The Croonian lectures on the chemical side of nervous activity : delivered before the Royal College of Physicians of London, in June, 1901 / by W.D. Halliburton.

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BY

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London

JOHN BALE, SONS & DANIELSSON, LTD.

OXFORD HOUSE

83-89, GREAT TITCHFIELD STREET, W.

1901

PREFACE.

THE lectures as now printed are somewhat fuller than those actually delivered. Analytical tables are for instance given, and details of experiments are described, which were only referred to in general terms in the spoken lectures. This little book aims at presenting in a systematic way the numerous researches relating to the nervous system which for several years past have been carried out in the Physiological Laboratory of King's College, London. Much of what appears has been published elsewhere, mainly in the *Journal of Physiology*, and in the *Philosophical Transactions* of the Royal Society, but I have never before had the opportunity of presenting the whole in a connected form. The fourth lecture is in the main identical with a paper recently presented by Dr. Mott and myself to the Royal Society.

I have to thank the Council of the Royal Society, the Editors of the *Journal of Physiology*, and of the *British Medical Journal*, Dr. Mott, and Mr. John Murray, for permission to reproduce some of the illustrations here given.

I have also to thank the Royal College of Physicians for allowing the funds of the Croonian Trust to be devoted to the expenses of many of the researches here described.

King's College.
December, 1901.

W. D. HALLIBURTON.

CONTENTS.

THE CROONIAN LECTURES

ON

THE CHEMICAL SIDE OF NERVOUS ACTIVITY.

LECTURE I.

The Chemical Composition of Nervous Tissues.

Mr. President and Fellows,—My first duty is to thank you for the honour you have conferred upon me in electing me Croonian Lecturer for the present year. I feel the responsibility a heavy one, and I can only trust that the subject I have chosen may be shown to possess important bearings not only to physiologists, but also to those who, like the majority of my hearers, devote their studies to the healing of the sick.

On looking through the list of previous Croonian lecturers, one is at once struck with the large number who have selected the nervous system as their subject. The reason for this is not far to seek. The subject is a complex one; hence its fascination both for physiologists and physicians. It is the ruling system of the body which regulates and controls the other processes which occur there. It is, moreover, the seat of mental phenomena, and it therefore touches deep questions of being and consciousness; its investigation appeals to all those who have sought to unravel the so-called mysteries of thought and reason. To discover the secrets of the mind has ever been the bent of philosophic thinkers, and to ascertain the way in which the mind works, investigators have directed their attention to the organ of mind, the brain. Some have traced the devious channels of this organ with the scalpel, others with the microscope; others again, have

employed the experimental method to establish the localisation of cerebral functions. All these investigations have gone hand in hand with the researches of pathologists and clinical observers, who in the sick room and at the autopsy have obtained little by little knowledge of the nervous lesions which lie at the root of mental and nervous diseases.

The mass of knowledge which has now accumulated as the result of all this work is very immense, and these lectures have frequently been the channel by which the fruits of such labour have been laid before the medical profession. The enormous benefit which has thereby accrued to suffering humanity it is hardly necessary to emphasise before my present audience. The feats of cerebral localisation alone, which have rendered brain surgery a matter of every-day experience, are in themselves sufficient as an illustration of this fact.

In spite, however, of this increase of scientific and accurate knowledge, and in spite of its paramount usefulness, we must still confess that any real information of the deeper meaning of volition and consciousness is lacking. We may theorise and argue as before, but any visible or tangible means of testing our theories in the light of experiment seems as far off as ever.

No scalpel however skilful, no microscope however powerful, no stain however delicate, has yet succeeded in unearthing that intangible something we call the mind. The methods of that new branch of physiology which is termed experimental psychology are also at present equally inefficacious to solve the problem.

We sometimes speak of physiology as the application of the laws of chemistry and physics to life. Since this conception of the aim and object of physiology has pervaded its disciples, the progress of physiological knowledge has been rapid and fruitful. When Helmholtz, Ludwig, and their contemporaries set themselves to rescue our science from the slough of mysticism, they placed it on a firm basis, and no doubt hoped in time to be able to expunge the word "vital" from their vocabulary. But all must admit that that time has not yet arrived. The neo-vitalists, it is true, have not the same contented and reverent frame of mind in relation to the meaning of the word vital as the older vitalists had. They admit its unsatisfactory and unscientific nature; they use it merely as a convenient expression for what

cannot at present be brought into line with the forces that operate in the inorganic world, and not merely as a cloak for their ignorance. The use of this word becomes most frequent when we have to deal with mental phenomena, and the question for the future is whether the manifestations of vitality are only physical and chemical after all, or whether there is really some other, and at present unknown force, or unknown aspect of known forces, which in the meanwhile we must be content to label as vital.

On such a fundamental question as this, there is obviously much room for differences of opinion. The answer is certainly in the region of the unknown; some may place it with du Bois Reymond in the region of the unknowable. That may be so; one hopes that it is not, for if we once admit that any subject is unknowable we place an impenetrable bar upon research, and remove any stimulus to investigate it afresh.

My object in these lectures is a much more commonplace one than the investigation of such fundamental problems. I propose to lay before you the principal known facts concerning the chemical structure of nervous tissues, and the changes of a chemical nature that occur in activity, death, and disease of these tissues. This is a side of the subject which has been comparatively neglected. A great deal of work has been done, it is true, but the amount is small in comparison with that which has been carried out from the anatomical and microscopic standpoint. The scalpel and the microscope have not succeeded in discovering the mind; we should not therefore expect to find it in a test-tube. Still, even in our test-tubes we shall find something that is of interest, and I hope also of practical importance. The chemical investigator has a great disadvantage when he sets himself to study the composition of living matter, for his reagents, however gentle they may be, kill the object of his research, and he can then examine not living protoplasm, but only its ruins. In spite of this, chemical physiology is advancing with rapid strides, and I am convinced that chemical pathology has a great future before it; and the more study I devote to the consideration of pathological problems, the more deeply do I feel that chemistry is a most powerful ally in solving them.

If in what I have now to say I dwell more particularly on my own researches and on those carried out by others in my

laboratory, I do so not because I consider them more worthy of attention or of greater importance than the work which has been pursued elsewhere, but because I shall be limited by time, and further, because I understand that one object of this lectureship is to give an investigator the opportunity of presenting his own original work in a connected and orderly way before the medical public. I would here at the outset express my thanks to those who have worked with me, and more particularly to Dr. Mott and Dr. Brodie.

For the last five years Mott and I have laboured together, and much of what I have to say is his work rather than my own. In being associated with him I have had the great advantage of working not only with a pathologist, but also with a practical physician, and this will enable me to insist more forcibly than I should otherwise have been able to do on the practical application of our investigations to the every-day life of the practising medical man.

In his Croonian lectures last year, Dr. Mott laid before you some of our joint work; my lectures will supplement and extend some of the points he dwelt upon then, in the light of the work we have carried out during the last twelve months. In order to render the subject more complete, it will of course be necessary for me to mention the work of many others, and I think that in the end our conclusion will be not one of satisfaction at the accomplishment of so much, but rather a realisation of the vast field still hardly touched, and an earnest hope that more labourers will enter and assist in the endeavour to reduce the gaps in our knowledge.

Before it is possible to pass on to a study of pathological questions, I must ask your indulgence if for a time I dwell upon some details in connection with the physiological side of the subject, and the first of these which I will take up is the general chemical composition of the various parts which make up the nervous system.

Relation of Water and Solids.

The first general impression one derives from a glance through any of the analytical tables in the text-books, is the great preponderance of water in most of our so-called solid tissues. Even

bone contains nearly 50 per cent. of water, and when Hamlet expresses the wish that this too solid flesh would melt, he spoke without the knowledge that his muscles contained only 25 per cent. of solid material. The nervous system is no exception to this rule. The amount of water varies; it is present in larger amount in early than in adult life, in grey than in white matter, in the brain than in the spinal cord, in the spinal cord than in nerves.

This is illustrated by the following tables :—

TABLE I.

INFLUENCE OF AGE ON THE PERCENTAGE OF WATER IN THE BRAIN TISSUE.[1]

	White matter	Grey matter
In fœtus	87	92
Age 20-30	69	83
Age 70-90	72	84

TABLE II.

PERCENTAGE OF WATER IN DIFFERENT PARTS OF THE NERVOUS SYSTEM OF THE ADULT.[2]

Grey matter of Brain	81-86
White matter of Brain	68-72
Brain as a whole	81
Spinal cord	68-76
Nerves	57-64

TABLE III.

PERCENTAGE OF WATER IN DIFFERENT PARTS OF THE NERVOUS SYSTEM.[3]

Grey matter of cerebrum	83·4
White matter of cerebrum	69·9
Cerebellum	79·8
Spinal cord as a whole	71·6
Cervical cord	72·5
Dorsal cord	69·7
Lumbar cord	72·6
Sciatic Nerves...	65·3

We thus see that water is most abundant in the grey matter, or in those regions of the nervous system where the proportion of grey matter is greatest. It cannot fail to be a striking fact

[1] Weisbach, *Hofmann's Lehrbuch d. Zoochemie*, Wien., 1876, p. 121.

[2] Table compiled from a number of analyses made by others.

[3] This table gives the averages of a large number of experiments made with the organs of human beings, monkeys, dogs, and cats, by myself. (*Journal of Physiology*, 1893, vol. xv., p. 90.)

that the grey matter, the region which is most active, and most important, contains somewhat less than 17 per cent. of solid materials.

Specific Gravity.

The question of the amount of water is closely related to that of specific gravity. The specific gravity of the brain has been the subject of researches by Bischof, Danilewski, and others, but I only wish here to dwell upon one aspect of the question which has interested me, and which was brought prominently before the medical profession in an address on "Sex in Education," by Sir James Crichton Browne,[4] some years ago. Among the differences between the brains of men and women, Sir James stated that he had found that the specific gravity of the female brain is less than that of the male brain. It was, however, pointed out in the correspondence that followed the publication of the address, that this generalisation rested on very few observations, the brains of two healthy men and one healthy woman having been investigated. Observations on the brains of lunatics, which were mainly used, can hardly be considered as likely to yield trustworthy results of what obtains in the normal state. I have accordingly thought it advisable to examine the brains in a larger number of cases. This has been carried out in my laboratory by Mr. Gompertz, B.Sc. The details of his experiments will be published elsewhere. All I shall do here is to mention his main conclusion. He finds that in adult men and women who suffered from no brain disease, that there are fairly wide variations in both sexes, but that the average is practically identical in male and female, and that there is no foundation for the belief that the variations constitute a sexual difference.

I may point out that a low specific gravity of the brain does not necessarily imply a poorer quality, for the part of the brain which is most important and most active--the grey matter—has a lower specific gravity than the white matter.[5]

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[4] *British Medical Journal*, 1892, vol. i., p. 949.

[5] This fallacy underlies a good deal of the statements made on this subject. J. P. H. Boileau (*Lancet*, 1882, vol. ii., p. 485), for instance, draws attention, in the examination he made of the brain of a highly-gifted man, not only to its great weight but also to its *high* specific gravity.

Solids of Nervous Tissues.

But coming now to the more important subject of the solids we find it is possible to divide them into the following classes:—

(*a*) Proteids. These comprise a very considerable percentage of the solids, especially in the grey matter (over 50 per cent.).

(*b*) Nuclein. From the nuclei of the cells.

(*c*) Neuro-keratin. From the supporting framework (neuroglia).

(*d*) Fats: including phosphorised fats (protagon and lecithin).

(*e*) Cerebrins or cerebrosides. Nitrogenous glucosides.

(*f*) Cholesterin.

(*g*) Extractives. Small quantities of numerous organic substances, of which creatine, xanthine, hypoxanthine, inosite, lactic acid, uric acid, and urea have been identified.

(*h*) Gelatin. From the adherent connective tissue.

(*i*) Inorganic salts. Of these, alkaline phosphates and chlorides are the most abundant, but the total ash is only about 1 per cent.

The following table gives some typical quantitative analyses which have been made of the proportion in which the principal solids occur in different nervous structures :—

TABLE IV.

Portion of nervous system	Proteids	Lecithin	Cholesterin and fat	Cerebrin	Neuro-keratin	Other organic matters	Salts
Grey matter of ox brain (Petrowsky)	55·37	17·24	18·68	0·53	6·71		1·45
White matter of ox brain (*ibid.*)	24·72	9·90	51·91	9·55	3·34		0·57
Spinal cord (Moleschott)	23·8	75·1					1·1
Human sciatic nerve (Josephine Chevalier)..	36·8	32·57	12·22	11·30	3·07	4·0	—

Some of the substances just mentioned require fuller notice, and in particular we must consider the proteids, protagon and lecithin, and the cerebrosides.

Proteids of Nervous Tissues.

The large amount of proteid matter, next to the high percentage of water, is the most striking fact in the preceding table.

The highest percentage is found where one would expect it, namely, in the grey matter, where the protoplasmic structures, the nerve-cells, occur.

The following table is a compilation from my own analyses :—

TABLE V.

	Water	Solids	Percentage of proteid in solids
Grey matter of cerebrum ..	83·467	16·533	51
White ,, ,, ..	69·912	30·088	33
Cerebellum	79·809	20·191	42
Spinal cord as a whole ..	71·641	28·359	31
Cervical cord	72·529	27·471	31
Dorsal cord	69·755	30·245	28
Lumbar cord	72·639	27·361	33
Sciatic nerves	65·349	38·684	29

This table illustrates the fact that the amount of grey matter, of water, and the percentage of proteid in the solids vary directly the one with the others. This is very well seen in the different regions of the spinal cord. The percentage of proteid matter in the white matter of the brain is a little higher than in the spinal cord. This is the only exception to the general rule, and perhaps may be explained by the high percentage of neuro-keratin in white matter.[6]

The earliest to study the nature of the proteids was Petrowsky.[7] He investigated the question previous to our modern ideas concerning proteids, and described them as consisting of a globulin somewhat resembling myosin, and an albumin especially abundant in grey matter. Baumstark[8] in a more recent research speaks of the chief proteid matter in nervous tissue as resembling casein. There is a certain amount of truth in this, for it is a nucleo-proteid. A few years later I took up the matter, and the following were my chief conclusions.[9]

The proteids present are three in number. They differ in temperature of heat coagulation, in the readiness with which they are precipitated by neutral salts, and by acetic acid; one of them

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[6] The percentage of neuro-keratin is in grey matter, 0·3; in white matter, 2·2 to 2·9; and in nerve, 0·3 to 0·6 per cent. (Kühne and Chittenden, *Zeitsch. f. Biol.*, xxvi., p. 291).

[7] *Pflüger's Archiv.*, vii., p. 367.

[8] *Zeitsch. f. physiol. Chem.*, ix., p. 145.

[9] *Journ. of Physiology*, xv., p. 106.

contains phosphorus and is a nucleo-proteid, so differing from the other two, which are globulins. The most important characters of these proteids are the following :—

(*a*) This proteid is a globulin ; it may conveniently be termed neuro-globulin *a*. It is coagulated by heat at the low temperature of 47°, and is analogous to similar globulins which are found in all cellular structures, such as cell-globulin of lymph-cells, paramyosinogen or musculin of muscle, hepato-globulin *a* of the liver, and kidney globulin. Indeed, this proteid seems to be as constant a constituent of protoplasmic structures as the nucleo-proteids are.

It is precipitated by a comparatively small percentage of such neutral salts as magnesium sulphate. It is not precipitated by weak acetic acid. It contains no phosphorus in its molecule.

In view of the subject of hyperpyrexia, a pathological problem we shall be considering later, I would ask you to make a mental note of the low coagulation temperature of this proteid.

(*b*) This proteid is a nucleo-proteid. It can be readily prepared from nervous tissues by making a saline extract, but under these circumstances it is mixed with the other proteids. It may, however, be prepared in large quantities by precipitating an aqueous extract of brain by weak acetic acid. The supply obtainable from white matter is small.

It is coagulated by heat at 56 to 60°. Like globulins it is precipitable by saturating its solutions with neutral salts ; but more salt is necessary than in the case of neuro-globulin *a*.

It contains 0·5 per cent. of phosphorus.

After subjection to gastric digestion, an unsoluble residue of nuclein remains behind.

Dissolved in dilute sodium carbonate and injected into the vascular system of rabbits it causes, like other nucleo-proteids, extensive intravascular coagulation.

(*c*) This proteid is a globulin. It may conveniently be called neuro-globulin *β*, and is closely analogous to the hepato-globulin *β* of liver cells. It is coagulated by heat at 70 to 75° C. ; it is precipitable by neutral salts, but requires complete saturation with magnesium sulphate to precipitate it entirely. It is not precipitable by weak acetic acid like the nucleo-proteid just described, and contains no phosphorus in its molecule.

The only other research with which I am acquainted on the

proteids of nervous tissues is one by P. A. Levene.[10] He has particularly directed his attention to the nucleo-proteid of the brain, and although he separated it out from the organ by a method different from that which I employed, he was evidently dealing with the same substance; the amount of phosphorus being 0·5 per cent., which is the same number I obtained. The purine bases obtainable from cerebro-nucleo-proteid are guanine, adenine, small quantities of xanthine, but no hypoxanthine.

Protagon and Lecithin.

In the year 1865 Liebreich[11] separated from the brain a material he termed *protagon;* he further found that when decomposed by baryta-water it yields two acids—stearic acid and glycero-phosphoric acid—and a base called choline.

Hoppe-Seyler, and Diaconow[12] working under Hoppe-Seyler's direction, denied the existence of this substance protagon, and considered that it was a mere mechanical mixture of the phosphorised fat called *lecithin*, with a nitrogenous non-phosphorised substance called *cerebrin*. Lecithin yields the same three decomposition products that were obtained from protagon by Liebreich. Diaconow's elementary analyses were, however, far from convincing.

The subject in this country was taken up by Gamgee and Blankenhorn[13]; and the result of their work has been that Liebreich's discovery has been fully verified. They showed that protagon is a perfectly definite crystalline substance of constant elementary composition. They also showed that even prolonged treatment with alcohol and ether will not extract lecithin from protagon, as alleged by Diaconow. When protagon is digested with alkalis it yields cerebrin or cerebrins and the decomposition products of lecithin.

This work has been confirmed by Baumstark,[14] Ruppel,[15] and Kossel and Freytag.[16]

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[10] *Archives of Neurology and Psychopathology*, v. ii., p. 1-14, 1899.
[11] Liebreich, *Annalen der Chem. u. Pharm.*, cxxxiv., p. 29.
[12] Diaconow, *Centralbl. f. d. Med. Wissensch.*, 1868, p. 97.
[13] Gamgee and Blankenhorn, *Journ. of Physiology*, ii., p. 113.
[14] Baumstark, *Zeitch. f. physiol. Chem.*, ix., p. 329.
[15] *Zeitsch. f. Biol.*, xxxi., p. 86.
[16] *Zeitsch. f. physiol. Chem.*, xvii., p. 431.

