverse half of the Wedensky effect contains as many full tetani as the first half. If we examine the corresponding tracings in Charts 3, 4, 10, and especially, 26, we see that the second half is deficient in full tetani relatively to the first. In the case of Chart 2 the drum was brought to a standstill after the inscription of each tetanus, until a new position of the secondary coil should be sought out; in the case of the records on the other charts, the drum was going steadily all the time. Thus in the first case the nerve got a more or less prolonged rest after each period of activity, in the other cases the nerve had less time to rest. Chart 24, tracing 1, shews the effect of giving a rest of about 10 seconds between the inscription of each tetanus, and, as we see, the second half takes up as much space on the chart as the first half.

(c). Lag of Wedensky Effect with Rise of Temperature.

Another phenomenon, though this time not such an unequivocal instance of fatigue, may be described here. If one cools a suitable preparation by stages, keeping

the nerve at a constant temperature at each stage and testing for the Wedensky effect, one gets eventually at a low temperature the effect established. Chart 27, shews such an experiment. As we go from tracing 1, taken at 0°C , to tracings 2, 3, 4 and 5, taken respectively at -1° , -1.5° , -1.8° and -2.4° , we see the Wedensky condition slowly appearing, until in tracing 5 it is fully present. If, however, ^{now} we raise the temperature by stages and test at each stage (see tracing 6, and Chart 28, tracings (i), (ii) and (iii)) the Wedensky effect still lingers on at a temperature at which it was formerly not elicitable. I have never yet observed the Wedensky phenomenon in a cooled preparation with the nerve at a temperature above 0°C , except in such cases where the nerve had been already cooled to a low temperature, meantime being frequently stimulated, and then again slowly raised in temperature. In such cases the effect may lag on until the nerve has reached a temperature of 0° or $+1^{\circ}\text{C}$. Before this can be taken as a pure case of fatigue however, one would require to cool the nerve without stimulating and then, when the temperature had once more been raised to 0°C or over, find that no trace of the Wedensky effect still persisted.

(d). Blocking Experiments.

It was interesting to try the effect of blocking the impulses on their way along the nerve to the muscle, and then after a few seconds removing the block.

Such experiments were first carried out by Bernstein (38) and Wedensky (39) by means of a constant galvanic current (p. 36); and as their results were taken to prove non-fatigability of the nerve, one of the most convincing proofs of fatigue would be to shew that under these conditions the nerve can be made to shew alteration.

For supplying the constant current a small accumulator of 4 volts potential was used. By means of a rheocord a current of any potential from 0 to 4 volts could be sent through the nerve. Non-polarisable electrodes were employed for making contact with the nerve. The current was arranged in the ascending direction. On testing the efficacy of the block it was found that a constant current of between 2 and 3 volts potential was sufficient to keep the muscle at rest when strong tetanisation of the central end was employed. The effect of a block of this strength was however sufficient to induce polarisation within the nerve of such extent that it did not recover for some seconds afterwards.

On cooling the nerve the Wedensky condition was established, and when this was present a strength of stimulation was selected which just gave a descending tetanus. See Chart 29, upper line. (In the Charts shewing fatigue by blocking the lower time-marker registers the time of tetanic stimulation, the upper the time of application of the constant current).

To prevent any possible effect from progressive fall of temperature, the flow of the cold fluid was so arranged that the temperature should begin to slightly ascend. Repeated tetanisation with the same strength of stimulus now caused a tetanus which gradually began to increase in height. Previous observation with the constant current had shewn that for these descending tetani a much lower potential is sufficient to act as a block. Consequently when the tetanus^{that} was being repeated was on the point of becoming a complete tetanus, a block of between 1 and 2 volts was put in, and the current was kept running for about 8 seconds. On removing the block the tetanus was now found to be lower and more broken. The block was again put in for another 8 seconds; on removing it the tetanus had become a typical descending tetanus. The block was again put in, this time for about 10 seconds, and on removal the muscle reacted only by isolated twitches. During the whole time the nerve was being continuously stimulated.

As in these experiments, however, there is always the possibility that the nerve is being polarised by means of the constant current, this cannot be taken as conclusive proof of fatigue of the nerve. One thing in the above experiment points to the fact that the fall in height of the tetanus is not due to polarisation, and that is that the muscle response after the second application of the block took the

form of a descending tetanus. Had the nerve been polarised recovery would have at once set in on removal of the block, and the tetanus — as control experiments have shewn — would have immediately begun to ascend. The amount of polarisation depends on the potential of the current used to form the block, and in this case the potential was low. Wedensky who in his experiments used a blocking current of 2 volts potential improved on Bernstein's method by regularly changing the direction of the constant current. Although he does not specifically mention the length of time that he allowed the current to run in one definite direction, one gathers that it was for many minutes and not merely for some seconds; (Bernstein had allowed the blocking current in his experiments to run for hours in one direction). The method requires to be very carefully used to give reliable results.

The best examples of fatigue by this method are seen in Chart 30. As we shall find in next section the whole of the Wedensky phenomena can be established by freezing the nerve. When the conductivity has returned the Wedensky phenomena can be elicited at a much higher temperature than was before possible (see p.), while recovery to the normal "non-cooled" condition of the nerve proceeds at a very rapid pace provided the temperature is high. Tracings 2, 3, 4, 5 and 6 were taken during

this process of recovery from freezing, and in each case we see the very marked effect that stimulation of the nerve had upon its functioning capacity. The drum was travelling at a rate of one revolution in 100 seconds.

To clear up any doubt as to the possible action of the constant current in polarising the nerve, the tetanisation and the passage of the constant current ought to be carried out in the following relative order:-

where the upper line represents the time of the block and the lower the time of tetanisation. As yet I have not succeeded in getting tracings taken in this way. Fortunately however, we are not limited to the method first introduced by Bernstein in order to prove fatigue. In the next sub-section fatigue of the nerve through activity is proved beyond a doubt.

(e). Temporary Break-down in the Nerve through Activity.

As was said on page 75, in a certain stage of cooling, initial tetani may be got with all intensities of stimulations. A further illustration of the fact may be seen in Chart 31. Here at a temperature of somewhere between -2.8° and -3.2° (tr. No. 2), initial tetani are got up to a strength of 800. Meanwhile as tracing 3 shews, peripheral stimulation of

the nerve by means of the thermometer electrodes reduces full contractions of the muscle. In this condition of the nerve, as numerous experiments have shewn, the conductivity is just on the point of disappearing. The rate of stimulation was now changed from 96 to 60 per second and the initial tetani instead of becoming full tetani are only with difficulty elicitable at an intensity of 500.

This introduces us to a new fact, viz. that when the conductivity disappears it goes away in definitely regular "march" or order. If we once more examine tracing 2 we notice that the initial tetani gradually climb in height until the intensity 800 is reached, while in tracing 4 the initial tetanus at 500 is considerably lower than that at 1000, and at 100 an initial tetanus is no longer elicitable.

Chart 32 shews the same thing. Here the rate of tetanisation was 30 per second, and in tracing (1) the gradual climb in height until an intensity of 800 is reached is quite plain. In tracing (2) a small initial tetanus is just elicitable at 100, the tetanus at 200 is higher, while that at 300 is higher still. In tracing (3) there is a faint response at 200, none at 300, and only at 500 is the initial tetanus of any size. The climb in the next two tetani is beautifully shewn. Further attempts after the lapse of a minute or so to elicit responses of the muscle by means of central stimulation failed. The conductivity was

gone entirely.

Meantime, between the inscription of the two tracings (2) and (3), stimulation at a very slow rate (somewhere between 4 and 10 a second) by manipulating the contact spring with the hand, caused a tetanus with the single responses of the muscle plainly evident, such as one gets in genesis of tetanus; so that the refractory period of the nerve lay somewhere between $1/30$ th. and $1/10$ th. second.

Now if we remember, Boruttau and Fröhlich⁽⁵⁶⁾, from the evidence at their disposal, formed a theory (see p. 49) which accounted for the fact that the conductivity disappears at the same moment for both strong and weak stimuli. Here we see that the conductivity for strong stimuli is longer retained than the conductivity for weak stimuli, so that the theory of these two observers accounts for a fact which does not really exist. Knowing as we do that in the case of an initial tetanus only the first excitation of a series of excitations entering the cooled part of the nerve, manages to get through, we have no difficulty in explaining the present result. When the nerve is in this lethargic condition a strong excitation can plough its way through, while a weak one is stopped on the way. An excitation of intermediate strength is so cut down in passing through the affected portion of the nerve that on emerging at the other end it is sub-maximal in effectiveness.

The proof of this is seen in Charts 35 and 36, which are records taken on the following day from the same preparation that gave the results we have just been discussing. In these two charts we see the result not merely of rhythmical stimulations at a rate of 30 per second, but also of single stimuli at various intensities. First of all we see in tracing (13) the Wedensky condition just present, in that tetani are got on stimulation at the first three intensities. Initial twitches are then got which actually climb in height until a maximum is attained at 1000. The nerve was then given a slightly longer rest, and a single stimulus at 1111 was sent through. This, as we see, evokes a higher twitch of the muscle. Meantime a single stimulus at 600 is submaximal, while in the following series single stimuli at 600 are seen to evoke less complete muscle contractions than single stimuli at 1000. A minute or two afterwards (see tr. 14) tetanisation at a rate of 30 per second produces an irregular result, until we reach ^{an} intensity of 900; single stimuli following immediately afterwards are effective only when they get above 500 in intensity, and then the muscle twitches begin to climb. In this tracing we can at least guess at a process of tardy recovery from excitation going on ^{inside} the nerve.

Tracing (15), taken a minute or two later, shows once more the result of single stimuli. At 1000 the

make shock is more effective than the break. Tracing (16) is pretty much a repetition of the previous tracing; the temperature had been allowed, however, to rise. Chart 36 is very interesting. The first five tracings were taken at a temperature of 0° . In (17) and (18) we see the result of single stimulations; the contrast with (19), (20) and (21), where rhythmical tetanisation was used is great. The fatigue of tetanisation at 500 is sufficient to prevent subsequent tetanisations from stimulating the muscle until we reach 900 when a faint twitch occurs; at 1000, too, only a faint twitch occurs. A rest of a minute or so is sufficient to allow the nerve to conduct the first of the next series of stimuli at 1000; this causes such a breaking up in the nerve that by the time a second tetanisation occurs at the same intensity the tissue is not yet prepared to conduct even the first excitation through. In tracing (22) taken at 1°C the refractory period is less than $\frac{1}{30}$ th. second; the nerve is beginning to recover its property of conducting weak excitations. Before it was taken out of the cold chamber it conducted at an intensity of 100 and caused tetanus of the muscle; whence we conclude that the conductivity returns in the reverse order to that in which it disappears, viz. first of all strong stimuli become effective, and then weak.