Protagon is prepared by digesting brain with alcohol at 45° C., the extract is filtered while warm, and then cooled to 0° C. Protagon crystals mixed with cholesterin are thus deposited. The cholesterin is dissolved out by ether. The protagon is then collected, redissolved in warm alcohol, and allowed to recrystallise on cooling. Its formula is $C_{160}H_{308}N_5PO_{35}$.

An elaborate research by Thudichum[17] led him to the conclusion that there are three groups of phosphorised substances in the brain, which he termed kephalins (very soluble in ether), myelins (far less soluble in ether), and lecithins (characterised by their extreme instability). In each of these ill-defined groups there are several members, the empirical formulæ of which have been calculated.

Thudichum's work has, however, been so far confirmed by that of Kossel, in that he has shown that protagon is not a single substance, but that there is more than one protagon. They yield either one, two or three derivatives of the cerebrin group, and lecithin in addition. There are also several lecithins containing different fatty acid radicles.

The *cerebrins* are nitrogenous substances which are found in large quantities in the white substance (especially in the medullary sheaths), and also in egg-yolk, pus corpuscles, spleen cells, &c. Many members of the group have been separated and analysed since they were first studied by Müller. These have received various names (phrenosin, kerasin, pseudo-cerebrin, &c.), but at present the subject is one which has not advanced into the area of practical medicine. Suffice it to say that their other name of cerebroside indicates that they are glucosides, and that the sugar obtained from them has been identified as galactose almost simultaneously in this country and in Germany.[18]

Though these substances possess an attractiveness due to the fact that they are still on the borders of an unknown country, it is refreshing to turn next to the other material derived from protagon, namely, lecithin, for here we have to deal with a substance of which the composition is known, and the decomposition products of which possess a distinct physiological and pathological importance.

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[17] Thudichum, *Report of Med. Officer of Privy Council*, 1874, p. 113 *et seq.*

[18] Brown and Morris, *Proc. Chem. Soc.*, London, 1889, p. 167 ; Thierfelder, *Zeitsch. f. physiol. Chem.*, xiv., p. 209.

It is a complex fat of wide distribution, being a constituent of protoplasm. The tables of analyses already given show its quantitative importance in nervous structures. When it is decomposed, either in the laboratory or in the body, it breaks up into three substances, as shown in the following equation :—

$$\underset{\text{[lecithin]}}{C_{44}H_{90}NPO_9} + 3H_2O = \underset{\text{[stearic acid]}}{2C_{18}H_{36}O_2} + \underset{\text{[glycero-phosphoric acid]}}{C_3H_9PO_6} + \underset{\text{[choline]}}{C_5H_{15}NO_2}$$

There are other lecithins present in smaller quantities which yield other fatty acids on decomposition. To give the foregoing lecithin its chemical name we must call it choline-distearyl-glycero-phosphoric acid. The choline radicle is united to the acid by means of the oxygen of the hydroxyl, it is therefore not a salt but an ether-like combination, thus :—

$$\begin{array}{l} CH_2O - C_{17}H_{35}CO \\ CHO - C_{17}H_{35}CO \\ CH_2O - PO - O.C_2H_4 \\ \qquad\quad | \qquad\quad (CH_3)_3 \\ \qquad\quad OH \qquad HO \end{array} \Big\} N.$$

The same facts can be put more simply by comparing an ordinary fat with lecithin. An ordinary neutral fat such as those which are found in adipose tissue or milk contains only three elements, carbon, oxygen and hydrogen. Lecithin contains the same three elements with nitrogen and phosphorus in addition. An ordinary neutral fat on decomposition links to itself the elements of water, and then splits up (is hydrolysed) into glycerin and a fatty acid ; thus stearin yields stearic acid and glycerin ; palmitin, palmitic acid and glycerin ; and olein, oleic acid and glycerin.

Fat + water

Fatty acid Glycerin.

Lecithin yields not only a fatty acid and glycerin, but in addition to these substances it gives rise to phosphoric acid, which contains all the phosphorus of the lecithin, and choline, an alkaloid which contains all the nitrogen of the lecithin.

Lecithin + water

Glycerin Fatty acid Phosphoric acid Choline.

In this connection choline is of considerable importance, because it can be readily detected, and the presence of choline may be usually taken as evidence that lecithin, and so nervous material generally, has undergone decomposition. Choline is an ammonium base, which has the following constitution.

$$N\begin{cases}(CH_3)_3\\CH_2-CH_2OH\\OH\end{cases}$$

Its name was originally given to it because it was first separated out from the lecithin of the bile; but its chemical name is trimethyl-oxyethyl-ammonium hydroxide. It was at one time thought to be identical with the base neurine which Liebreich separated from nervous tissues, and the two are closely related, choline readily becoming converted into neurine under the influence of certain bacterial agencies. Neurine has also been obtained by Brieger from the putrefactive decomposition of flesh, Neurine only differs from choline by two atoms of hydrogen and one of oxygen; and its structure—

$$HO.N.(CH_3)_3C_2H_3$$

justifies its chemical name, which is trimethyl-vinyl-ammonium hydroxide. These two alkaloids are also closely related to two others, namely, betaine, the non-toxic alkaloid of the common beet, and muscarine, the highly poisonous alkaloid of the toad-stool, *Agaricus muscarius*.

It will be beside my present purpose if I pursue this aspect of the subject more fully; the list of chemical substances I have given is a long one, and no doubt there is considerable theoretical interest attaching to many of them. I have purposely selected the proteids and the phosphorised fat for fuller consideration because they are the most abundant, and, so far as our present knowledge goes, the most important substances which are present.

The Cerebrospinal Fluid.

Any account of the chemical structure of the brain would be incomplete without some reference to the cerebrospinal fluid, and I propose to conclude my first lecture by a brief description of this remarkable fluid.

Most of our knowledge of the cerebrospinal fluid has been derived from an examination of the contents of meningoceles, and

in cases of hydrocephalus. The fluid removed by the first tapping at all events may be regarded as fairly normal. A few years ago, however, I had the opportunity of examining the fluid from a remarkable case which was under the care of Dr. St. Clair Thomson. The patient was a young woman who had for years suffered from continuous dripping from the nose; this was not amenable to any treatment. At first it was thought to be a case of nasal hydrorrhœa, but certain characters in the affection convinced the observer that this could not be so, and that the fluid, which dropped from one nostril only, was cerebrospinal fluid. This was supported by the results of the chemical examination of the fluid.

The escape of cerebrospinal fluid from the nose has long been known to follow traumatic injury to the cribriform plate of the ethmoid bone, but the possibility of its spontaneous escape from the nose does not appear to have been fully established before the present instance. However, considerable research into the literature of the subject has shown that there are several cases recorded in which, though no history of injury existed, the flow of fluid from the nose was of such a character that they must have been similar to the present case, although in the majority of instances the true nature of the fluid escaped observation. Many of these patients exhibited cerebral symptoms in the course of the disease, and some ultimately died from inflammation of the cerebral meninges, which had probably spread from the nose through some opening in the bony lamina that normally separates the cranial and nasal cavities.[19]

The first enquiries we instituted in Dr. Thomson's case related to the quantity of fluid formed. One portion, collected by the patient herself in the course of an hour, measured 4 cc. Another portion, collected under the supervision of Dr. Thomson in ten minutes, measured 3·9 cc.

If the first portion is taken as a measure of the rate of secretion, the amount formed in the day will be 96 cc. Taking, however, the second observation as being more accurate, the

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[19] "Observations on the Cerebrospinal Fluid in the Human Subject," by St. Clair Thomson, M.D., Leonard Hill, M.B., F.R.S., and W. D. Halliburton, M.D., F.R.S., *Proc. Royal Society*, vol. lxiv., p. 343.

A full account of the case is given in Dr. Thomson's book, "The Cerebrospinal Fluid." Cassell and Co., 1899.

amount formed in the twenty-four hours will be over half a litre (561·6 cc.). It is possible that this estimate is too high, as doubtless the patient, being under the observation of a physician, would be somewhat excited, and the consequent alteration of the circulation would, as we shall immediately see, cause the flow to become more abundant.

In a monograph on the cerebral circulation[20] Leonard Hill put forward the view that the rate of secretion of the cerebrospinal fluid, when the cranio-vertebral cavity is opened, depends directly on the difference between the pressure in the cerebral capillaries and that of the atmosphere. At the same time it was shown that cerebral capillary pressure varies directly and absolutely with vena cava pressure. Thus the cerebral capillary pressure can be raised with great ease by any agency which causes a rise of pressure in the vena cava or cerebral veins. On the other hand, cerebral capillary pressure varies directly, but only proportionately, with aortic pressure, for between the aorta and the capillaries there lies the peripheral resistance.

It follows from the above that the easiest methods of raising the cerebral capillary pressure in man are: (*a*) By compression of the abdomen. (*b*) By the assumption of the horizontal posture. In this position, however, the rise of venous pressure may be compensated by the fall of arterial pressure, which normally occurs when the body is at rest. This is, no doubt, the case during sleep. (*c*) By straining or forced expiratory effort, with the glottis closed.

By all these methods the vena cava pressure is considerably raised; and by the last method the venous inlets into the thorax may be completely blocked, and the pressure in the cerebral capillaries raised to something like aortic pressure.

It is true that by such a forced expiratory effort the aortic pressure is lowered. Nevertheless, the total effect on capillary pressure is a very great rise, for a fall of aortic pressure of 25 mm. of mercury produces a fall in cerebral capillary pressure of less than 5 mm. of mercury, while a rise of vena cava pressure of 25 mm. of mercury produces a rise of cerebral capillary pressure of 25 mm. Hg.

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[20] "The Physiology and Pathology of the Cerebral Circulation," by Leonard Hill. London Messrs. Churchill, 1896.

Dr. Thomson's case gave us a unique opportunity of testing the correctness of these views on the living human subject, and our experiments entirely confirm them. As will be seen from the following figures, the flow of cerebrospinal fluid is accelerated by all those circumstances which raise the cerebral capillary pressure. The increase in flow is, moreover, accompanied by a decrease in the percentage of solid matter.

The fluid passed while the patient was making forced expiratory efforts was nearly double in quantity that which flowed while she was sitting quietly. Abdominal compression also raised the rate of flow, by increasing the vena cava pressure and so leading to increase of the cerebral capillary pressure. In all cases increase of volume is accompanied with fall in the percentage of solids in the fluid.

The following table illustrates these points:—

TABLE VI.

Condition of patient	Amount of fluid collected in ten minutes	Percentage of solids in the fluid
1. Sitting quietly	2·378 cc.	1·1
2. During straining	3·912 cc.	0·43
1. Sitting quietly	2·188 cc.	1·14
2. Abdomen compressed	3·009 cc.	0·68
1. Sitting upright	1·670 cc.	1·11
2. Lying down	3·245 cc.	1·03

Cavazzani,[21] from experiments on dogs, found that the cerebrospinal fluid collected in the morning was more alkaline than in the evening, and contained more solid residue. He considers that this is related to the activity of the nervous system, and that it confirms Obersteiner's theory of sleep. He obtained corresponding results in the case of a man with traumatic fistula of the frontal bone.

We considered it worth while to repeat this observation.

The qualitative examination of the fluid collected first thing on several mornings gave the same results as that of specimens collected the last thing in the evening. Both were distinctly alkaline, but no estimation of the relative alkalinity was made. The following table gives in percentages the results of the quantitative analyses:—

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[21] "Sul Liquido Cerebrospinale," *La Riforma Medica*, Anno VIII., 1892, vol. ii., p. 591.

	Morning fluid	Evening fluid
Water	99·004	99·027
Solids	0·996	0·973
Organic solids	0·118	0 100
Inorganic solids	0·878	0·873

The evening fluid is thus slightly poorer in both classes of constituents than that of the morning; the difference is chiefly due to an alteration in the organic solids. This is just what we should expect, as the decreased capillary pressure during sleep would lessen the rate of exudation of water. Without committing ourselves to any theory on nervous activity or sleep, we may say that our experiments confirm those of Cavazzani.

All our experiments, therefore, show the close correspondence between the amount of the fluid and the height of cerebral capillary pressure. But in spite of this, cerebrospinal fluid is not a simple pressure exudation from the blood. The idea that it is a secretion was first propounded by Carl Schmidt many years ago, long before the birth of Heidenhain's theory that all lymph must be regarded as a secretion, in the formation of which the endothelial cells of the capillaries play a selective action.

Schmidt propounded his doctrine on the strength of his analyses of the saline constituents of the fluid; he stated that potassium salts are more abundant than those of sodium. But this has not been confirmed by subsequent investigators. Thus Yvon[22] gives the following numbers, NaCl 7·098, and KCl 0 033 per 1,000. F. Müller[23] gives the relationship of NaCl to KCl as 21·5 : 1. My own figures[24] in cases of meningocele show in 100 parts of chlorides that 95·15 consist of sodium chloride, and 4·85 of potassium chloride.

The amount and proportions of the salts are thus about the same as in blood, lymph, and transudations generally.

But examination of the organic solids shows Schmidt's contention that cerebrospinal fluid is a secretion to be correct, though the grounds on which he supported his idea are incorrect.

The fluid stands apart from all other similar fluids in the following particulars :—

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[22] *Journ. de Pharm. et de Chemie*, fourth series, xxvi., p. 240, 1877.

[23] *Mittheil. a. d. Würzburger med. Klinik*, i., p. 267.

[24] *Journ. of Physiology*, x., p. 232.

(1) Its clear watery character.

(2) Its low specific gravity, 1,004 to 1,007.

(3) It only contains a trace of proteid; the characters of this are those of globulin, whilst in some cases a small admixture of proteose is present. Fibrinogen and albumin are absent.

(4) The presence in it of a substance which reduces Fehling's solution, but does not ferment with yeast, nor does it give any osazone crystals. The substance is therefore not sugar.[25] It is possibly an aromatic substance, allied to pyrocatechin, but this requires renewed investigation.

The following analyses (in parts per 1,000) of the fluid from spina bifida cases may be next given[26]:—

TABLE VII.

In parts per 1,000	Case 1	Case 2	Case 3
Water	989·75 ..	989·877 ..	991·658
Solids	10·25 ..	10·123 ..	8·342
Proteids	0·842 ..	1·602 ..	0·199
Extractives } ..	9·626 ..	0·631 ..	3·028
Salts }		7·890 ..	5·115

The percentage of organic solids is thus as a rule a little higher than in the absolutely normal fluid. In cases of hydrocephalus the percentage of solids is rather greater (see next table).

TABLE VIII.

In parts per 1000	Case 1	Case 2	Case 3
Water..	986·78 ..	984·59 ..	980·77
Solids	13·22 ..	15·41 ..	19·23
Proteids and extractives ..	3·74 ..	6·49 ..	11·35
Salts	9·48 ..	8·92 ..	7·88

In cases of chronic hydrocephalus, the fluid removed by the first tapping has the normal qualitative characteristics of cerebro-spinal fluid; but that removed by subsequent tappings resembles a dilute transudation from the blood, and if inflammation supervenes this becomes more marked; the proteids become more abundant, and resemble those found in blood and lymph; the

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[25] Nawratski, in a recent paper (*Zeitsch. f. physiol. Chem.*, xxiii., p. 523. 1897), has affirmed, principally from observations on the cerebrospinal fluid of the calf, that the reducing substance is dextrose. This is directly contrary to the observations of all other writers on the subject, and to my own experiments.

[26] W. D. Halliburton, "Report of Spina Bifida Committee," vol. xviii. of *Clin. Soc. Transactions*.

amount of reducing substance increases, and is here probably mixed with sugar. This is illustrated in the following table :—

TABLE IX.

CASE OF CHRONIC HYDROCEPHALUS.

	Specific gravity	Percentage of proteids	Reducing substance
First tapping ..	1006 ..	0·045 ..	Traces
Second tapping ..	1010 ..	0·069 ..	Fairly abundant
Third tapping ..	1010 ..	0·272 ..	More abundant

Returning now for a moment to normal cerebrospinal fluid, we find that its osmotic relationships are different from those of ordinary lymph.

Thus Zanier[27] finds in the ox that the fluid is hypertonic compared to the serum of the same animal. Widal, Sicard, and Ravant[28] arrived at the same result by the cryoscopic method. This character separates it from other serous fluids, and various drugs pass from the cerebrospinal fluid into the blood.

M. Lewandowsky[29] has performed somewhat similar experiments ; he regards the fluid as a specific product of the brain, and only to a small extent as a simple transudation from the blood.

The experiments of F. Ransom[30] with tetanus toxin and antitoxin show that these organic materials will pass from the cerebrospinal fluid to the blood, though they pass in the opposite direction from the blood to the lymph.

It is an interesting question whether the fluid has the same composition in all parts, for the fluid has a double origin. It is found in the lymph channels and spaces of the brain and cord tissue, and the perivascular lymphatics have been shown to open into the subarachnoid space. In the second place, it is found in the cerebrospinal cavity (ventricles of brain and central canal of cord), and it can hardly be doubted that it is here formed largely by the secretory epithelial cells which cover the choroid plexuses.[31] We can only surmise that this double method of formation may

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[27] *Centralbl. f. Physiol.*, x., 1896.

[28] *La Presse medicale*, October 24, 1900, p. 128.

[29] *Zeit. klin. Med.*, xl., p. 480.

[30] *Zeitsch. f. physiol. Chem.*, 1900, xxxi.

[31] *See* article on Meningitis, by Dr. Lees and Sir T. Barlow, in Allbutt's "System of Medicine."

imply a difference in the composition of the fluid formed. The fluid as usually examined must be a mixture of the two, and I cannot see that we have at present any certain method of collecting the two fluids separately.

I will conclude with one more question, and that is, whether choline occurs in normal cerebrospinal fluid. The importance of this question arises from the fact that choline is a substance which lends itself readily to detection, and its presence is a valuable indication of a break-down of nervous tissue. In a fluid which plays the part of a lymph, we naturally look for the products of disintegration of any tissue. Mott and I have shown that in diseased conditions in which the katabolic side of nervous action is preponderant, choline is found in great abundance. This is a point I shall have to dwell upon more fully later. For the present it is sufficient to say that in the normal fluid so little is present that it may be regarded as absent for all practical purposes. Still it is present. This Gumprecht[32] has shown to be the case; he worked with larger quantities of fluid than were used by Mott and myself. Although the quantity in the normal fluid is so small, it is not without interest, for it furnishes evidence that in the metabolism of the nervous tissues lecithin as well as proteid is in a condition of unstable chemical equilibrium.

[32] *Verhandl. des Congr. f. innere Med.*, Wiesbaden, 1900, p.p. 326-348.

LECTURE II.

Metabolism in Nervous Tissues.