Because of their importance I have described these tracings at unusual length. They illustrate a

case where the conductivity has been caught in the act of disappearing, and kept in this critical condition for many minutes together. It will be noticed that no point did I stimulate with the peripheral electrodes, so as to test the condition of the muscle. Had I done so the conductivity, as I have proved again and again, would infallibly have disappeared at once. In fact if there is one certain method of suddenly abolishing the conductivity of a nerve when the Wedensky condition is well established it is to tetanise at the peripheral side of the cooled portion. When the conductivity in such a case goes away it is not away for good. After a rest of a minute or two, though still cooled to the same extent as before, the nerve recovers and functions again just as before. Chart 38 is a record of the first occasion on which I discovered this apparent anomaly, and at the time of its occurrence it puzzled me greatly. Since then I have found the same thing occur again and again, so much so that I had entirely ceased to use the peripheral electrodes when the nerve was in this critical condition.

If we imagine for a moment what happens when a single excitation entering a cooled portion of the nerve is only able to emerge from it cut down to a fraction of its original efficacy, we see that the molecular commotion in each minute length of the cooled portion declines and declines in extent the

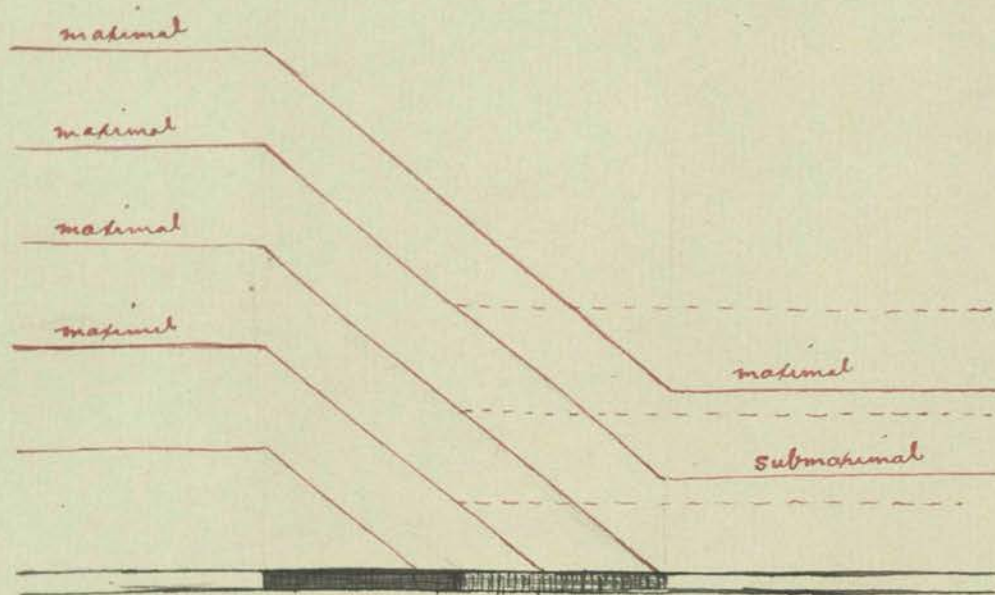


Fig.

further the excitation penetrates towards the peripheral end. Associated with this diminished disturbance of the more peripheral portions of the cooled part is ^abetter recovery than in the more centrally lying parts, which are shattered to such an extent that they do not recover for some little time. If we can reverse the direction of our attack, so to speak, and fire into the nerve from the rear as well as from the front, it breaks down, and becomes demoralised. This we do when we stimulate peripherally. Only if the nerve gets sufficient time and freedom from disturbance to recuperate again can it once more take part in conducting impulses. We are now in a position to see why the Wedensky effect is got better by cooling a long than a short piece of nerve (see p. 78). In the case of a winter nerve there corresponds, roughly speaking, to any definite temperature at which the nerve is examined, a definite gradient of fall in the amount of the excitatory process. When the original excitatory process is high (see diagram, fig.) it succeeds in getting through a long length of cooled nerve, and still remains maximal. When it is low it may be cut down to submaximal or even subminimal strength. On the other hand if it has to traverse a shorter portion of nerve the extent to which it is cut down is less. We can also understand why irregularities appear on

one of the descending tetani of a Wedensky effect. The first two or three excitations get through the nerve, diminished in effectiveness, it is true, but still of considerable magnitude. As they go through they break down the nerve more or less, and the part that suffers most is the central part. Ultimately there comes a time when the excitations are stopped on the way and do not reach the peripheral part, which then gets breathing space for a moment, and recovery spreads from the peripheral end centralwards. When the recovered portion meets the excited portion, the stimuli once more get through and excite the muscle. A second response then occurs, and thus it goes on till the central part of the cooled portion, by being kept in a state of continuous disturbance, refuses entirely to conduct excitations till it gets a rest again. We can also see how the "refractory period" as ordinarily understood, varies with the intensity of the stimulation. A strong excitation on its way through the nerve causes more breaking up than a weak excitation, and the corresponding period of recovery is greater. Finally we see that the refractory period can be prolonged to some seconds, if not to minutes, in duration. It all depends what we mean by "refractory period". If we mean the period of time that elapses before a second stimulus following another of equal intensity can be effective, then the re-

:fractory period can be prolonged to at least $\frac{1}{30}$ th. second. If, however, we include in our definition the period of time that elapses, before a slight stimulus which previously would have been effective, can be effective after the application of a stimulus of undefined magnitude, then the refractory period can certainly be prolonged to many seconds and probably to minutes.