In my first lecture I dwelt upon the general composition of nervous structures, and in addition to giving you a long list of the chemical substances found there, with tables of quantitative analyses, devoted some time to a description of the proteids, and phosphorised constituents of nervous material.

But living material is never at one moment the same chemically as it was the moment before, or will be the moment after. This condition of unstable chemical equilibrium, indicative of continual and continuous intra-molecular re-arrangements, is summed up by the convenient word metabolism; the assimilative changes which lead to the building up of protoplasm and the repair of waste is generally termed anabolism, while the contrary phase, the disintegrative, which is the result of the wear and tear associated with activity, is called katabolism.

I propose to ask you to-day to follow me in the enquiry as to the evidence we possess of metabolic activity in nervous tissues. This will involve the discussion of such questions as fatigue and sleep. I am afraid we shall meet with considerable disappointment in the answers we shall be able to furnish to our enquiries, for it is in this part of our subject that our knowledge is scantiest.

To ascertain the chemical composition of the brain when it is dead is a task of some difficulty, but it is easy when compared with the endeavour to determine what chemical changes it undergoes while it is alive.

We are only on the threshold of our chemical enquiries; still the time cannot be far distant when we shall have crossed it and opened the door to more certain knowledge. We shall find here that the experiments made for us by nature, which we call

diseases, will come to our aid, for in pathological conditions we have not the nicely balanced equilibrium between anabolism and katabolism which characterises the physiological state, but as a rule the katabolic side predominates and so we are enabled to grasp it.

Very often for the purposes of teaching and illustration we compare the nervous system to a telegraphic system penetrating to every part of a country and serving for the regulation and ordering of the various occurrences which take place there. Messages fly to and from distant parts, and are received, co-ordinated or started at central offices, which we may compare to the groups of nerve-cells we call nerve-centres. In such a telegraphic system, the most active parts are the offices; it is there we look for evidence of action in the shape of fatigue in the operators, or wear and tear of instruments. The wires are comparatively speaking passive transmitters; they undergo but little change and do not manifest signs of fatigue.

So it is in the nervous system; the signs of action are to be found in the beginnings and endings of the nerve-fibres, the cells of brain and cord, and the end organs in muscle and other peripheral structures. Any evidence of fatigue in the more passive transmitters, the nerve-fibres, is very difficult to discover. This coincides with the arrangements of the vascular supply of such parts. The nerve-centres are richly supplied with blood-vessels, which furnish them with an abundant supply of nutrient material. Cerebral anæmia[1] rapidly produces pathological changes in the nerve-cells, and death quickly supervenes. But in a nerve the blood-vessels are comparatively insignificant, and a nerve can be removed from the body, and be made to manifest activity for many hours subsequently.

The necessity for oxygen, and the fact that it is used up during the activity of the brain, can be very strikingly demonstrated by the experiment which Mr. Leonard Hill has performed with the help of methylene blue. Ehrlich was the first to show that if a solution of this pigment is injected into the blood stream of an animal the blood is rendered blue, but the organs, especially those which, like glandular organs, are in a state of activity, are

[1] For recent work on this subject see Leonard Hill, F.R.S., *Phil. Transactions of the Royal Society*, 1900, vol. B., 189, p. 69.

colourless. On exposure to oxygen after the organs are removed from the body, they also become blue. The meaning of this is the seat of oxidation is in the tissues and not in the blood. Though methylene blue holds its oxygen more firmly than oxyhæmoglobin does, the tissues are nevertheless able to take oxygen from it and form a colourless reduction product; but after the tissues are removed from the body and consequently are losing this vital avidity for oxygen, they become blue once more on exposure to the atmosphere.

Now, in an anæsthetised animal the brain is inactive, and the brain, like the blood, has a blue tint. If, however, a spot of the cerebral surface is stimulated that part of the brain is thrown into action, oxygen is used up, and the methylene blue is reduced, and in consequence that area of the brain loses its blue colour. If the animal is so deeply narcotised that the brain does not discharge an impulse, the part stimulated remains blue.

In any plan of research on changes in nerve we must be largely guided by what is already known of the tissue to which it is so closely related, namely, muscle.

When a muscle is active, the changes it undergoes are numerous and easy to detect. The naked eye can see its shortening; the microscope reveals changes in its constituent sarcous elements. The production of heat is so prominent that a temporary rise of temperature can be ascertained to occur even with such a rough instrument as a thermometer, though for the finer changes in the temperature of small muscles a thermopile is necessary. Accompanying these manifestations of a transformation of energy, the galvanometer shows us a variation in its electrical condition; and the basis of all the other changes is the sudden and massive increase of its normal chemical tone. Oxygen is used up, carbonic acid is given off in large amount, and the reaction of the muscle on prolonged activity becomes acid; this acidity is in part due to the formation of acid phosphates, and in part to the production of sarco-lactic acid.

Turning to nervous tissues, what a contrast we have. When active, no change is visible to the highest powers of the microscope; the refractive index of the axis cylinder remains unaltered;[2] the most delicate thermopile fails to detect any rise of tempera-

[2] Grose, *Pflüger's Archiv.*, xlvi., p. 56.

ture, and the chemical changes which occur are proved to take place rather by circumstantial than by direct evidence. The only change in a nerve which can be detected by physical means is the electrical variation.

The chemical changes that occur on the death of a muscle are in part an exaggeration of those which take place when it is active during life. This is a guide to us when we seek to determine the corresponding facts in nerve. Rolleston[3] showed that in nerve there is on its death a rise of temperature. Now this can only be due to increased chemical action, and probably of the same kind as, though greater in degree than, that which occurs during life. Moreover nervous tissues become acid when they die.

But in order to systematise the description of these changes, it will be best to consider them under the following headings :—

(1) The reaction of nervous tissue.

(2) The hypothetical production of carbonic dioxide during the activity of nerve.

(3) Evidence of metabolic activity in nervous structures derived from the examination of cerebrospinal fluid, and saline extracts of nervous tissues.

(1) Reaction of Nervous Tissues.

Heidenhain[4] and Gscheidlen[5] both state that the normal reaction of the axis-cylinder is alkaline ; but on death or on long-continued activity the reaction becomes acid. They further state that the grey matter is acid even during life. O. Langendorff[6] found the reaction of the central nervous system alkaline during life ; the alkalinity rapidly diminishes after death, or on stoppage of the circulation. S. Moleschott and Battistini[7] found both central and peripheral portions of the nervous system acid, especially the grey matter ; this was increased by activity.

I am convinced that these conflicting statements are in part due to the fact that the nervous structures in question were not

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[3] *Journal of Physiology*, xi., p. 208.

[4] *Centralbl. f. d. med. Wiss.*, 1868, p. 833.

[5] *Pflüger's Archiv.*, viii., p. 171.

[6] *Neurol. Centralbl.*, 1885, No. 14. *Centralbl. f. d. med. Wiss.*, 1886, No. 25.

[7] *Arch. ital. de biol.*, viii., p. 90. *Chem. Centr.-Bl.*, 1887, p. 1224.

always examined in the perfectly fresh condition, and they may also be partly explained by the use of different indicators of acidity by the various observers.

In my own work, I have found in animals that the fresh tissues are invariably alkaline, but on exposure they become rapidly acid, especially the grey matter. In the human brains I received from the *post-mortem* room the reaction of the grey matter was always, and of the white matter often, acid to litmus. This I attribute to changes after death, for at least twenty-four hours had always elapsed since death. The acidity is due to lactic acid; but according to Müller and Gscheidlen it is not sarco-lactic acid but the fermentation lactic acid. Müller also obtained traces of formic acid.

In order to test the question of whether acidity develops on activity, Dr. Brodie and I have been recently investigating what occurs in a non-medullated nerve. This appeared to us the best means of attacking the problem, for the possibly masking effect of a large mass of myelin would then be absent. The splenic nerves of the dog, which are large and easily dissected out, were used, but we found that after faradisation for six hours the reaction never became acid.

(2) The Hypothetical Production of Carbon Dioxide during the Activity of Nerve.

This is an interesting branch of the subject, which we owe to Dr. Waller. Dr. Waller uses as his object of attack isolated nerves, usually the sciatic nerves of frogs. He stimulates them in the usual way by induction shocks, and he takes their electrical response as his guide to their activity. He has in this way studied the influence of numerous reagents and drugs upon nerve, and the presence and extent, or the absence of the galvanometric answer show whether any particular reagent increases, diminishes, or annuls nervous action. Among the reagents which he thus investigated carbonic acid is one, and his results with this gas are most instructive. Large doses of carbonic acid act like an anæsthetic, and completely abolish the electrical response, but the nerve soon recovers when the poisonous gas is replaced by air. Very small doses of carbonic acid increase its activity, and the swing of the galvanometer

needle is increased when the nerve is thrown into action. A nerve thus forms a very delicate test object for this gas, far more delicate, in fact, than most chemical reactions are. When a nerve is excited to activity the electrical responses improve, just as when a muscle is made to undergo a succession of contractions the beneficial effect of contraction manifests itself by what is technically called the "staircase phenomenon." This beneficial effect of previous action is exactly similar to what is produced by minute doses of carbonic acid gas, and Dr. Waller argues from his experiments that they prove what cannot be directly tested by the rougher methods of chemical analysis, namely, that activity is associated with the discharge of carbon dioxide. I shall have to return to this question in our subsequent discussion of fatigue.

(3) Evidence of Metabolic Activity in Nervous Structures derived from the Examination of Cerebrospinal Fluid and of Saline Extracts of Nervous Tissues.

We are so accustomed to associate the word metabolism with the activity of the proteid constituents of protoplasm, that we are sometimes apt to forget that other materials frequently exhibit a similar alternate or simultaneous series of anabolic and katabolic phases. In nervous structures this is particularly true for the complex phosphorised molecules of protagon. Gumprecht has shown that perfectly normal cerebrospinal fluid contains minimal traces of choline, a substance derived from the decomposition of lecithin, the main constituent of protagon. This trace of choline represents the small temporary balance on the wrong side of the account. Even under normal physiological conditions, the products of katabolism can be detected; this becomes much more pronounced in diseased conditions. This point I am reserving for fuller study in my next lecture. Exactly similar evidence is obtained by making saline extracts of perfectly fresh nervous tissues. Violent reagents which break up the nervous tissues will naturally lead to the appearance of large quantities of choline in the extract mixed with numerous other substances. But physiological saline solution, the most harmless of all reagents, will even at room temperature extract choline

from perfectly fresh tissues in sufficient quantities to render its detection by both chemical and physiological tests an easy task (*see* figs. 12 and 13). It is mixed in these extracts with other materials, some of which are basic, but these have not been accurately identified. The presence of choline in such extracts indicates that in the tissues some of the protagon has undergone katabolic changes, and so we have a very positive sign of chemical activity in the living tissues. In addition to this fact there is a further one, namely that the most active part of the nervous system, the grey matter yields most choline to solvents.[8]

Fatigue.

Experimental methods have shown that in a muscle-nerve preparation, fatigue is due to the accumulation of the products of muscular activity, and that it may be artificially induced by irrigating such a preparation with a dilute solution of sarcolactic acid, and removed by neutralising this with salt solution containing a trace of alkali. It has further been shown that the muscular fibres are not to any great extent the seat of fatigue, and that the nerve-fibres are practically inexhaustible. By a process of exclusion the seat of exhaustion has thus been localised in the intramuscular nerve-endings. When a muscle is fatigued in the intact body, there is, however, another factor to be considered beyond the mere local poisoning of the end-plates. This is the effect of the products of muscular katabolism passing into the circulation and poisoning the central nervous system. Mosso, by the use of the instrument he calls the ergograph, has demonstrated that the fatigue products cause most of their injurious effects by acting on the central nervous system and diminishing its power of sending out impulses. Moreover, the introduction

[8] That this matter is attracting considerable attention just now is shown by the numerous researches published during the last year or two. In addition to my own paper on the "Physiological Effects of Extracts of Nervous Tissues," *Journ. of Physiology*, xxvi., p. 229, the following may be mentioned: Cleghorn, *American Journ. of Physiol.*, ii., p. 471; *Journ. of the Boston Society of Medical Sciences*, iv., p. 289, 1900; Osborne and Vincent, *Journ. of Physiology*, xxv., p. 283; Hunt, *Proc. Amer. Physiol. Soc.*, 1899; Gumprecht, *loc. cit.*; Gulewitsch, *Zeit. physiol. Chem.*, xxvii., p. 50. All these observers agree on the presence of choline, though some doubt whether it is the most important substance which goes into solution.

of the blood of a fatigued animal into the cerebral circulation of a normal one will give rise in the latter to all the symptoms of fatigue. The blood still remains alkaline; the toxic material cannot therefore be free lactic acid, and lactates do not produce the effect. Mosso has put forward the theory that the poisonous substance is basic in nature, but we have really no accurate knowledge of its chemical nature.

Among these facts, the most striking is the non-fatiguability of nerve-fibres. This does not mean that the nerve-fibres undergo no metabolic changes when transmitting a nerve impulse. It means that the change is so slight, and the possibilities of repair so great, that fatigue in the usual acceptation of the term cannot be demonstrated. This is an illustration of the wonderfully economic way in which Nature often works.

That a change does occur in a nerve-fibre is evidenced by the electrical variation it undergoes and which can be detected by the galvanometer or the electrometer. Further, we have already seen Dr. Waller believes he has shown that carbonic acid is evolved by the axis cylinder. How, then, can we account for the fact that fatigue cannot be shown to occur? To meet this difficulty Waller has tentatively suggested a most ingenious explanation, which it will be well to give in his own words.[9] He says: "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 offshoot 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. You have seen further that such nerve is inexhaustible, yet that it exhibits very clear symptoms of chemical change after action. 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."

[9] "Lectures on Physiology," First Series, "Animal Electricity," 1897, p. 70.

A year or two after these words were written Miss Sowton,[10] at Dr. Waller's suggestion, undertook a piece of work in order to test the truth of this hypothesis. If the absence of fatigue is due to the presence of the fatty sheath, fatigue ought to be demonstrable in nerve-fibres in which the fatty sheath is absent. She selected the olfactory nerve of the pike as the non-medullated nerve with which to try the experiment, and her results confirmed Dr. Waller's expectation; the galvanometric replies of the nerve become somewhat feebler after repeated stimulation.

It appeared to me advisable to test the question in another way. Some doubt has recently been cast on the trustworthiness of the electrical response as a sign of nervous activity.[11] I do not pretend to be able to pronounce an opinion on this subject, but as the doubt has arisen, the greater becomes the necessity for a fresh method of attacking the problem. The splenic nerves appeared to be the most convenient large bundles of non-medullated fibres for the purpose. Dr. T. G. Brodie has been associated with me in carrying out the investigation. A dog is anæsthetised with morphine and A.C.E. mixture, the abdomen opened, the spleen exposed, and the splenic nerves which lie by the side of the main splenic artery are laid bare. It is quite easy to dissect out a length of nerve sufficient for the experiment (1½ to 2 inches). The nerve is then cut as far from the spleen as possible, and the spleen is enclosed in an oncometer, similar to that employed by Schäfer in his work on the spleen.[12] On stimulating the nerve with a weak faradic current the organ contracts, and the recording lever falls. The diminution of the size of the spleen is quite visible to the naked eye, however, without the use of any apparatus. The next thing to do is to put a block on the course of the nerve, which will prevent the nerve impulses from reaching the spleen. Here we met with some difficulty. Curare and atropine are both ineffective; the constant current has a great disadvantage; non-medullated nerves are so much affected that very feeble constant currents (one-third of a Daniell cell) will completely block the transmission of impulses, and not only that,

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[10] *Proc. Royal Society*, vol. lxvi., p. 379, 1900.

[11] *See* Professor Gotch's article "Nerve" in Schäfer's Text-book of Physiology.

[12] Schäfer and Moore, *Journ. of Physiology*, xx., p. 1.

but the nerve remains blocked after the current is removed. After the current has been allowed to flow for two minutes the nerve remains impassable to nerve-impulses for an hour or more, and then slowly recovers. If, therefore, faradic excitation of the nerve is kept up all this time and fails to excite the contraction of the spleen after the removal of the constant current, it is impossible to say whether this is due to fatigue of the nerve-fibres on the proximal side of the block, or whether it may not be due to the fact that the block created by the constant current is still effective.

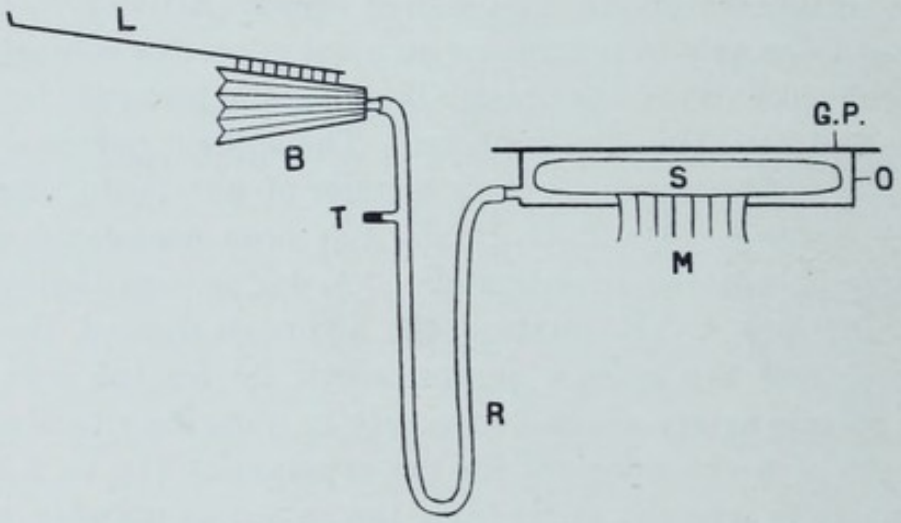

FIG. 1.—Apparatus for obtaining splenic curves. S, spleen in oncometer O, which is made of gutta percha, and covered with a glass plate (G.P.) luted on with vaseline. M is the splenic mesentery containing vessels and nerves; this passes through a slit in the base of the oncometer and is made air-tight with vaseline. The oncometer is connected to the flexible bellows (B) by the india-rubber tube (R), the side tube (T) being closed during an experiment by a piece of glass rod. The recording lever (L) writes on a revolving drum.

Our best results were obtained by using cold instead of a constant current as our blocking agent.

Fig. 1 is an outline drawing of the apparatus used.

Fig. 2 shows the arrangement adopted in connection with the nerve. The nerve (N) rests on a metal tube (T) through which water can be kept flowing. E is the situation of the electrodes. If the nerve is excited, the spleen contracts and the recording lever (in fig. 1) falls. If now water at 2 to 3° C. is kept flowing through T, the nerve-impulses are blocked by the cold, and cannot reach the spleen. Immediately the cold water is replaced

by warm water at 30° C., the nerve again becomes passable by nerve-impulses, and the spleen contracts once more.

If now the water in T is kept at the low temperature mentioned, and the nerve is being excited with strong induction shocks all the time, the spleen remains irresponsive; the nerve-impulses are able to reach T but not to pass it. If then warm water is passed through T, and the block produced by the cold is thus removed, and the spleen continues to be irresponsive, we have a proof that the piece of nerve between E and T has been fatigued. But our experiments have shown us that non-medullated nerve is just as difficult to fatigue as medullated nerve. Even after six hours' continuous excitation the nerve is just as excitable as it was at the start, and a full splenic contraction is obtained when the cold block is removed.

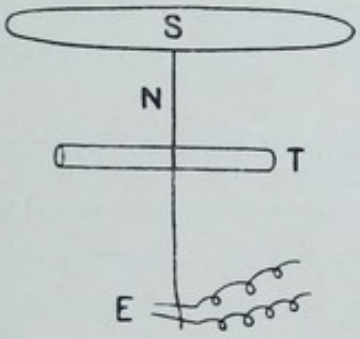

FIG. 2.—Arrangement of apparatus in connection with the splenic nerve. S is the spleen, and N the main bundle of nerves. The nerve rests on the metal tube (T) through which water at the required temperature is kept flowing, and on the electrodes (E) which come from the secondary coil of an inductorium.

We have made similar experiments with vaso-motor nerves, such as the cervical sympathetic nerve in the rabbit, and the sciatic nerve in a curarised dog, and have obtained corresponding results. This confirms the work previously published by Eve.[13] Eve excited the cervical sympathetic for twelve hours, and found no loss of excitability at the end of that time. Eve stimulated the nerve below the upper cervical ganglion, and the main object of his work was to ascertain whether any histological evidence of fatigue could be found in the cells of the ganglion. The only change he could find there

[13] *Journ. of Physiology*, xx., p. 334.

was a somewhat diffuse staining of the cells by methylene blue, which he attributes to the formation of acid substances in the cells. A blue stain of similar appearance may be induced in the motor cells of the spinal cord, after exhaustion is produced in them by giving strychnine. In such experiments the spinal cord becomes as a rule distinctly acid to litmus paper. Max Verworn[14] has more recently employed strychnine as a means of producing fatigue. He considers that the only specific effect of this alkaloid is increase of reflex activity, and he attributes the subsequent paralysis to vascular conditions and the accumulation of fatigue products, among which he places carbon dioxide in the first rank. Eve, on the contrary, did not find that carbonic acid alone produces the effects.

We must conclude from such experiments that Dr. Waller's theory is unproved, and that while fatigue is demonstrable in nerve-cells, it has never yet been shown to occur in nerve-fibres of either the medullated or non-medullated variety.[15]

Micro-chemical Methods.

I must now make an excursion into the histological side of the subject, but this is really a branch of chemistry also, for all the staining properties of tissues are micro-chemical reactions. Of the numerous methods recently invented for the study of the structure of nerve-cells, two stand out as pre-eminently useful. One of these is Golgi's silver-chrome method, and the other is Nissl's methylene blue process.

Golgi's method and its numerous modifications result in a black staining of the nerve-cells and their branches. The neuron theory, about which you heard so much from Dr. Mott[16] last year, rests mainly on preparations made by this method. The method, valuable as it is, is not, however, capable of demonstrating changes of a pathological kind in the cells. Cells which show even profound changes by other methods, may look quite normal when the silver-chrome process is employed.

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[14] *Arch. für (Anat. u.) Physiol.*, 1900, p. 152.

[15] A possible explanation of the effects described by Miss Sowton is given in a paper which Dr. Brodie and I are sending to the *Journal of Physiology*, in which the whole question is discussed more fully.

[16] "The Degeneration of the Neurone," Croonian Lectures, by Dr. Mott, 1900.

Nissl's method is far more delicate and is extremely simple. Let me very briefly describe it, and the principal results which have accrued from its use.

The Significance of Nissl's Granules.

If portions of the brain or spinal cord are fixed in absolute alcohol, and sections obtained from the hardened pieces are stained by means of methylene blue, the nerve-cells exhibit a characteristic appearance. The nucleus and nucleolus take up the blue stain, and throughout the cell body a number of angular-shaped masses, which are termed *Nissl's granules*, are also stained blue. These extend some distance into the dendrons, but not into the axon. The substance of which they are composed is termed *chromatoplasm*, or *chromophilic material*. The existence of granules in cells which have an affinity for basic dyes like methylene blue is not at all common; the granules in the majority of the white blood corpuscles, for instance, have an affinity for acid dyes. Micro-chemical methods have shown that the main constituent of the Nissl granules is nucleo proteid. The name *kineto-plasm* has been given to it by Marinesco in order to express the idea that it forms a source of energy to the cell. It can hardly be denied that the substance of which the granules are composed, forming as it does so large a proportion of the cell contents, and made of a material in which nuclein forms an important constituent, is intimately related to the nutritional condition of the neuron. Some have even compared it to the granular material which is present in secreting cells; in these cells before secretion occurs the granules accumulate, and during the act of secretion they are discharged and converted into constituents of the secretion. It is stated by some observers that the Nissl granules are used up during the discharge of energy from nerve-cells, and that this may be regarded as a visible sign of fatigue; it certainly is the case that if the cells are examined after an epileptic fit, in which there has been a very massive discharge of impulses, the Nissl granules have disappeared, or at least broken up into fine dust-like particles, so that the cell presents a more uniform blue staining. It is, however, doubtful whether this is due to a transformation associated with intense activity, or whether it may not be caused

by venosity of the blood. The cells are very sensitive to altered vascular conditions; anæmia, for instance, produces a similar change accompanied with swelling of the cell, and swelling and, in extreme cases, extrusion of the nucleus.

Since attention has been directed towards the Nissl granules, a literature which has become alarmingly vast during the last few years has sprung up in relation to them. This is quite easy to understand, for neurologists have by this sensitive test been able to identify changes in the cells which could not be detected by the previous methods of staining. Thus the cells have been examined in various diseases, and after being subjected to the action of various poisons. In a new subject of this kind there is, as would be expected, considerable divergence of views, and even the fundamental question has not yet been answered satisfactorily whether the Nissl granules are present as such in the living cell, or whether they are artifacts produced by the fixative action of strong alcohol. The fact that they cannot be demonstrated when the cells are stained by the injection of methylene blue into the circulation before the animal is killed is a very strong piece of evidence in favour of the latter view; though it may be urged against this, that methylene blue used in this way is a poison and so not calculated to give us normal results. But, whichever view is correct, the method is a valuable one, and Nissl's views on this question appear to be indisputable; they are briefly as follows: Healthy cells fixed and stained in a constant manner will appear the same under constant optical conditions, and the appearances then seen form the equivalent of such healthy cells during life. It follows that if cells prepared by the same method and examined under the same conditions show a difference from the equivalent or symbol of healthy cells, the difference is the measure of some change that has occurred during life.

Chromatolysis is the term applied to designate the disappearance or disintegration of the Nissl granules. The process generally begins at the periphery of the cell and in the dendrons, but in advanced cases the whole cell may be affected. We have already alluded to the fact that chromatolysis occurs in various abnormal states, and the diminution of the chromophilic nucleoproteid indicates a diminution of the vital interaction of the highly phosphorised nucleus with the surrounding cell protoplasm. Chromatolysis alone is not indicative of cell destruction,

and a cell may recover its function afterwards. The integrity of the nucleus and of the fibrils appears to be much more important to the actual vitality of the cell.

When a nerve-fibre is cut across the distal segment undergoes Wallerian degeneration; this is an acute change. But the nerve-cell and the piece of the nerve-fibre still attached to it do not remain unaffected. If regeneration of the fibre and restoration of function take place no change is observable. But before regeneration occurs (and in the central nervous system regeneration never occurs) the cell and its processes undergo a slow chronic wasting; one of the earliest signs of this *disuse atrophy* is chromatolysis. Warrington[17] has recently shown another interesting fact, namely, that section of the posterior roots of the spinal nerves causes chromatolysis in the anterior nerve-cells of the same side; this indicates that loss of sensory stimuli produces a depression of the activity and metabolic functions of the spinal motor cells. This accords quite well with the physiological effects observed under these conditions. The drawings in the accompanying plate (Plate I.) show the normal appearance of a nerve-cell when stained by methylene blue (A). B is a cell which shows chromatolysis as the result of status epilepticus, and C shows much the same condition as a result of cerebral anæmia. D illustrates the effect of prolonged narcosis, to be referred to later in this lecture (see p. 40).

Sleep and Narcosis.

We have now considered the question of fatigue in many of its aspects. Let us next turn our attention to a brief consideration of sleep, Nature's great restorer for exhausted "nerves." Theories to account for sleep have been numerous, and some of these are chemical. Thus certain observers have considered that sleep is the result of the action of chemical materials produced during waking hours, which have a soporific effect on the brain; according to this theory awakening from sleep is due to the action of certain other materials produced during rest, which have the opposite effect. Obersteiner has gone so far as to consider that the soporific substances are reducing in nature, and

[17] *Journ. of Physiology*, xxv., p. 462, 1900.

PLATE I.—NISSL'S GRANULES.

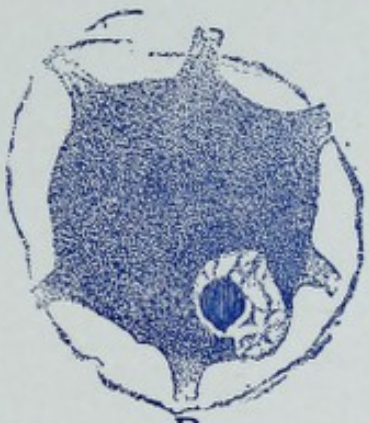
A. Normal pyramidal cell of human cerebral cortex.

B. Swollen œdematous pyramidal cell from a case of status epilepticus. Notice diffuse staining and absence of Nissl's granules; the nucleus is enlarged and eccentric. The lymph space around the cell is enlarged.

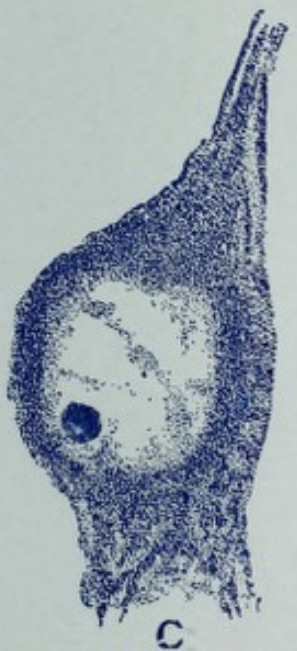
C. Pyramidal cell of dog after ligature of vessels going to the brain and consequent anæmia. Notice the great swelling of the nucleus and advanced chromatolysis, most marked at the periphery of the cell. Figs. A, B, and C are after Mott; magnification in each case 700 diameters.

D. Skeleton condition of cerebral cell in a rabbit produced by six hours' anæsthesia with ether. After HAMILTON WRIGHT.

A



B



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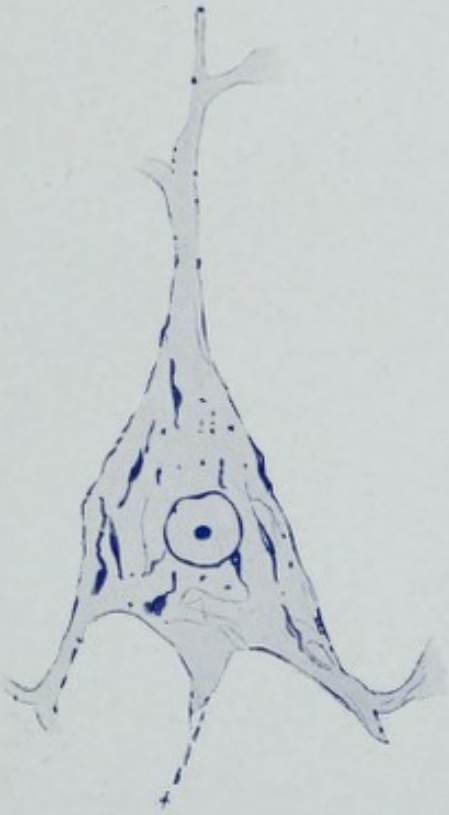

D

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others regard them as alkaloidal (leucomaines). These theories all rest on the flimsiest foundations, and none has yet been found to stand experimental tests.

The nerve-cell, like so many other cells of our bodies, exhibits a rhythmical tendency. In the heart the alternate periods of rest and action are both short; in many other involuntary muscles, such as those of the spleen and intestine, the periods are longer. If we place nerve-cells in the same category, the periods are variable in length, but as a rule are longer still. Some years ago Dr. Sydney Ringer showed that certain mixtures of inorganic salts are capable of maintaining the rhythmic activity of cardiac muscle, cilia and similar structures. Quite recently these experiments have been repeated and confirmed in two American laboratories, one under Professor Howell at Baltimore, the other under Professor Loeb at Chicago. Although all the essential facts of Dr. Ringer's work have been shown to be correct, they are now in the light of the modern theory of solutions interpreted differently. Loeb, in particular, has shown that ionic action is at the root of the matter. There are differences noted in different forms of contractile tissue, but in all there must be a nicely balanced admixture of the ions in order that rhythmic action may be maintained. Some ions (like OH) have little or no effect; others (like Na) stimulate; and others (like Ca) inhibit action. An optinum saline solution is one in which the stimulating ions are mixed in due proportion with those which restrain activity. The inorganic constituents of the body would perhaps have been the last, one would have anticipated to act in this way, but there are many unlikely things that happen during life. If we in the future find that nerve-cells are to be considered on all fours with contractile cells, it is possible that it will be found that the phenomenon of alternate periods of action and rest can be explained by the comparatively simple action of inorganic substances or of their constituent ions.

This, however, is after all not much more than a wild guess[18] and we must return to the safer regions of experiment. The introduction of the Golgi method opened a fresh field for investi-

[18] That such a view is not entirely outside the bounds of probability is shown by the fact that certain electrolytes, notably bromides of the halogens, possess the power of modifying the normal rhythm of the brain-cells.

gators, and several have sought to find by that method a condition of the neurons produced by narcotics, which is different from that which obtains in the waking state.

Demoor and several others found that in animals in which deep anæsthesia has occurred that the dendrites exhibit what he calls moniliform swellings, that is, a series of minute thickenings or varicosities. On the strength of this observation, he has formulated what we may call a bio-physical theory of sleep. In the waking state, the neighbouring nerve-units are in contact with each other; transmission of nerve-impulses from neuron to neuron is then possible, and the result is consciousness; during sleep the dendrites are retracted in an amœboid manner; the neurons are therefore separated, and the result is unconsciousness.

Lugaro, on the other hand, takes the precisely contrary view. He was not able to discover moniliform enlargements and his bio-physical hypothesis is that the interlacing of dendrites is much more intimate during sleep than during consciousness. He therefore explains sleep by supposing that the definite and limited relationships between neurons no longer exist, but are lost and rendered ineffective by the universality of the connecting paths. It is not very difficult to explain such divergence of views, for they both depend mainly on observations made by a single method; and the method itself is open to objection. It is one which gives even in the same brain most inconstant results, and is not calculated to show much more than a mere outline of a few of the cells and their branches. So much doubt has arisen of late in regard to the trustworthiness of the method, that many neurologists are beginning to doubt whether the neuron theory implying absolute non-continuity of nerve-units has been satisfactorily proved, and there is a tendency to return to the idea of a connecting network not very different from that originally put forward by Gerlach.

A more satisfactory investigation of the effect of anæsthetics on nerve-cells has been carried out by Dr. Hamilton Wright, Director of the Pathological Institute of the Federated Malay States. He began this work some years ago, and carried out the majority of his experiments in my laboratory, and since his arrival in the Straits Settlements has completed them, and published his results in two papers in the *Journal of Physiology*.[19]

[19] Vol. xxvi., p. 30, 1900; vol. xxvi., p. 362, 1901.

He used rabbits and dogs, and subjected them to ether and chloroform narcosis for periods varying from half an hour to nine hours. In both animals he found that the nerve-cells are affected, but in rabbits much more readily. This accords quite well with what is known regarding the susceptibility of rabbits as compared to dogs towards the influence of these narcotising agents. In a rabbit the nerve-cells, especially of the cerebrum, show changes even after only half an hour's anæsthesia, but in dogs at least four hours' anæsthesia must be employed. By the

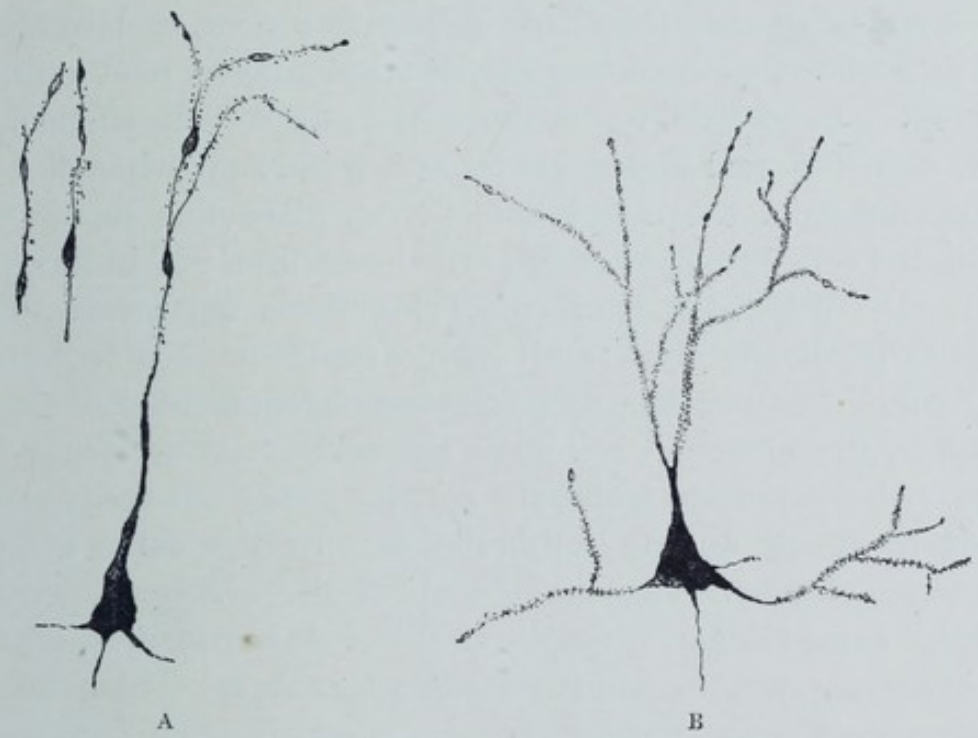

FIG. 3.—Moniliform enlargements on dendrites of nerve-cells, rendered evident by Cox's method. A, in a cortical cell of a rabbit; B, in a corresponding cell of a dog's brain, after six hours' anæsthetisation with ether in each case. HAMILTON WRIGHT.