On page 92 we referred to certain results which Kronecker⁽⁶⁷⁾ got on using very rapid rates of stimulation. "The production of a note indicating 20,000 vibrations per second was effectual for the excitation of the nerve, the muscular response being at first a tetanus, but on repetition it became an initial twitch, and subsequently failed". This looks very much as if he had actually fatigued the nerve. Engelmann⁽⁶⁸⁾ seems to have got the same effect; he is reported by Gotch () as finding "that failure of excitability occurred in nerve stimulated by rapidly recurring currents". [It will be convenient here to shortly describe the series of Charts, 32 to 37 inclusive, because of their intrinsic interest. The curves in all these charts were got from one preparation, which remained alive for 9 days. The sister-preparation, which was not used for tetanisation experiments lived for 11 days, and at the end of that time quite moderate stimulation of the central end of the nerve evoked contractions of the muscle. How

long the nerve lived I am unable to say. The muscle at least, was dead on the 13th. day. The frog from which the preparations came was a large female of the *Esculentia* species, and had been kept for five weeks continuously among melting ice. The dissected preparations were carefully preserved at the same low temperature when not in use.

I have been unable by reference to literature on the subject to find any precise information as to the actual length of time that dissected nerve-muscle preparations have lived after death. Beidermann says that they may live "for days" provided they are kept in the proper kind of fluid. The fluid in this case was Ringer's solution. I am of opinion that the previous treatment of the frog, and the temperature at which the preparations were kept, contributed in high degree towards the result.

The question is interesting not merely from the special point of view of nerve physiology, but from a general standpoint. Doctors Herring and Simpson⁽⁸⁰⁾ found that the dissected nerves and muscles of an artificially cooled mammal remain irritable for two or three hours after removal from the body. It is possible that if the animal were kept alive for a more or less prolonged period at a low temperature, the preparations might survive even longer. Experiments of this kind could best be carried out on hibernating mammals. The relationship between low

temperature and storage of oxygen (see II, Sect.C) is worthy of being kept in mind.

But to return to the charts. In Chart 33 we see (tr. (1)) the effect of a night's steeping in Ringer. On the previous day one had got initial tetani at a rate of 30 vibrations per second. The nerve has now largely recovered, and at a temperature of -1.2° tetanisation with 128 stimuli per second evokes full tetanus of the muscle. Nevertheless, the temperature being kept constant, the nerve recovers less and less completely after each round of stimulation, until in tracing (4) an initial tetanus is got at an intensity of 300. On reducing the rate of stimulation to 80 per second and the temperature by $\frac{1}{10}$ th. degree^(tr.5) the initial tetanus is shifted back to 800; on repetition (tr. 6) it returns to 400. Tracings (7), (8) and (9), shew a gradual prolongation of the refractory period through use, the temperature being constant. In tracings (10, (11) and (12), tetanisation is carried out at a rate of 30 per second. Charts 35 and 36 we have already discussed. Chart 37, lower line, shews that with a rest of 60 hours in Ringer at 0°C the Wedensky effect had almost passed off for a rate of 214 per second even through the temperature of the nerve is reduced to the freezing point. Conf. p.]

Section I. Freezing of Nerve.

(1). General Considerations.

Already a number of experiments have been carried out on the freezing of poikilothermic animals. They have shewn that, provided great care is taken in the subsequent process of thawing, a vertebrate may be frozen hard without being killed. Sir John Franklin⁽⁸¹⁾ on his polar voyage in the year 1820 saw carp, after being frozen as ^hard as stone, once more come to life on warming at the fire. Laboratory experiments by Preyer⁽⁸²⁾ shewed that frogs may be ~~had~~^h frozen; and, provided the temperature of their interior has not reached -2.5°C , again restored to complete vitality. Kochs⁽⁸³⁾ carried out similar experiments, and concluded that if the interior of the frogs was completely frozen they could not again live.

By far the most reliable experiments have, however, been carried out by Raoul Pictet⁽⁸⁴⁾. The animals with which he experimented were protected by means of wood from contact with the metal walls of the cooling chamber into which they were brought. Different animals reacted differently to low temperature. Fishes were cooled down in a block of ice to -15°C , still remained alive after careful warming, although others similarly treated could be pounded into powder just like ice. On the other hand after a tem-

temperature of -20°C the fishes remained permanently dead. Frogs withstood a temperature of -28°C , millipedes of -50°C , and snails a temperature of -120°C , without being killed; while bacteria survived after being cooled to below -200°C . As we are aware from Macfadyen's researches⁽⁸⁵⁾, the lower limit in the case of the latter organisms has not been reached at a temperature of -252°C , i.e. only 21°C above the absolute zero.