Golgi method (Cox's modification) the moniliform enlargements can be seen. These become more numerous, larger, and encroach more and more on the dendritic stems, the longer the anæsthesia is kept up. The accompanying illustrations show the appearances seen.

Lugaro's failure to find these appearances is doubtless due to his not having maintained the anæsthesia long enough in his dogs.

Wright started his work with a bias in favour of Demoor's bio-physical theory, but he soon found that the theory was

untenable; the results of his observations have shown him that the action of anæsthetics is bio-chemical rather than bio-physical, and he has been led to this conclusion by the employment of other histological methods, particularly the most sensitive one we possess, namely, the methylene-blue reaction.

Owing to the chemical action of the anæsthetic in the cells, the Nissl bodies have no longer an affinity for methylene-blue, and the cells consequently present what Wright calls a rarefied appearance; when this becomes marked the cells appear like the skeletons of healthy cells (*see* Plate I, D). In extreme cases the cells look as though they had undergone a degenerative change, and after eight or nine hours' anæsthesia in dogs, even the nucleus and nucleolus lose their affinity for basic dyes. The change, however, is not a real degeneration and passes off when the drug disappears from the circulation. Even after nine hours' anæsthesia the cells return to their normal condition within forty-eight hours, the cells stain normally, moniliform enlargements have practically disappeared, and no nerve-fibres show a trace of Wallerian degeneration. The pseudo-degenerative change produced by the chemical action of the anæsthetic no doubt interferes with the normal metabolic activity of the cell-body, and this produces effects on the cell-branches. In the early stages of Wallerian degeneration, the branch of the nerve-cell which we call the axis-cylinder, presents swellings or varicosities, produced by hydration or some similar chemical change. The moniliform enlargements seen during the temporary pseudo-degenerative effects produced by anæsthetics are comparable to this.[20] These enlargements are therefore not the primary cause of loss of consciousness, but are merely secondary results of changes in the cell-body. When a tree begins to wither the earliest apparent change is noticed in the branches most remote from the centre of nutrition, the root; as the changes in the centre of nutrition become more profound the larger branches become implicated, but the seat of the mischief is not primarily in the branches. This illustration may serve to render intelligible what is found in nerve-cells and their branches.

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[20] Some observers look upon the varicosities as artifacts. If they are they ought to have been found in all Wright's specimens, for the method of preparation was the same throughout.

Whether the appearances found in dogs and rabbits are applicable to the human subject is another question. I am inclined to think that we may safely regard them as such; there is no reason why an anæsthetic should act differently in different animals. The resistance of the animal is a variable factor, and this causes a variation in degree only; the effect is probably the same in kind for all animals, man included.

But I feel with Dr. Wright that we should be very chary in concluding that the artificial sleep of a deeply-narcotised animal is any criterion of what occurs during normal sleep. The sleep of anæsthesia is a pathological condition due to the action of a poison. The drug reduces the chemico-vital activities of the cells, and is, in a sense, dependent on an increasing condition of exhaustion, which may culminate in death. Normal sleep, on the other hand, is not produced by a poison, or at any rate we have no evidence of any poison; it is the normal manifestation of one stage in the rhythmical activity of nerve-cells, and though it may be preceded by fatigue or exhaustion, it is accompanied by repair, the constructive side of metabolic activity.

LECTURE III.

Pathological Side of the Subject.

My first and second lectures have been concerned with physiological considerations. I propose in this and the next lecture to turn to more strictly pathological questions. I have already stated my belief that the comparatively new subject of chemical pathology has a great future before it, and this is as true for nervous diseases as for diseases of other parts of the body. Up to the present time, comparatively few pathologists have worked at the chemical side of nervous disease, and a few years ago, when Dr. Mott and I started our work, this branch of investigation was practically untouched.

The researches which we may consider to be in an approximately complete condition are three in number. They are:—

(1) The bearing of chemical research on hyperpyrexia.

(2) The chemical pathology of General Paralysis of the Insane.

(3) The chemistry of Wallerian degeneration.

I propose to consider the first two of these to-day, and to reserve the third for my final lecture.

In my account of these investigations I shall find it necessary to repeat to some extent what Dr. Mott stated in his Croonian Lectures last year. It is almost impossible to avoid this, if the presentation of the subject is to be complete. I shall endeavour, however, to make this part of what I have to say as short as possible, and dwell more fully on our work of the year that has intervened since Dr. Mott addressed the College.

The Coagulation Temperature of Cell-globulin and its Bearing on Hyperpyrexia.

When in 1893 I published some work on the proteids of nervous tissues,[1] I little thought that the subject would turn

[1] *Journ. of Physiology*, 1893, xv., p. 90.

out to be one of practical pathological interest. The history of physiology is full of such surprises. Physiologists *quâ* physiologists work rather from the academic than from the practical standpoint; but as time passes nearly every piece of work which they do is discovered by pathologists to have practical bearings. The first workers at the obscure subject of the chemistry of cell-nuclei little thought they were paving the way for the proper understanding of the uric acid problem. The earlier workers at the physiology of the thyroid gland did not foresee the application of their work to the correct knowledge of cretinism and myxœdema, and to the cure of these conditions. Many other instances might be given, but my hearers will themselves be able to supply them.

It is well known that there are various factors that influence the temperature of heat-coagulation of proteid substances. Among these the rate of the rise of temperature is one of some importance. This was clearly shown in the work of Corin and Ansiaux[2] on serum, and in that of Hewlett[3] on white of egg. These observers found that if the temperature is maintained long enough below the point at which heat-coagulation is usually stated to occur, not merely opalescence, but the formation of flocculi will take place.

In performing the process of fractional heat-coagulation with saline extracts of various organs and tissues, I[4] have shown that in nearly all of them a proteid is present which coagulates at an extremely low temperature (45° to 50° C.). This proteid is a globulin, and it appears to be as characteristic of protoplasmic structure as nucleo-proteid. Whether this proteid is the same in extracts of all tissues is uncertain, but the term cell-globulin may be provisionally employed to designate it.

The next question which arises is whether the behaviour of saline extracts of cellular tissues is a trustworthy guide to teach us the condition of the proteids as they actually exist in protoplasm. As a help in answering this question, I must allude to

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[2] *Bulletin de l'Acad. roy de Belgique*, xxi., p. 3, 1891.

[3] *Journ. of Physiology*, xiii., p. 494, 1893.

[4] The papers relating to this subject are numerous, and were all published in the *Journal of Physiology*. A summary of them will be found in my article on the Chemistry of the Tissues and Organs in Schäfer's "Text-book of Physiology," vol. i.

the work of Brodie and Richardson,[5] and Vernon.[6] These investigators show in the case of muscle that the shortening which occurs during heat-rigor takes place in a series of steps; the temperatures at which these steps occur are the same as those at which the individual proteids separate out during the fractional heat-coagulation of an extract of muscular tissue. Thus in mammalian muscle, the two principal shortenings occur at 47° and 56° C., the coagulation temperatures of the two principal muscular proteids. In frog's muscle there are three steps, at 40°, 47° and 56° C. respectively, which correspond to the three proteids that can be separated out in a saline extract of this variety of muscular tissue.

Brodie also showed another important point, namely, that after the first step has occurred in the shortening, the muscles lose their irritability. In other words, in order to destroy the vitality of muscular tissue, it is not necessary to raise the temperature sufficiently high to coagulate all its proteids, but that when one of the muscular proteids has been coagulated the living substance as such is destroyed; the proteids of muscle cannot be regarded as independent units; the unit is protoplasm, and if one of its essential constituents is destroyed, protoplasm as such ceases to exist.

I think we may safely infer that what is true for muscle is in the main true for other tissues, and that the results which have been obtained by the examination of saline extracts of these tissues can be safely applied to the elucidation of the composition of the protoplasm of which they are composed.

We can now pass to the application of these general principles to nervous tissues, especially in connection with the question of hyperpyrexia. Mott's histological observations have proved that in cases of death from hyperpyrexia the nerve-cells show a disappearance of the Nissl granules; both cell-bodies and dendrons show a diffuse blue staining with methylene blue. He has proved that this is not due to the toxin of the disease, but to the high temperature. It is a familiar fact that very high body temperature is incompatible with life. Marinesco[7] has

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[5] *Phil. Trans.*, cxci., B. 127, 1899.

[6] *Journ. of Physiology*, xxiv., p. 239, 1899.

[7] *Recherches sur les lesions des centres nerveux consécutive à l'hyperthermie Expérimentals et à la Fièvre*, *Revue Neurologique*, 1899. *See* also

pointed out in experiments on hyperthermia in animals that a temperature of 47° C. is immediately fatal; a temperature of 45° C. kills in an hour or two; a temperature of 43° C. kills after a longer lapse of time. Moreover, the occurrence of death is coincident with the break-down of the nerve-cells in the manner just indicated. It is possible that analogous changes occur in other parts of the body also, but these do not seem to have been specially investigated. The nerve-cells are undoubtedly essential to healthy life, and lend themselves very readily to microscopic investigation by the methylene-blue process. A temperature of 47° C. leads to a practically instantaneous disappearance of the chromatophile granules; the same change occurs at 45° C. in a few hours; at 43° C. a longer lapse of time is necessary.

The coincidence of the fatal temperature (47° C.) with that of the coagulation temperature of cell-globulin is very striking, and we argue that, as in muscle, the coagulation of even the lowest coagulating proteid of nerve-cells must produce a destruction of the life of their protoplasm, and so a distinct chemico-physical cause can be found for death due to hyperpyrexia. Still a temperature as high at 47° C. (117° F.) in man is unknown, and we thought it possible that the proteid in question would coagulate at a lower temperature, if it was kept at that temperature a sufficient length of time. We proceeded to put the suggestion to the test of experiment, fully anticipating, in the light of the work of Hewlett and others alluded to before, that the supposition would turn out to be correct.

We made saline extracts of the grey matter of the brains of cats and human beings, and found that the first crop of flocculi separates out at 47° C. when the rate of heating is rapid. But if the temperature of the water bath is maintained at 44° C. for two hours, precipitation of the proteid occurs at this temperature. At 42° C. (108° F.), three to four hours' heating brought down the coagulum at that temperature. At 40° to 41° there was no coagulation even after eight hours' heating.

We proved the same point in another way. A cat was after anæsthetisation rapidly killed by bleeding to death; the brain was

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Goldscheider and Flatau, *Normale und Pathol. Anat. der Nervenzellen*, Berlin, 1898.

removed and divided into two equal halves. A saline extract of one half was made immediately; the other half was heated to 47° C. for an hour and then similarly treated. The extract of the first (the normal) half contained 0·674 per cent. of proteid, and fractional heat coagulation revealed the presence of proteids which came down at 47°, 56° to 65°, and 72° C. respectively (*see* p. 9). In the extract of the second (the heated) half the total percentage of proteid was only 0·144; the 47° C. coagulum was absent, but the other two were obtained.

In the next experiment the half-brain was heated to 42° instead of 47° C. It was kept at 42° C. for five hours. The extract of the normal half-brain contained 0·483 per cent. of proteid and gave the usual three crops of coagula; the extract of the half-brain which had been heated to 42° C. contained only 0·226 per cent. of proteid, and the coagulum at 47° C. was absent.

The chemical examination of brain tissue as fresh as possible thus gave results which exactly correspond to those obtained in the experiments with saline extracts.

We have not repeated Marinesco's experiments on hyperthermia in animals, but for histological purposes we have performed the experiment of exposing the brain *in situ* immediately after death to an elevated temperature. In the case of one cat the brain was kept at 44° to 45° C. for one and a half hours, and in another at 42° to 43° C. for three and a half hours. In each case, and particularly in the first, the cells exhibited chromatolysis.[8]

We may therefore sum up by saying that our experiments fully confirm our hypothesis that the physico-chemical cause of death from hyperpyrexia is the coagulation of cell-globulin. When this constituent of cell-protoplasm is coagulated, the protoplasm as such is destroyed. The temperatures at which such coagulation is most readily produced is 47° C. But temperatures as low as 42° C. will have the same effect, provided the heating is continued long enough. These chemical changes in the brain substance are demonstrable by experiments with

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[8] Full details of all these experiments and observations will be shortly published in a paper by Dr. Mott and myself in the forthcoming volume (vol. ii.) of Mott's *Archives of Neurology*.

saline extracts of that tissue, or with the "surviving" brain of animals just killed. They are coincident with the histological (chromatolytic) changes in nerve-cells, which can be rendered evident by the use of the methylene-blue method. The expression *coagulation necrosis* employed by Marinesco for this appearance is therefore justifiable. Marinesco and others who have employed exclusively histological methods of research, have naturally failed to grasp the chemical meaning of their

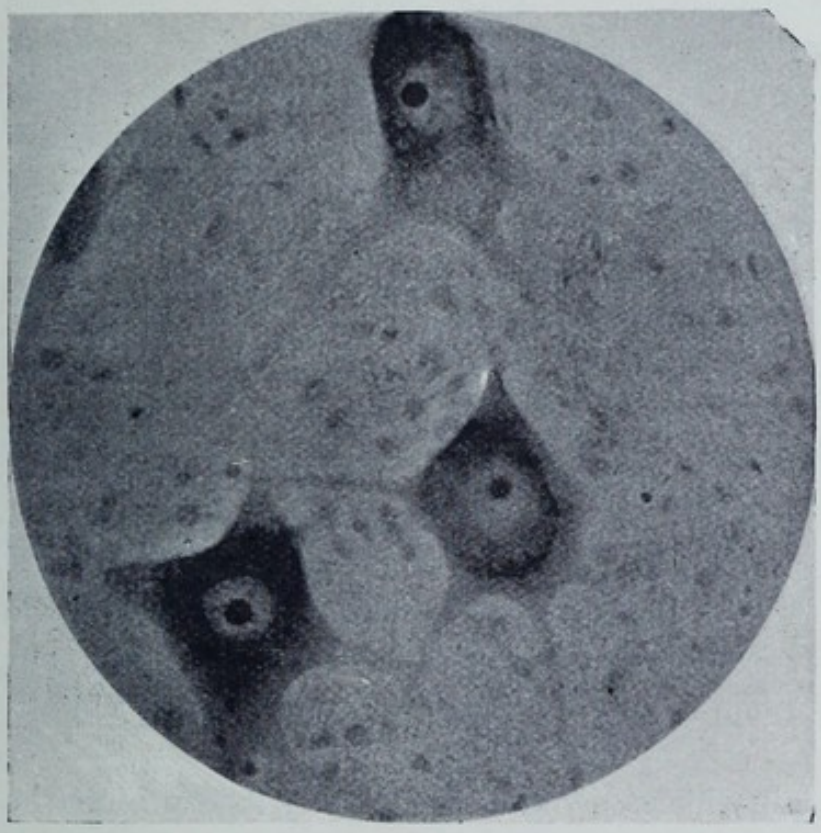

FIG. 4.—Section of spinal cord from a case of hyperpyrexia, the temperature being 109° F. before death. The whole of the cells throughout the central nervous system showed a diffuse homogeneous staining with methylene blue. The Nissl granules had entirely disappeared from the processes and body of the cells, and the stainable substance had a fine, dust-like appearance. Magnification 400 diameters. MOTT.

observations, and have consequently missed the connection of the temperature necessary to produce these changes with that of the coagulation temperature of cell-globulin. Lastly, though the nerve-cells are those which have been more specially investigated by these methods, it is by no means improbable (looking at the wide distribution of cell-globulin) that many other cells of the body are affected by high temperatures in

a corresponding manner; some varieties of what is called "cloudy swelling" are without doubt instances of coagulation necrosis. The accompanying figure (fig. 4) illustrates the histological changes in nerve-cells referred to.

Chemical Pathology of General Paralysis of the Insane.

General Paralysis of the Insane is a para-syphilitic affection like tabes, with which it is, pathologically speaking, identical. It is a premature, primary, progressive decay of the neuron, affecting especially those structures which have been developed latest. To the naked eye the extensive degenerative and wasting process which occurs, especially in the frontal and central convolutions, is perfectly evident. Microscopical examination of the diseased brains reveals degenerative changes in the cells; and the perivascular lymphatics are seen (by Marchi's method of staining) in acute cases to contain phagocytes filled with black-stained fatty matter. During the course of the disease there are frequently seizures of an epileptiform or apoplectiform kind, and after recovery of the patient from each of these fits he is, as a rule, worse mentally. Each fit apparently indicates the breakdown of a new focus of cerebral matter.

The place of the atrophied brain substance within the cranium is taken by excess of cerebrospinal fluid. It is often possible to obtain as much as one or two hundred cubic centimetres of this fluid.

The main object of our research was to examine the cerebrospinal fluid, and to attempt to discover in it some substance or substances derived from the disintegration of the brain-matter, which passing thence into the general circulation would produce auto-intoxication, and thus account for some of the symptoms of the disease. How far this object has been attained, will be discussed at the conclusion of this lecture.

Our methods and results have already been published in full in the *Philosophical Transactions of the Royal Society*,[9] and all I shall attempt here is to describe these in more general terms, in order to pave the way for the consideration of the subject of nervous degeneration in my final lecture.

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[9] Mott and Halliburton, "The Physiological Action of Choline and Neurine," *Phil. Trans.*, Series B, vol. cxci., pp. 211-267, 1899.

Normal cerebrospinal fluid is alkaline to litmus and contains a very small percentage of solids (*see* first lecture). The fluid from cases of General Paralysis is not only more abundant, but is also richer in solids; this is principally due to excess of proteid material. The average percentage of proteid in eight specimens was 0·24, that is about three times as much as is found in cerebrospinal fluid in cases of spina bifida. It is alkaline like the normal fluid. Fibrinogen is absent as in the normal fluid;

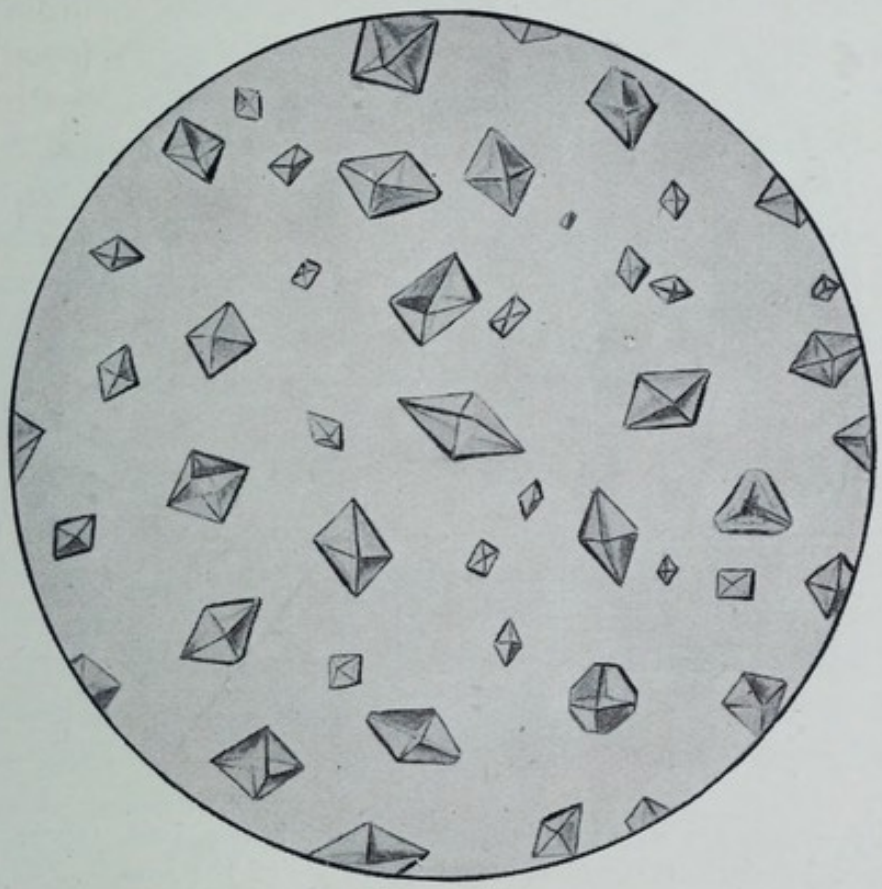

FIG. 5.—Crystals of the platino-chloride of choline prepared from a solution of choline hydrochloride. Crystallised from 15 per cent. alcohol.

proteoses and peptone are also absent. There is a small quantity of albumin present; in the normal fluid albumin is absent. The most abundant proteid, however, is nucleo-proteid; in one case sufficient was present to produce intravascular coagulation, when 10 cc. of the fluid were injected into the jugular vein of a cat.

Another marked distinction between the normal and the pathological fluid is the presence in the former of a reducing substance, and the absence of this, as a rule, in the latter; in only two out of fourteen cases was it found.

But the most noteworthy distinction between the two fluids is the presence of abundance of choline in the specimens removed from cases of General Paralysis. This can be identified chemically by numerous tests, of which the most important are the characters (solubility, crystalline form, &c.), of the platino-chloride. The accompanying figures show the shape of these yellow octahedral

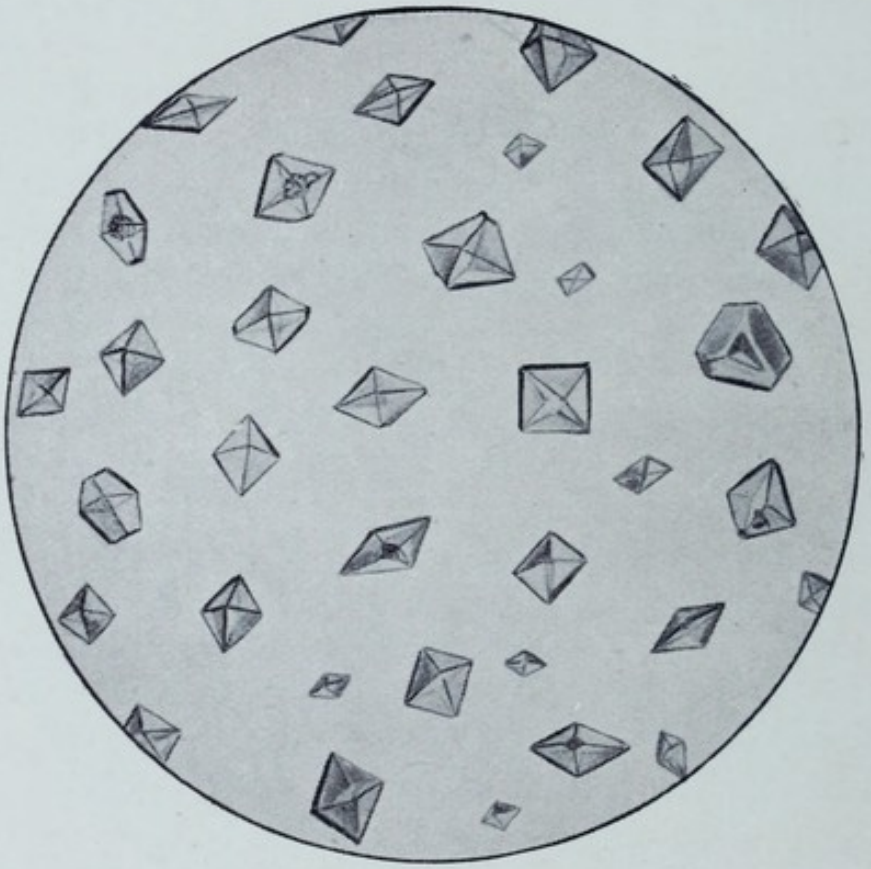

FIG. 6.—Crystals of the platino-chloride of the base separated from cerebrospinal fluid in cases of General Paralysis of the Insane. Crystallised from 15 per cent. alcohol. The identity of this base with choline is not only shown by the form of these crystals, but by its solubilities and by other numerous reactions described in full in the paper referred to.

crystals. The chemical examination of the fluid supports our contention that the cerebrospinal fluid acts as the lymph of the brain, and when the disintegration of the cerebral tissue is increased, as in General Paralysis, the fluid contains the products of such disintegration (*e.g.*, choline, nucleo-proteid). The greater number of the specimens of cerebrospinal fluid we have examined were removed from the cadaver as soon as possible after death. We have also had the opportunity of examining four specimens

removed during life by lumbar puncture from cases of General Paralysis.[10] The results obtained with these are identical with those obtained from the *post-mortem* specimens. We have also secured on several occasions blood removed for remedial purposes from such patients by venesection, and we regard one of the most important outcomes of our work, the discovery that the blood also contains the same toxic material during a seizure. It is not present in the urine.

The presence of choline can be shown not only by the chemical reactions just referred to, but also by physiological tests. This necessitated the study of the physiological action of choline, an undertaking of a prolonged nature involving experiments on the heart, blood-vessels, respiratory movements, and so forth. To this we added a similar investigation on the physiological action of the closely-related alkaloid neurine. The substance separated out from the cerebrospinal fluid and blood of these patients acts precisely like choline in every particular. The action of neurine is different, and this alkaloid is much more toxic. The absence of neurine from the cerebrospinal fluid was also demonstrated by chemical testing.

The principal physiological actions of choline or choline hydrochloride, using small doses (1 to 5 cc. of 0·2 per cent. solution) injected intravenously in anæsthetised dogs and cats are the following. We used weak solutions, for we sought as far as possible to note the effects produced by solutions of such strength as would be comparable to the amount of the base presumably present in pathological cerebrospinal fluid.

(1) Choline produces a temporary fall in arterial blood-pressure.

(2) This is in some measure due to its action on the heart.

(3) It is also, and probably mainly, due to dilatation of the peripheral vessels, especially in the intestinal area.

(4) The kidney and limbs undergo a passive lessening of volume secondary to the fall in general arterial pressure.

(5) The spleen contracts markedly, and when this passes off, its normal curves are greatly exaggerated.

(6) We obtained no evidence of any direct action of the base on the cerebral vessels.

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[10] We are indebted for these to Dr. John Turner, Essex County Asylum.

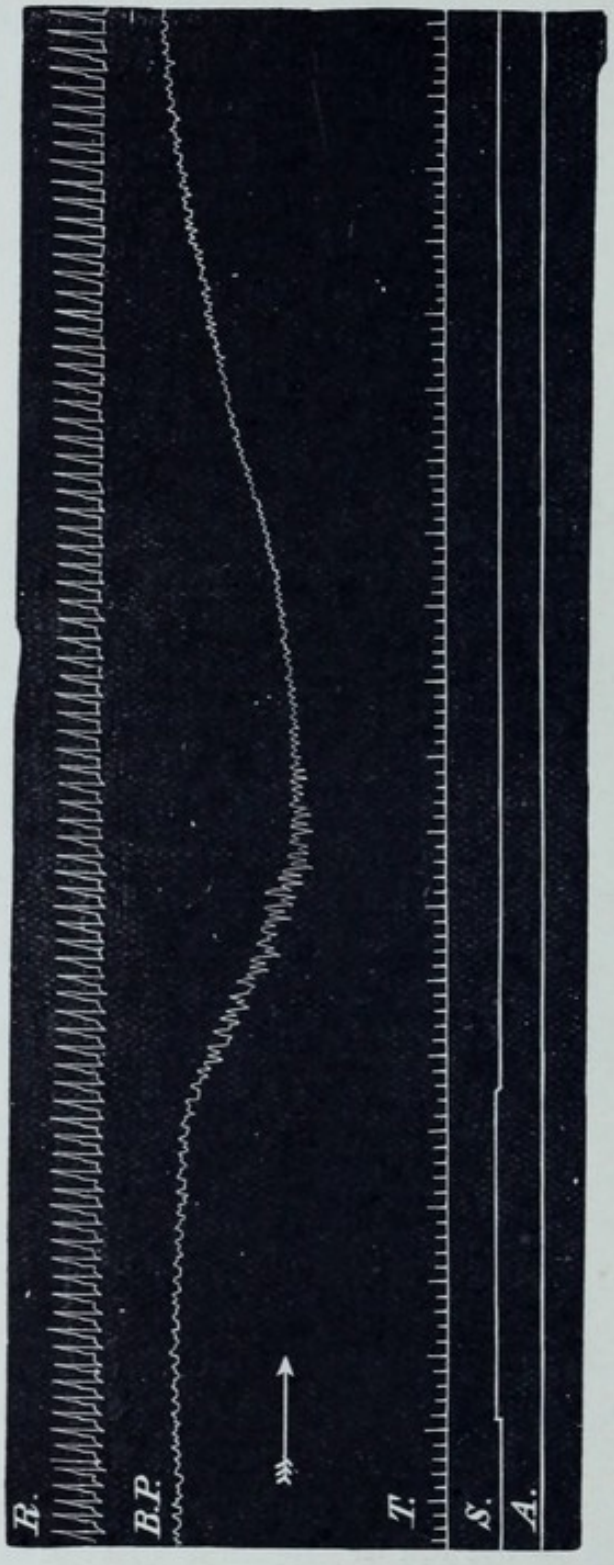


FIG. 7.—Half the original size. *R.* is a tracing of the respiratory movements taken by the tambour method; *B.P.*, a tracing of the blood pressure from the carotid artery; *A.*, abscissa of the blood pressure; *T.*, time in seconds; *S*, signal, the alteration of level of which indicates the period during which the material was injected into the jugular vein. The same letters have the same significance in subsequent tracings. The arrow in each case indicates the direction in which the tracing is to read; this in nearly all cases is from left to right.

Fig. 7 shows the effect in a dog of injecting 6 cc. of a 0·2 per cent. solution of a choline hydrochloride. There is a fall of arterial pressure but no effect on respiration.

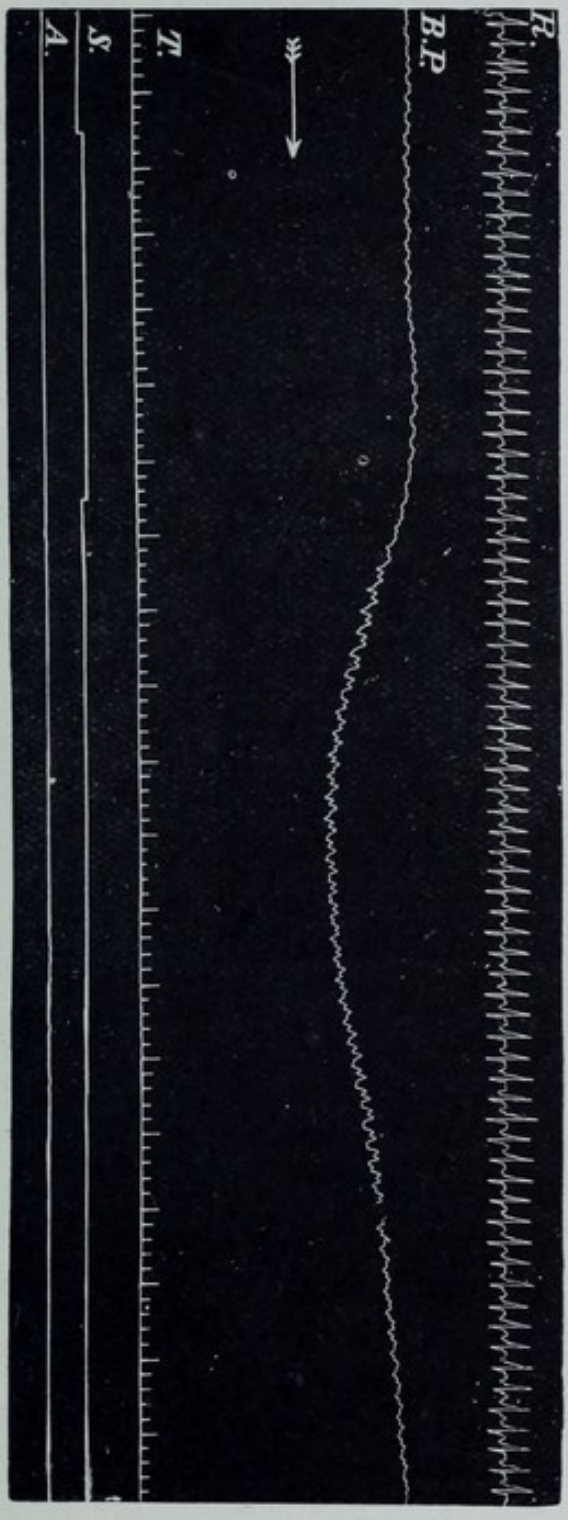


FIG. 8.—Half the original size. Similar effect in a dog produced by the injection of 10 cc. of cerebrospinal fluid removed from a case of General Paralysis of the Insane.

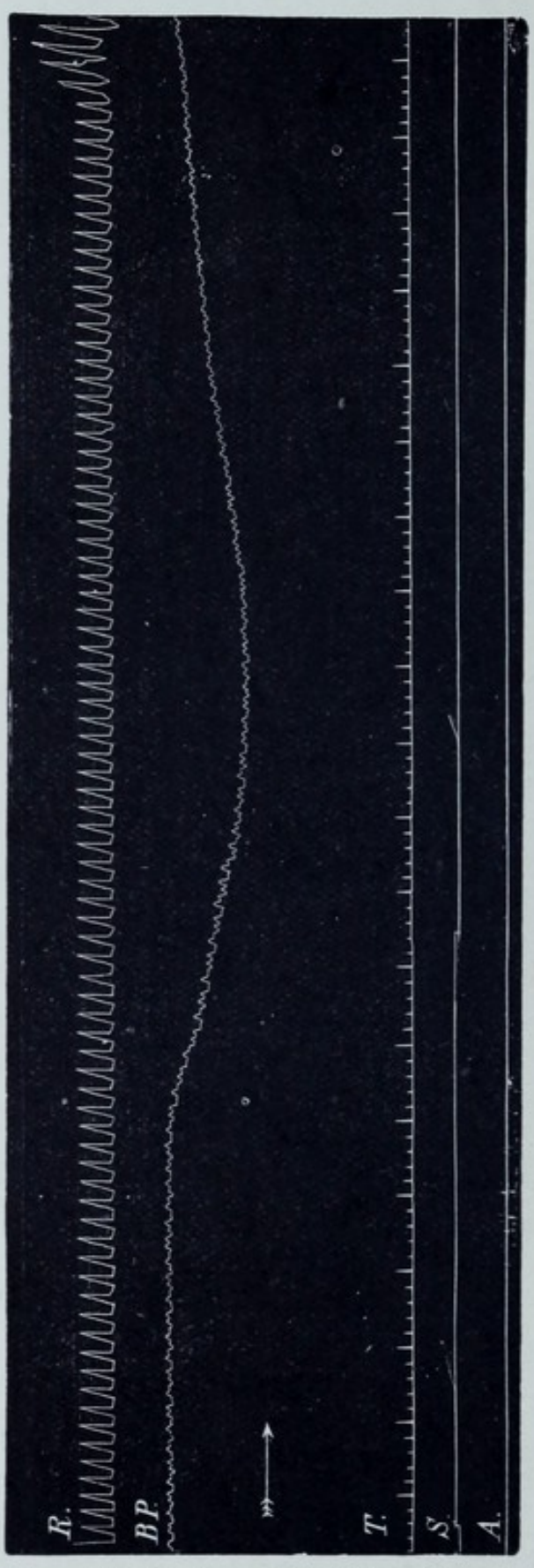


FIG. 9.—Half the original size. Effect in a dog of saline solution of the alcoholic extract of 70 cc. of blood removed during a seizure from a patient suffering from General Paralysis of the Insane. As in figs. 7 and 8, there is no effect on respiration (*R.*), but there is a well-marked temporary fall of arterial blood pressure (*B.P.*). In the same blood, choline was identified chemically.