I have twice had an opportunity of seeing frogs frozen hard. In one case I had five in a tin vessel which was standing on a block of ice. To insure that the temperature should be low, some salt was strewn on the ice. Next morning the frogs were found stuck fast to the bottom of the tin and as hard as stones. On careful thawing three of the five lived. Preparations from the dead ones taken even three days after death shewed the Wedensky effect quite well. The dead animals had been lying on ice during the intervening time.

On another occasion owing to a severe frost during the night a number of my frogs were found frozen in the morning. Some which had been sitting on a stone slab were quite hard, others which had clustered in a mass together were only partially frozen. In these a thin sheet of ice had formed over the skin. Underneath this the animals themselves were semi-frozen. They could execute movements of

some muscles; on being pithed and dissected their tissues were found to cut like paper. A blunt pair of scissors was as efficacious as a sharp pair for dissecting them. Hard lumps of ice could be picked out of the peritoneal cavity, the muscles were hard and firm, but on being cut reacted by movement. Needless to say the Wedensky condition in these frogs was very readily elicited. On this occasion the frogs which were hard frozen on the slab of stone did not recover.

(2). What happens when a Nerve Freezes?

In experimenting with low temperatures I have again and again had experiments interrupted by the freezing of the nerve. The muscle would suddenly begin to twitch or then pass at once into a condition of prolonged tetanic contraction, followed after a minute or so by convulsive irregular movements. These continued for two or three minutes the muscle contractions becoming feebler and feebler until they finally ceased. (See Charts 5, 9, 15 and 21). In other cases again the muscle would give one or two little twitches and conductivity would be found abolished. Central stimulation of the nerve was then entirely inadequate to cause contraction of the muscle. On being raised out of the cold chamber the nerve was hard and stiff and had a white opaque appearance quite different from the glistening sheen of ordinary moist nerve.

*Some nerves may
freeze & not
twitching
A kept nerve
sometimes loses then
a fresh nerve.*

It was my habit to touch the frozen nerves & -
pencil to see if it was rigid. When I stopped touching
the nerves as a matter of routine.

At first I found that no recovery from freezing occurred even when the nerve was left to soak all night in Ringer. One day, however, I found to my surprise that a preparation had quite recovered by next day, although the conductivity when last I tested it was gone. I remembered that I had simply raised this preparation out of the cold chamber when it froze, and had not touched it, as had been my wont in order to see if the nerve was stiff. After this I refrained from touching any frozen nerve until I was quite sure that it had thoroughly thawed again; although the conductivity is still temporarily abolished yet the nerve if carefully handled does not die. After a night's soaking in Ringer it conducts quite well next day. We consequently conclude that a nerve ~~frozen~~ is very susceptible to mechanical injury, but is not killed by the freezing.

During the process of freezing the conductivity is not necessarily at once abolished. Stimulation (see Chart 17, tr. 1) of the central end of the nerve may produce contraction of the muscle, but the property of conducting is very soon lost. If the process is interrupted and the nerve at once raised out of the cold chamber the conductivity after a minute or two comes back in an imperfect manner (Ch.17, tr.2), but in any case much sooner than if the nerve be left to freeze to the bitter end. A rest in Ringer brings the conductivity back completely.

*Cf. cases where
freezing power
at not too
low temp.*

Improvement
Increase in conducting power of nerve

Once or twice it was noticed that the mere stimulation of the nerve, when the temperature was very low, sufficed to send the muscle at once into a prolonged tetanic contraction, (see e.g. Ch.9); as if the molecular commotion inside the nerve had suddenly "shaken into frost", a nerve already below the temperature of the freezing point. More frequently however, a few preliminary twitches of the muscle heralded the onset of the freezing (Charts 5 & 37). One thing, I got to look upon as pathognomic of the near approach of freezing and that was a sudden increase in the excitability of the nerve. Chart 5 shews this well. There the tetani registered at 500 and 300 just before freezing has set in, shoot up above the level of the preceding tetani. This, however, I afterwards found not to occur in "cooled" nerve. It occurs almost without exception in non-cooled nerve. (See

See especially
one chart in
later series

9

The registered temperature at which freezing sets in varied in my observations from about -2.5°C to -5°C . Notwithstanding that the thermometric observations are by no means to be regarded as exact, I am of opinion that different nerves freeze at different temperatures, or rather that the muscle twitchings caused by freezing commence when the nerve are at different temperatures.

for length of
time before
muscle begins
to twitch after
n. dipped in c. c.

It was for long difficult to know what actually causes the muscular twitchings when a nerve freezes. Prolonged observation proved that as soon as twitch-

Ready to disappear if I am asked after a preliminary freezing.

ings set in the nerve is already opaque in appearance, so that it is probable that the freezing occurs from without inwards. Although unlikely on physical grounds it is possible that a cylinder of ice forms round the nerve and mechanically nips it. The retention of the conductivity during the first stage of the freezing led me to think that this might be the case, and from the form of the freezing curves in such charts as 5, 9, 15 and 21, I imagined that possibly the tetanic contraction of the muscle occurs during the process of mechanical compression of the nerve, while the irregular twitchings might be due to molecular rearrangement going on inside the fibres.

*This in line with
idea that nerve
not yet completely
cooled.*

On this hypothesis I started experiments to see if the conductivity is still retained so long as the muscle is in steady tetanic contraction and the twitchings have not begun to appear. Such experiments led to no special results, for at one time the nerve would still conduct when the muscle began to twitch *irregularly*, at another conductivity would be abolished by the time that the irregular movements of the muscle set in. They had one result, however; it was found that if the freezing was interrupted half way by warming the nerve again, the conductivity, if abolished, very soon returned, but on a second cooling the conductivity seemed to go away without further freezing of the nerve.

Chart 39 shews this well. A non-cooled preparation which did not shew the Wedensky effect at a rate

of 170 per second, was allowed to freeze partially (tr. 1). It was thawed and found to have apparently made a perfect recovery. It was lowered into the cold chamber again (tr. 2) and tetanised as the temperature fell. Four tetani were elicited. After that the conductivity disappeared. A second warming (tr. 3) brought the nerve back again to the normal. On being once more cooled (tr. 4) the conductivity was gone. And so on it went. When the temperature of the nerve was high conductivity was present. Simple cooling, without freezing, sufficed to remove the conductivity.

5 4 3 2 1 0 32
show this effect



Chart 40, a continuation of the same experiment shews a new phenomenon. On being raised up for the fifth time (tr. 8) the muscle responses to tetanisation of the nerve are at first single twitches. A little later they become tetani.

Result of reverse
stimulation.

Broken tetanus
at 1000

Chart 42 (upper line) shews the same kind of thing. A winter preparation, which had given the tracings seen in Chart 41, had been allowed to partially freeze. When the temperature was raised to $+6^{\circ}\text{C}$ the muscle responses were initial tetani; some time later, at a temperature of $+10^{\circ}$ the responses are full ~~of~~ tetani; on being cooled to $+2^{\circ}\text{C}$ the conductivity is gone, and twitches of the muscle only begin to appear at a temperature of $+5.5^{\circ}\text{C}$.

Broken tetanus.
Pupph' start.

We consequently conclude that partial freezing of a nerve followed by immediate thawing, alters its

structure in some way. While the nerve conducts quite well at high temperatures, on the other hand at intermediate temperatures — not necessarily very low — the conductivity may be abolished.

Further experiment shewed that after partial freezing followed by thawing, the conductivity which is apparently perfect at a high temperature is not really so. Charts 43 and 44 form the record of an experiment on a non-cooled nerve. In tracing 2 the premonitory increase in excitability just before freezing (conf. p. 126), is well seen. While the muscle was still twitching the preparation was raised up. When the temperature has reached $+8^{\circ}$ to $+9^{\circ}$ tetanisation at a rate of 170 per second causes single twitches (tr. 3, 4 and 6). On the other hand tetanisation at a rate of 30 produces full tetani (tr. 7); so does tetanisation at about 50 per second (tr. 8); while tetanisation at 80 per second brings forth the curious result seen in tracing 9. We are reminded once more of the descending tetani of a Wedensky effect.

After soaking all night in Ringer at room temperature the nerve was found to have so far recovered (Ch. 45, tr. (4)) that tetanisation at the rate of 170 per second causes full tetani and no longer single twitches. Only when the temperature was lowered to $+3^{\circ}$ did these tetani give place to single twitches (tr. 5). The change established in the nerve by

freezing, like the winter condition of a cooled preparation, tends to pass off when the preparation is soaked in Ringer.

The next question came to be; "Is this change which freezing brings about, the same as the winter condition of a nerve?" Charts 46 and 47 help us to an answer. In tracing 4 we see the freezing of the nerve, preceded by the usual increase in the excitability. In tracing 6, tetanisation at a rate of 170 per second produces an almost typical Wedensky effect. In tracing 10, we see, near the end, an apparent fatigue effect, and when the nerve is thus conducting badly, the simple device of sending the tetanising currents through the peripheral electrodes for a second or two, suffices to entirely abolish the conductivity, and so completely that the nerve was incapable of conducting for many minutes afterwards. In tracing 13, we see the same result repeated. The correspondence with the phenomenon described on page 116 is almost complete. Here it would seem, we have an exaggerated winter condition of the nerve.

Stimulated by this suggestion I began to investigate the effect of freezing in a more careful manner than I had done. In order to limit the site of the freezing to a small^{er}/length of the nerve I used the smallest brass chamber ($\frac{1}{4}$ "). The freezing curves now began to shew more variations. Charts 48, 49 and 50 shew typical examples, but although I at first thought

that a "family likeness" could be detected in the curves given by preparations from the same frog, further investigation shewed that this was not so constant as I had thought.

In order to still further limit the site of the freezing I had a glass tube of thin bore made, on to which I could lower the nerve. By running the cold fluid through the tube a minute portion of the nerve could be frozen. By afterwards running warm fluid through, the nerve could be thawed again. Further, when the nerve was once frozen and thawed again, it could be lowered into one of the brass cooling chambers and the reactions of the nerve thus tested at different temperatures. The nice adjustment of the apparatus, too, rendered it possible to freeze the nerve more than once at exactly the same spot.

When a minute portion of the nerve was frozen the freezing curves took on a different aspect. Examples are to be seen in Charts 51, 52, 54, 55, 57, 58, 60 and 62. An unbroken tetanus was never obtained at the start. Twitching of the muscle is visible in all the curves from the very commencement. Thus we may conclude that the initial prolonged contraction seen in Charts 5, 9, 21 etc. are simply due to stimulation in a large number of nerve fibres at the same time, so that the effect is artificial, and does not denote any change of a different kind from that indicated by the subsequent twitching of the muscle.