(7) The action on the splanchnic vessels is due to the direct action of the drug on the neuro-muscular apparatus of those vessels, for after the influence of the central nervous solution has

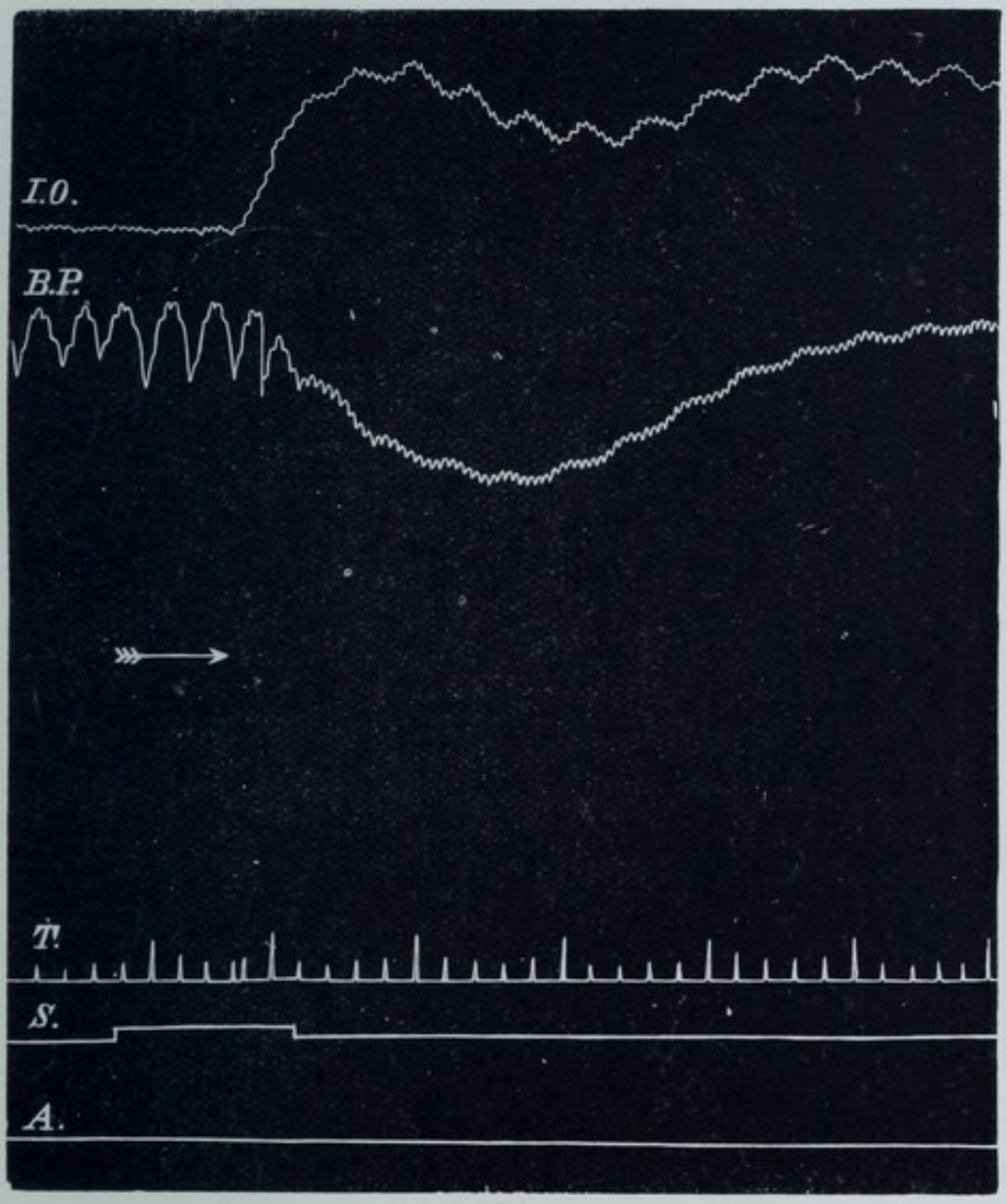


FIG. 10.—Original size. Effect of injecting 5 cc. of a solution of choline hydrochloride (0·2 per cent.). The fall of blood pressure is accompanied by a dilatation of the intestinal vessels; this is shown by the rise of the lever of the Marey's tambour connected with the intestinal plethysmograph (*I.O.*). Dog.

been removed by section of the spinal cord, or of the splanchnic nerves, choline still causes the typical fall of arterial pressure. The action of peripheral ganglia may be excluded by previously poisoning the animal with nicotine.

(8) There is little or no effect on the respiration.

(9) Section of the vagi makes no difference in these experimental results.

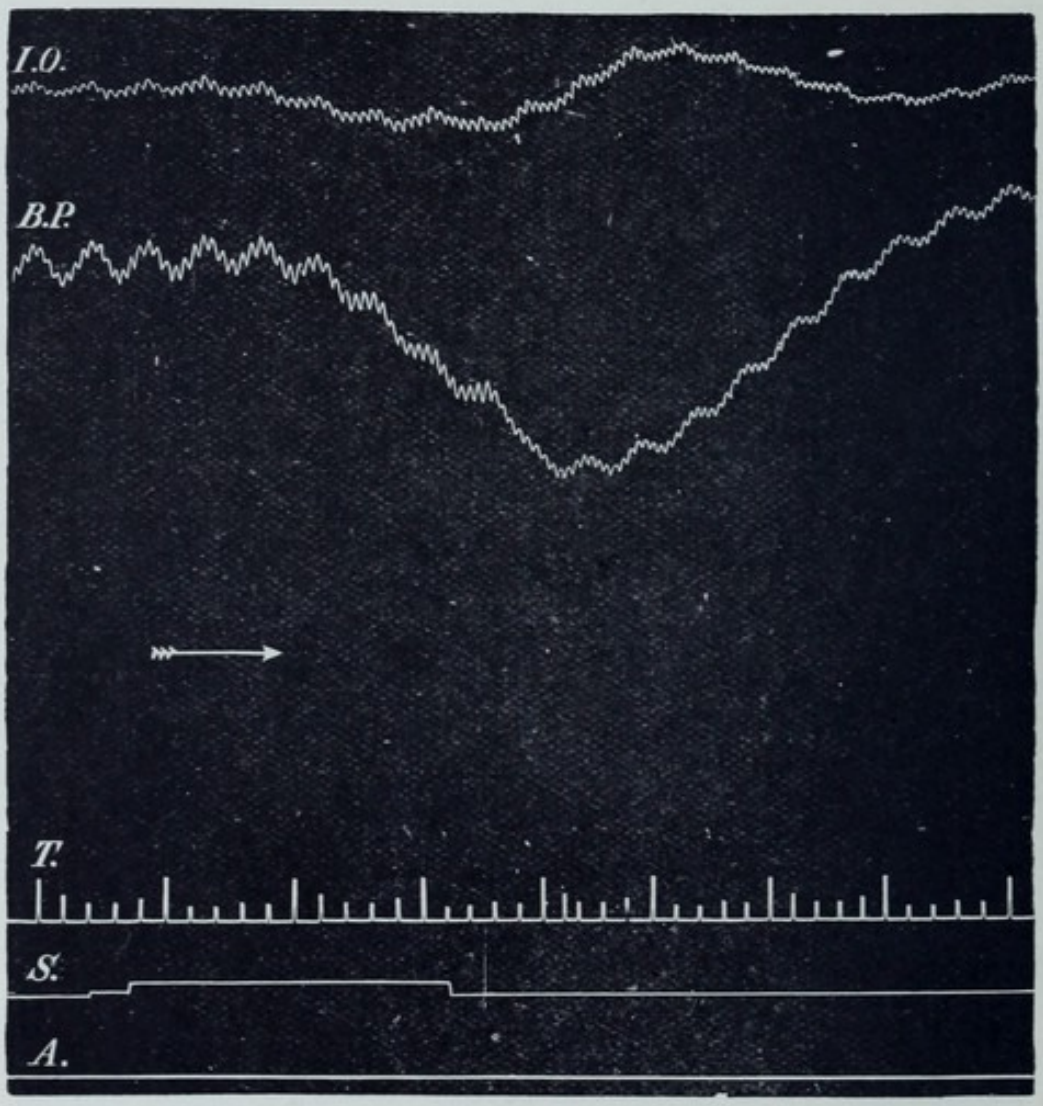
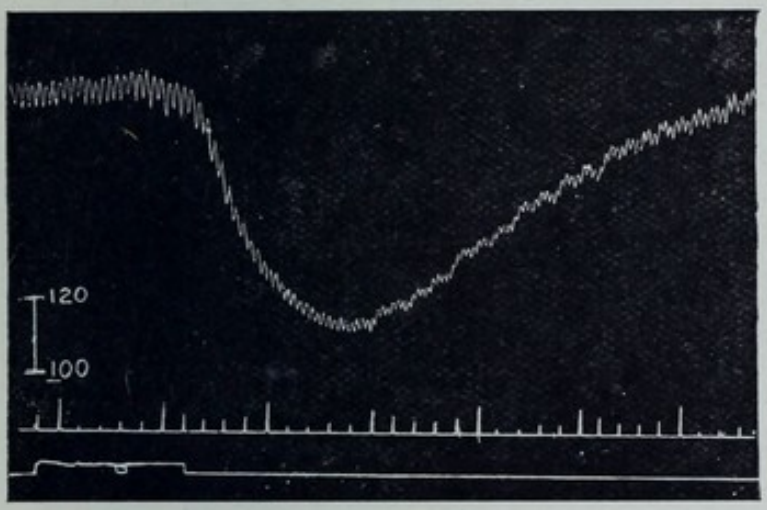


FIG. 11.—Original size. Tracing of intestinal oncometer (*I.O.*) and arterial blood pressure (*B.P.*) in a cat; 10 cc. of cerebrospinal fluid from a case of General Paralysis were injected; the same effect was obtained in the same animal by injecting 2 cc. of a 0·2 per cent solution of choline. The fall of blood pressure is at first mainly cardiac in origin, for the oncometer tracing first follows the fall of arterial blood pressure passively; it, however, soon rises, indicating dilatation of the peripheral vessels.

(10) Previous atropinisation of the animal causes a great difference; it abolishes the fall of arterial pressure, though there is still some dilatation of splanchnic blood-vessels. In fact, very often injection of choline after atropine produces a rise instead of a fall of blood pressure.

I need not detain you by entering into details concerning the action of neurine. Suffice it to say that it is much more poisonous, affects the heart more, causes peripheral constriction rather than dilatation of blood-vessels, stimulates and then paralyses the respiratory movements, and has a curare-like action on voluntary muscles.

Illustrations of the numerous tracings we have taken in working out these facts are given in the paper already referred to. I reproduce here a few of the most typical. (These at the actual lecture were shown as lantern slides.)



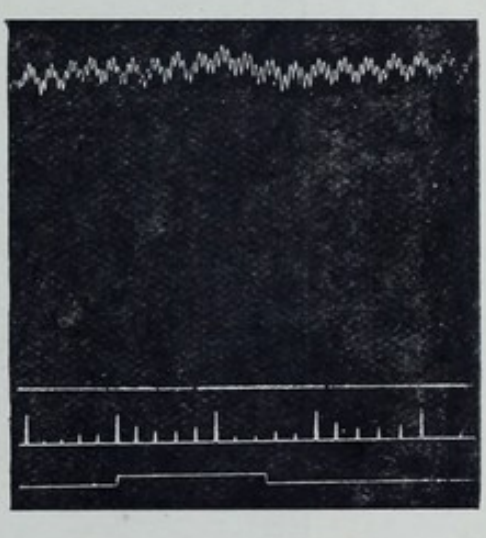


FIG. 12. FIG. 13.

FIG. 12.—Effect on injecting extract of cat's brain before atropine.

FIG. 13.—Effect after atropine of the same extract in the same animal (cat); the fall of blood pressure seen in fig. 12 is here replaced by a slight rise. With pure solutions of choline the rise is generally more marked.

Both these figures are considerably reduced in size; the amount of reduction can be judged from the measure of the height of blood pressure indicated in millimetres on the side of fig. 12.

Fig. 7 shows the effect of 5 cc. of a 0·2 per cent. solution of choline hydrochloride in physiological saline solution. The effect (fall of blood pressure, no effect on respiration) is just the same as that produced by the substance separated out from cerebrospinal fluid from cases of General Paralysis (fig. 8).

The next figure (fig. 9) shows the result of injecting into a dog the saline solution of the alcoholic extract of 70 cc. of blood removed during a seizure from a patient suffering from the same disease. Normal blood treated in this way, and normal cerebrospinal fluid produce entirely negative results.

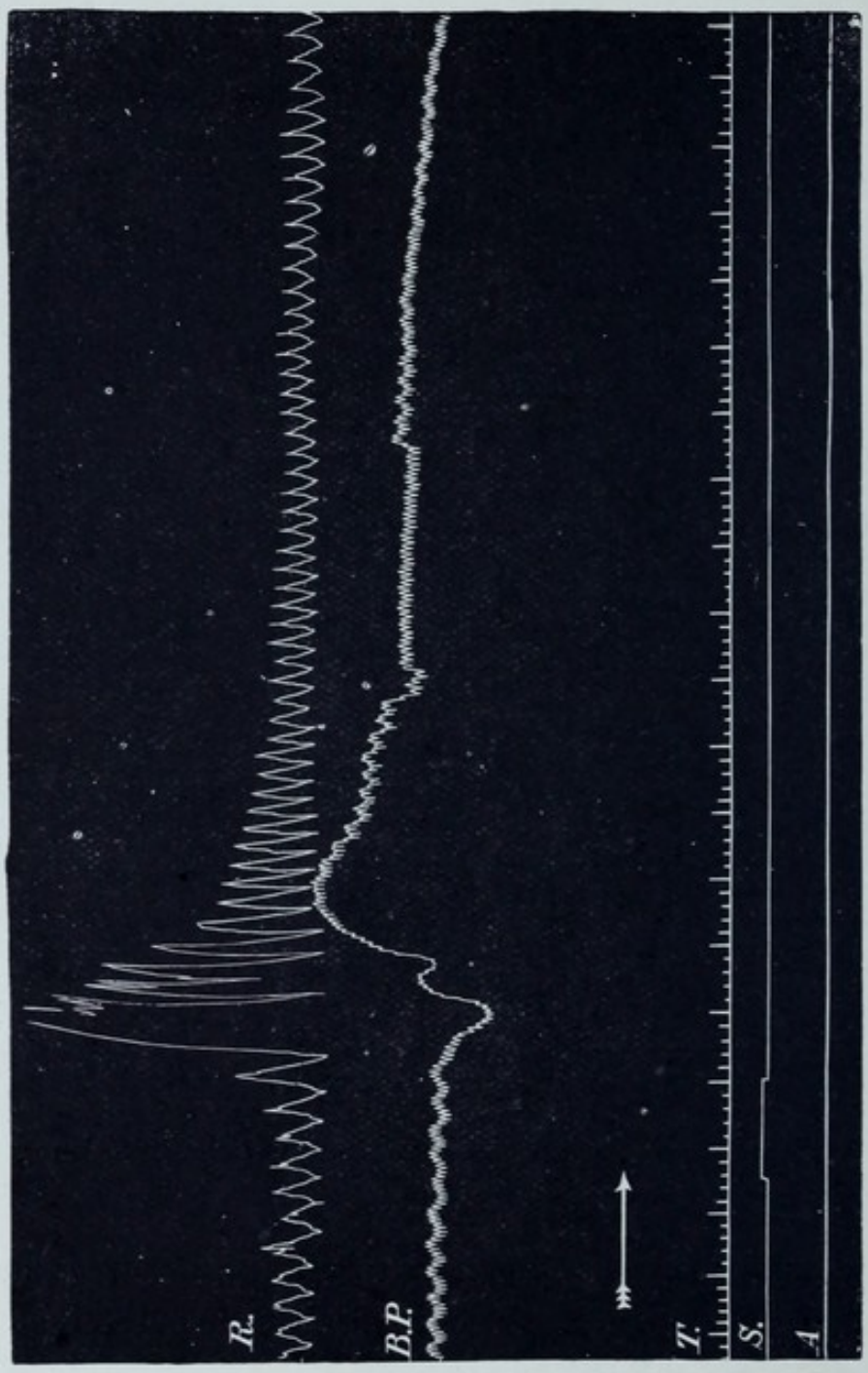


FIG. 14.—Half the original size. Effect of injecting in a cat 2·5 cc. of a 0·1 per cent. solution of neurine. In the tracing (*R.*) each upstroke is caused by inspiration. Note the increase in respiratory efforts followed by a decrease. In the blood-pressure tracing there is a fall followed by a pronounced rise.

The next couple of tracings (figs. 10 and 11) illustrate the dilating action of choline and cerebrospinal fluid respectively on the peripheral blood-vessels. The upper tracing in each case is that of the intestinal oncometer, rise of the lever of which indicates dilatation of blood-vessels.

The next tracings (figs 12 and 13), show how after atropine the fall of blood pressure is replaced by a rise. I give only one of a pair of tracings, that obtained with choline, obtained by making an extract of cat's brain; others obtained with cerebrospinal fluid are practically identical with it.

I might, however, go on multiplying graphic records to show that physiologically the material obtained from cerebrospinal fluid has exactly the same action as choline. But I will be content with giving two more, which show how different is the effect of neurine (figs. 14 and 15). The text beneath the figures gives the details; the tracings show the effect on blood pressure (a rise sometimes preceded by a slight fall, cardiac in origin), on respiration (exacerbation of movement), and on the intestinal blood-vessels (a constriction accompanying the rise of blood pressure).

The chief interest in our work has centred round the discovery that the cerebrospinal fluid of patients suffering from general paralysis contains toxic material. We believe that this toxic material, which undoubtedly originates from the disintegration of nervous tissue, is probably not a single substance. We have obtained chemical evidence of the existence of nucleo-proteid in the fluid, and though the amount of nucleo-proteid is not, as a rule, sufficient to cause massive intravascular clotting when the fluid is injected into animals, we consider that the presence of even small quantities continuously being poured out into the cerebrospinal fluid, collecting in the perivascular lymphatics and passing thence to the blood will produce harmful results. The idea has suggested itself to our minds that an increase in the coagulability of the blood in the small vessels of the cerebral region, which nucleo-proteid would produce, might form a determining factor in promoting venous stasis, and thus are caused the acute manifestations or seizures of apoplectiform or epileptoid nature which the patients exhibit.