Recovery not a simple for temp Ch. 2

When one froze the nerve in this more restricted fashion one was able to verify all the conclusions previously formed. After freezing, the conductivity of the nerve is abolished and does not return until the temperature of the frozen part reached a given height, or rather, it returns eventually at any temperature above the freezing point, but takes a shorter time to return the higher the temperature to which it is raised.

When the conductivity returns it comes back in the same fashion as we have seen in a fatigued nerve (p.) viz. first of all strong excitations get through while weak excitations cannot penetrate the affected portion till recovery has further advanced. This is admirably seen in the series of tracings in Chart 64 where the conductivity to tetanisations of different intensity is worked out as the temperature is brought from -2°C up to $+7^{\circ}\text{C}$. In tracing (4) the affected portion is just penetrable to the first stimulus at 1000, in tracing (5) a twitch occurs at 500, in tracing (7) this twitch is higher, a tetanus occurs at 1000, in tracing (8) a twitch occurs at 400, and so on it goes till at 7° we see tetanus at 30.

The nerve corresponding to this chart had been frozen some 27 hours before (See Chart 62, lower line). After the muscle twitchings had ceased the nerve still conducted for a couple of minutes or so. Later, conductivity was found to be entirely gone, and in

spite of thawing (see upper line) it had not returned after a space of 8 minutes. The preparation was put back into Ringer and left to lie all night. Next day (Chart 62, upper line), the nerve functioned apparently normally at room temperature. The series of tracings on Chart 63, shews that the nerve had in reality almost completely recovered, for only when the temperature is reduced to -3°C (tr. 8) do initial twitches put in an appearance. Later, apparently from fatigue, the conductivity becomes abolished. (tr. 12)

One rather suggestive discovery made by these freezing experiments is illustrated on Chart 58. In tracing 2, we see the irregular tetanus that occurs on freezing. On the discovery that the conductivity was abolished, warm fluid was at once run in, and in a short time recovery set in rapidly. This is shown in tracing 3, where tetanisation at a rate of 30 causes at first an ascending tetanus. The refractory period is gradually becoming less and less as the tetanisation goes on. In spite of this rapid shortening of the refractory period, the great rate of recovery is more apparent than real for at the end of a minute and a half (tr. 4) tetanisation at 80 per second causes a descending tetanus, while two minutes later, tetanisation at 50 - 60 per second causes descending tetani. In tracing 7 we have beautiful examples of fatigue curves. Their regularity suggests that in this method of freezing followed by thawing we have a new and easy

means of studying fatigue processes in nerve.

I have not as yet been able to investigate fully the many new phenomena that occur after freezing of a nerve. So far it seems to be pretty certain that freezing produces in a short time the same change in nerve that prolonged cooling of the animal does. That the muscle twitchings which occur on freezing are not due to the mechanical action of ice crystals, seems to be proved by the fact that a second freezing of the nerve at the same spot as the first causes almost no muscle twitchings. Again when a non-cooled nerve is lowered on the cold glass tube twitchings occur almost at once. In the case of a cooled nerve on the other hand the twitchings are much longer in occurring. This, too, would point to a molecular re-alteration rather than to a crude physical stimulation. The cooled preparation would seem to be already in a state approximating to the frozen condition and consequently freezing causes a relatively smaller disturbance in the nerve.

The relation of oxygen supply to a nerve that had been frozen is an interesting question. According to Fröhlich the nerves of cooled frogs possess an extra reserve store of oxygen. Experiments on the asphyxiation of nerves that had been frozen, might give some result of value.

Since carrying out these experiments on the

freezing of nerve I became acquainted with the work of Boycott⁽⁸⁶⁾. During observations on the influence of low temperature on the conductivity of nerve he often had an opportunity of seeing the nerve freeze. After freezing he believes that conductivity and excitability are as a rule "permanently lost". "If, however, when a tetanus from freezing appears, the frozen part of the nerve is rapidly warmed to 20° or 30°, a certain degree of excitability and conductivity may still remain, which is probably due to some of the fibres not having been frozen or to a temporary block having been caused by the pressure of the ice crystals, but much stronger stimuli than were previously necessary are now required to elicit a response; moreover the remnants of irritability frequently disappear for good in the course of a few minutes. If the application of cold is persisted in and the nerve be thoroughly frozen no recovery seems to be possible". Curiously, enough, he observed the abolition of conductivity at a more or less moderate temperature after the nerve had been once frozen, for in a foot-note he says; "On two occasions in about 40 sets experiments a diminution, followed by an abolition, of the muscular contractions was observed in cooling, and an augmentation of the response on warming, but on both occasions the preparation had previously been in a cooling tetanus. It should be

mentioned that this is in no way the regular and characteristic sequel of a cooling tetanus, even excluding those cases where no further response was to be obtained after the tetanus had subsided".

The freezing point of nerve is given by Boycott as about -7°C , but his means of measuring the temperature was much less exact than mine. He seems to have almost entirely missed the interesting changes that occur in nerve through freezing.

Section, J. Preparation from an Artificially
Cooled ~~Animal~~ Mammal,

Seeing that the temperature conditions of mammalian nerve can be altered during life by artificial cooling of the animal it was very interesting to know if any condition resembling the Wedensky effect could be elicited from a cooled mammalian preparation. Through the kindness of Dr. Simpson, I was enabled to utilise the nerve muscle preparations from one of the animals in his cooling experiments. Investigation shewed that the tetani worked with a rapid rate of stimulation fell off in height very much more quickly than those obtained at a slower rate of excitation and the preparation as a whole could apparently be easily fatigued with rapid stimulation even at room temperature. Where the seat of this fatigue lies is a different question. Further experiments will be necessary.

Section K. SUMMARY of RESULTS.

It is difficult in short compass to put down the various new points in nerve physiology brought out by the present research. Generally speaking, a considerable amount of light has been thrown on the inner processes that go on in a nerve not only during the process of excitation but when the nerve is seemingly at rest.

The various steps by which this has been worked out are as follows:-

(1). A series of complex phenomena were discovered by Wedensky to occur in a narcotised stretch of nerve. Fröhlich found the same series of phenomena in asphyxia of the nerve, and was able to bring them into relation with an alteration in the refractory period. He showed that an asphyxiated or narcotised nerve undergoes fatigue.

(2) Investigation by Fröhlich and Boruttau showed that the Wedensky phenomenon may probably be related to a simultaneous alteration in all the "physiological constants" of nerve (refractory period rate of transmission of impulse, duration of electrical response, amplitude of excitation. etc.)

(3) The series of phenomena which occur in narcosis/

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narcosis and asphyxia have been found by me to occur in cooling of the nerve. Low temperature, generally speaking, has the same action on nerve as asphyxia or narcosis.

(4) Nerve can be fatigued by cooling. The significance of this discovery is heightened by the fact that chemical means were not used to bring about the fatigue.

(5) Nerves differ in the relative ease with which they may be made to exhibit the Wedensky phenomenon. The nerves of winter frogs are better objects for exhibiting the special effect by means of cooling than the nerves of summer frogs.

(6) The Wedensky phenomenon as brought out by localised low temperature is much more even and regular in its course than in the corresponding cases of asphyxia and of narcosis. This is due to the possibility of keeping the physical conditions more constant. The phenomenon consequently was more accurately and exhaustively examined than had previously been possible.

(7) By a combination of the principles stated in the last two paragraphs it was found that nerves differ to a great extent in the degree to which they exhibit/

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exhibit the Wedensky effect, under precisely similar physical conditions. The consequent possibility of exactly grading the relative extent of the "cooled" or "winter" condition of a nerve, and per contra of the "summer" condition, has for the first time been put forward.

(8) The "winter" condition in a dissected nerve is not permanent. After dissection a change goes on in the nerve, the rate of which depends on the temperature at which it is kept, whereby a nerve from a "winter" frog comes more to resemble the nerve of a "summer" frog. This discovery affects the value of much work previously done in the physiology of nerve.

(9) The changes undergone by a nerve in freezing have been studied more carefully than hitherto, and the conclusion arrived at that freezing is not necessarily fatal to the life of a dissected nerve. Further, the twitchings of the muscle that occur on freezing are in all probability due to a molecular re-arrangement of the fundamental conducting material, and not to any purely mechanical cause. If a nerve is frozen twice at the same spot, the second freezing causes little or no twitching of the muscle.

(10) In the freezing and subsequent thawing of a minute portion of the nerve a method has been discovered by which the typical "winter" condition can apparently/

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apparently be at once established, and in a much higher degree than in the living animal. This change, just like the real winter condition, passes off on further keeping, and more rapidly at a high temperature than at a low. This rapid and easy method of establishing the winter condition in a nerve promises to be of value for the investigation of further problems.

(11) An extension has been given to the significance of the expression, "fatigue" of nerve. It has been possible to examine the nerve in a condition in which the passage of an excitatory process through its structure, so breaks it up that it requires a prolonged rest to be once more able to function.

Some subsidiary points, not immediately connected with the action of low temperature on frog's nerve have been animadverted upon, e.g. the action of CO_2 gas on nerve, the importance of a blood supply for mammalian nerve, and the fatigability of preparations from artificially cooled mammals.

During the course of the exposition doubt has at times been cast on certain conclusions of previous investigators. The chief papers that have been subjected to criticism are :-

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Piotrowski ⁽¹⁰⁾	in reference to the action of CO. gas.
Bowditch ⁽⁴⁰⁾	} in reference to the fatigability of mammalian nerve.
Lambert ⁽⁴²⁾	
Eve. ⁽⁴⁴⁾	
Howell, Budgett & Leonard ⁽⁴⁵⁾	
Brodie & Halliburton. ⁽⁴⁷⁾	
Carvallo. ⁽⁵³⁾	in reference to fatigue of frog's nerve.
Boruttau & Fröhlich ⁽⁵⁶⁾	in reference to disappearance of conductivity.
Boycott ⁽⁶⁰⁾	} in reference to refractory period.
Gotch & Burch ⁽²¹⁾	
Boycott ⁽⁸⁶⁾	in reference to freezing.

In conclusion I wish to thank Professor Verworn for having suggested to me this research and Professor Schäfer for much kindly help and advice during its progress and for the many facilities afforded me for its prosecution in the Laboratory of the University.

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88. Biedermann, "Elektrophysiologie", Jena, 1895.
89. Halliburton, "Die Biochemie der peripheren Nerven", in "Ergebnisse der Physiologie", Jahrg. 4, 1905.
90. Biedermann, "Elektrophysiologie" in "Ergebnisse der Physiologie" Jahr. 2, 1903.