The other toxic material which we have succeeded in isolating, namely, choline, will not account for the majority of the

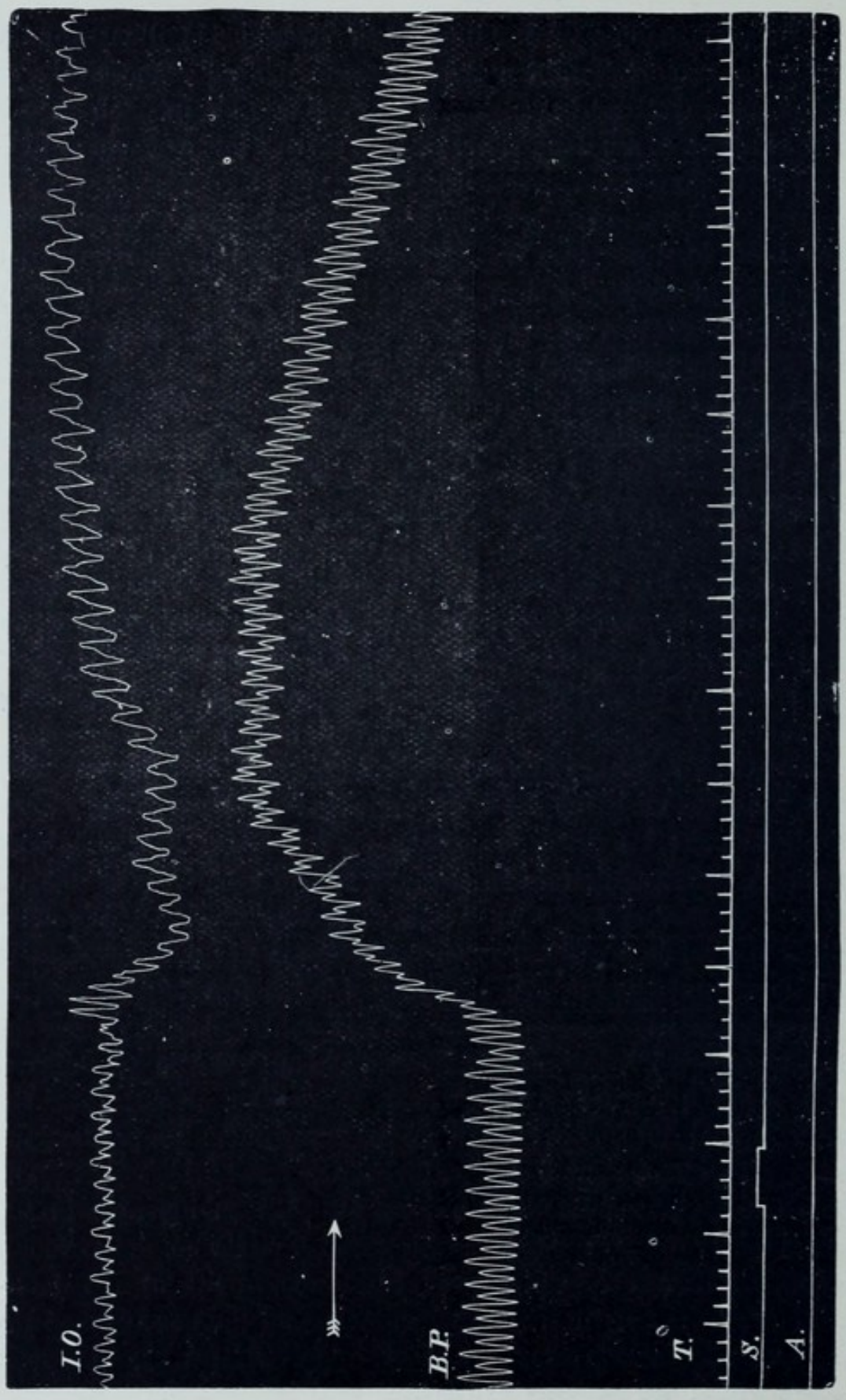


FIG. 15.—Three-quarters of the original size. Effect of injecting 1 cc. of a 0·1 per cent. solution of neurine. The preliminary fall of blood pressure is absent in this case; the rise is accompanied by the constriction of the peripheral blood-vessels, as shown by the fall of the recording lever of the intestinal oncometer (*I.O.*).

symptoms of the disease. In the completeness with which we have been enabled to identify (both on chemical and physiological grounds) choline in the cerebrospinal fluid and blood of these patients, we consider our work has been satisfactory only up to a certain point. As affording a complete explanation of the pathology of the disease it has been a disappointment; we regard the fact that choline does exist in these fluids chiefly as an indication of the disintegration of the brain-tissue, and if the majority of the symptoms are to be explained on the basis of auto-intoxication due to such disintegration, we must confess, if we except nucleoproteid, that any highly poisonous substance has eluded our grasp: certainly lactic acid and glycero-phosphoric acid are not very poisonous; they are even less so than choline.

Still, even choline has some effect. A feeble circulation and fatty degeneration of the heart are very frequent concomitants of the terminal stages of the disease, especially after a series of seizures, and the idea seemed feasible at one point in our research that choline might explain these. A single dose of choline in a dog or cat produces but little effect on the heart; still there is some effect, and it does not appear a far-fetched idea to suppose that the continual pouring of small doses of choline into the cardiac tissue might in time produce cardiac weakness, and even degeneration. This possibility still remains, but our study of the disease, and of the physiological action of choline, has shown us that choline will not explain the fits, but rather that the fits will explain the degeneration in heart and other muscles. Such fatty degeneration probably never occurs unless fits have occurred before death, and if similar fits occur in other diseases, as in the *status epilepticus* of epilepsy, there is the same fatty degeneration of the muscular tissues, even though no massive disintegration of nervous tissues has been present.

Take again the point of enfeebled circulation; the pulse is often small and might be supposed to be associated with low tension. By the use of the Hill-Barnard sphygmometer, Dr. Mott has tested this point on a large number of patients. In the first and second stages of the disease (*i.e.*, before the patient becomes bed-ridden) the arterial pressure is, as a rule, higher than normal; obviously choline alone will not explain this. At the beginning of epileptiform seizures the pressure is still high, but after prolonged convulsions the pressure falls considerably, to

rise again a few days after the convulsions have ceased. There can be no doubt that the convulsions are associated with the breaking down of nervous tissue, and we therefore think it probable that the choline so liberated is responsible for the fall of pressure which occurs then.

Our experiments with atropine are by no means uninstructive in connection with this subject. A small dose of atropine will modify the action of choline and produce a high instead of a low blood pressure. We do not, of course, mean to suggest that atropine or even a similar alkaloid is present in these patients, but these experiments do suggest that with a plurality of causes we undoubtedly obtain an intermixture or even a reversal of effects. No doubt with fresh light, which future work may throw on our own, it may be possible to see the *modus operandi* of these pathological processes more clearly, and our three years' work will not have been thrown away if it forms some sort of guidance to others, or to ourselves, in unravelling the plurality of causes to which we have just alluded, and which we must assume is present in General Paralysis as in so many other obscure diseases.

LECTURE IV.

The Chemistry of Nerve Degeneration.

We have now seen that in the disease called General Paralysis of the Insane, the degenerative changes that occur in the central nervous system are associated with the presence of the products of such degeneration in the cerebrospinal fluid. One of these products, choline, is derived from the break-down of lecithin, but we also noted that there are others, for instance, nucleo-proteid. Choline can be identified in the blood also of these patients. The tests on which one relies for the detection of this alkaloid are mainly two: the first is a chemical test, namely, the obtaining of the typical yellow octahedral crystals from the alcoholic extract of the blood. These crystals have not only a definite form, but their solubilities distinguish them from other somewhat similar crystals, as also does the fact that they yield a fixed percentage of platinum, and give rise to an odour of trimethylamine when decomposed by heat. The second test is a physiological one; a saline solution of choline, of choline hydrochloride, and of the residue obtained from the alcoholic extract of the cerebrospinal fluid and blood of these patients, produce a temporary fall of pressure when injected intravenously in animals. This fall is partly cardiac in origin, and partly due to dilatation of peripheral blood-vessels; the dilatation is due to the direct action of the alkaloid on the neuro-muscular mechanism of the blood-vessels themselves. There are many substances which produce a fall of arterial pressure, but choline is peculiar in the fact that after the administration of a small dose of atropine subcutaneously, it no longer produces a fall but a rise of blood pressure, or, at any rate, the fall is abolished.

In the investigation of the blood, as a rule, only a small amount of material has been at one's disposal, and in order to obtain satisfactory evidence of choline it is necessary to con-

siderably concentrate the alcoholic extract. It is therefore necessary to limit the number of tests to be performed. The two tests, however, appear to be, if positive, absolutely conclusive evidence of the presence of choline. The iodine test which has been employed by some investigators is not a very delicate one, especially in the presence of other organic substances. The great value and delicacy of the platinum test has been emphasised in the recent work of Gumprecht.[1]

Although the first paper,[2] by Mott and myself, dealt principally with General Paralysis, we indicated that the presence of choline in large quantities in the cerebrospinal fluid is not characteristic of this disease; we mentioned that in other diseases where great and evidently acute wasting of the brain tissue had occurred, choline was also present in excess in this fluid.

Since the publication of our work, the subject has been taken up by Gumprecht and our observations have been confirmed and extended by him. We stated that in the normal fluid choline was either absent or present in such small quantities that our tests were not sufficiently delicate to detect it. Gumprecht has shown that if sufficient fluid is employed it is possible to demonstrate the presence of minute quantities of choline in it; but this is enormously increased not only in General Paralysis, but in many other diseases of the nervous system; he directed his attention in particular to acute diseases like meningitis.

The presence of choline in the normal fluid is not devoid of physiological interest. It shows us that, under ordinary conditions, lecithin is in that condition of unstable chemical equilibrium which is indicated by the word metabolism. It is constantly breaking down, and being constantly built up afresh, and it is only in diseased conditions, in which the destructive side of metabolism preponderates over the constructive, that appreciable quantities of the products of its disintegration are present in cerebrospinal fluid and blood.

Physiological saline solution will extract a small amount of choline from perfectly normal nervous tissue at the ordinary temperature (*see* figs. 12 and 13); this is most apparent in

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[1] Gumprecht, *Verhandl. der Congr. für innere Medicin*, Wiesbaden, 1900, p. 326.

[2] *Phil. Trans.*, Series B., cxci., pp. 211-267, 1899.

situations where there is most activity, namely, in the grey matter. Within the last year or two numerous observers have shown this, and although they differ in opinion whether choline is the most important or abundant substance in such extracts, all are agreed on the main point as to its actual presence (*see* more fully, Lecture II., p. 27).

In normal blood much the same is true. In the work which we have done since the publication of our first paper we have devoted our attention to the blood rather than to the cerebro-spinal fluid. By concentrating large quantities of normal blood it is possible to show by the platinum test the presence of minute traces of choline; but in conditions in which extensive degenerative changes occur in the central or peripheral portions of the nervous system, the amount is considerably increased, and its presence can be shown in small quantities of blood; such quantities of normal blood yield wholly negative results. The largest yield of choline in a normal animal which we have met with was in a young kitten, and doubtless in young animals the myelinisation of the nerve-fibres which is taking place implies more active metabolism of the lecithin than in adult animals.

The general plan of our work has been (1) to examine the blood in various diseases of the nervous system in man; (2) to examine the blood in animals in which degeneration of the nerves has been made to occur by section of large nerves like the sciatics; (3) in these animals we have examined the nerves themselves in various stages of degeneration; this examination has been partly microscopic and partly chemical, and we have sought to correlate the two sets of changes, and in particular have endeavoured to ascertain the chemical meaning of the Marchi reaction, which is the method principally used to-day for the microscopic detection of degeneration in nerve-fibres.

Before passing on to consider our experiments, observations, and results in detail, it may be well at this point to again briefly allude to the composition of lecithin, and to the main features of the Marchi reaction.

Lecithin, the chief constituent of the medullary sheath, differs from ordinary fats in containing two additional elements, namely, nitrogen and phosphorus. An ordinary fat on decomposition breaks up into glycerin and fatty acid. Lecithin under

similar circumstances yields glycerin, fatty acid, phosphoric acid, and a base called choline ($C_5H_{15}NO_2$).

We have in our work endeavoured to follow the history of lecithin disintegration, not only in regard to the nitrogen it contains (the choline radicle), but also in respect of its phosphorus.

It is probable that in the body lecithin is not present in the free state, but in combination with a cerebrin or cerebrins to form a still more complex substance called protagon (*see* Lecture I., p. 10).

The Marchi reaction consists in placing small pieces of nervous tissue in a mixture of osmic acid and Müller's fluid, after previous hardening in Müller's fluid. Under these circumstances healthy nerve-fibres are stained a greenish-grey colour, but degenerated nerve-fibres are stained an intense black. In the later stages of degeneration, when the fatty products of the decomposition of the fibres have been absorbed, this black staining is naturally no longer observable. It is important also to observe that ordinary neutral fats, such as are contained in adipose tissue, give the Marchi reaction. It was knowledge of this fact that led us in part to the present investigation, and our expectation has been fully confirmed that the cause of the Marchi reaction in degenerated nerve-fibres is the replacement of the phosphorised fat by non-phosphorised fat.

Before the commencement of our joint work, Dr. Mott[3] had made some preliminary experiments in this direction, which were continued in conjunction with Dr. Barratt.[4] Spinal cords on one side of which degeneration had occurred, due to a lesion in the opposite cerebral hemisphere, were divided longitudinally into two halves; each half was extracted with ether in a Soxhlet's apparatus. The residue of the ethereal extract from the degenerated side was more abundant, but contained less phosphorus than on the healthy side; on the healthy side the residue consisted chiefly of protagon crystals. The degenerated half of each cord was also more watery.

Another worker who has made experiments in the same

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[3] Clifford Allbutt's "System of Medicine," vol. i., "Pathology of Nutrition."

[4] *Pro. Physiol. Soc.*, February; *Journ. of Physiology*, xxiv., p. iii.

direction is A. Noll.[5] He showed that in Wallerian degeneration of peripheral nerves, the amount of protagon diminishes until at last none at all is obtainable.

After these introductory remarks we may now pass to the full consideration of our own results; these may be most conveniently arranged under the following heads:—

(1) Examination of the blood in cases of nervous disease in man.

(2) Experiments upon animals. (*a*) Examination of the blood in cats in which Wallerian degeneration had been produced by section of nerves; (*b*) chemical examination of the degenerated nerves; (*c*) histological examination of the degenerated nerves.

(3) General conclusions.

The histological part of the work has been carried out entirely by Dr. Mott. The experiments in which living animals were employed were carried out entirely by myself at King's College. In this part of the work cats were exclusively employed. The animals were anæsthetised with the A.C.E. (alcohol, chloroform, ether) mixture during the operation, and rigid antiseptic precautions adopted.

(1) Examination of the Blood in Cases of Nervous Disease in Man.

The fact that we had examined the blood from several cases of General Paralysis has already been alluded to in the third lecture. The blood was removed by venesection as a remedial measure during seizures, and gave both the chemical and physiological tests for choline.

We have also described[6] the results of examining in a similar way the blood from a case of Beri-beri. This is a tropical disease, which is accompanied by extensive degenerative changes in nerves and muscles, and great vascular depression. A saline solution of the residue from the alcoholic extract of the blood, produced on injection a marked physiological result like that caused by a large dose of choline. The amount of material at our disposal did not enable us to make a thorough chemical

[5] *Zeitsch. f. physiol. Chem.*, xxvii., p. 370, 1899.

[6] *British Medical Journal*, July 29, 1899.

examination of the blood. Using the platinum test we only obtained some ill-formed crystals of a light yellow tint, which we were unable to assert positively consisted of the double salt of choline.

Venesection is so seldom employed as a therapeutic measure that since then we have had comparatively few opportunities of examining the blood removed during life. The specimens we have examined are the following:—

(1) Blood from a chronic case of Beri-beri.

(2) Blood from an acute case of the same disease.

(3) Blood from a case of the same disease, but we were not informed whether it was acute or chronic.

In all these three cases the blood was removed during life; it was immediately mixed with excess of alcohol, and forwarded to us. We have to thank Dr. Patrick Manson, F.R.S., for the first two specimens, and Dr. Hamilton Wright, Director of the Pathological Laboratory of the Federated Malay States, for the third specimen.

(4) Blood from a case of disseminated sclerosis (early stage) which was under the charge of Dr. Mott. This was removed during life by venesection.

(5) Blood from a case of combined sclerosis. This was removed very soon after death. We have to thank Dr. Batten for supplying us with this specimen.

(6) Blood from a case of alcoholic neuritis, removed very soon after death by Dr. Mott.

It will be seen that the diseases from which these patients suffered were very various, but all possess in common the feature which is also present in General Paralysis, of an extensive degenerative change in either the central or peripheral parts of the nervous system.

Before passing on to the study of the results, it will be best to state rather more fully the methods adopted.

The blood was mixed with six or eight times its volume of absolute alcohol, and filtered. The alcoholic filtrate was evaporated to dryness at 40° C., and the residue taken up with absolute alcohol. After filtration, the alcoholic solution was again evaporated to dryness, and the residue again taken up with absolute alcohol. This was repeated twice more, in order to ensure the absence of potassium salts. The final alcoholic solu-

tion was divided into two parts, A and B. Part A was used for chemical examination. Part B was used for the physiological test.

To part A, platinum chloride dissolved in alcohol was added, and the precipitate that formed was allowed to settle, and washed by decantation with absolute alcohol. It was then dissolved in 15 per cent. alcohol. It did not all dissolve, so the platinum chloride must have precipitated substances other than choline. The solution was freed from the insoluble residue by filtration, and then evaporated in a watch-glass to dryness at 40° C. Microscopical examination of the watch-glass with a low power showed whether or not the octahedral crystals were present, and a rough quantitative estimation of the choline was made by noting whether or not the crystals were abundant. Weighings were made in some cases, after redissolving and recrystallising, but as will be more fully explained when we come to the consideration of cats' blood, we do not regard our numbers as very trustworthy. For practical purposes the rougher method of a microscopic survey gives excellent comparative results, provided the same quantity of the original blood is taken in each case. The quantity we used was 10 cc. Using this quantity, normal human blood gives practically negative results, though a few occasional and small octahedra can generally be discovered on careful examination.

Part B was again evaporated to dryness, and the residue dissolved in physiological saline solution. After filtration, the solution was used for injection. It was free from proteid, but was usually somewhat opalescent. We have not determined what this opalescence was due to, though it appears possible that it may be caused by the presence of small quantities of lecithin, derived, like its decomposition product, choline, from the degenerated nervous tissue. No fat particles could be seen with the microscope. The fluid had a neutral reaction. A cat was then anæsthetised with A.C.E. mixture; the carotid artery of one side was connected with a mercurial kymograph for the registration of the arterial blood pressure. The external jugular vein of the opposite side was exposed, and into this the fluid was injected. The volume of fluid injected was in each case 5 cc., and the amount of the original blood to which this corresponded was noted. Concentration was usually carried out, so that the

injection of 5 cc. corresponded to 20 or 30 cc. of the original blood; the actual amounts will be found in the letter press underneath the tracings reproduced in the subsequent pages. After noting the effect of such injection, 0·5 cc. of a 0·5 per cent. solution of atropine was injected subcutaneously into the cat, and a few minutes allowed to elapse. In order to test whether the animal was fully atropinised, the vagus of one side was stimulated, or 2·5 cc. of a 0·2 per cent. solution of choline hydrochloride was injected into the jugular vein. When inhibition of the heart by vagus stimulation was not obtained, or when choline hydrochloride failed to produce its usual fall of arterial pressure (or this was replaced by a rise), the experiment was continued. The second part of the physiological test was then performed, and consisted in again injecting the saline solution from the blood suspected to contain choline. If the injection now produced no fall of arterial pressure, or this fall was replaced by a rise, the identification of choline by physiological means was completed.

Choline was present in all the cases enumerated. The two tests fitted together with great accuracy. If the chemical test is performed first, one can prophesy from the amount of crystals the result of the injection. If, on the other hand, the physiological test is performed first, one can prophesy from the fall of pressure whether an abundant or a scanty crop of crystals will be obtained.

The case where the least result was obtained was the acute case of Beri-beri; this is what would have been anticipated. Here the fall of pressure was insignificant, and the crystals required searching for. The case where the best result was obtained was from the Beri-beri blood received from the Malay States (No. 3 in our list). Here the crystals thickly coated the watch-glass, and the fall of pressure is shown in one of the accompanying tracings.

The tracings which follow give some of our results in the performance of the physiological test. They show the effects of injecting the material from the case of Beri-beri just alluded to (fig. 16), from that of combined sclerosis (fig. 20), from that of disseminated sclerosis (fig. 19), and from that of alcoholic neuritis (fig. 18). In the first case, also, the effect after the injection of atropine is shown (fig. 17). This is typical of what occurred in all

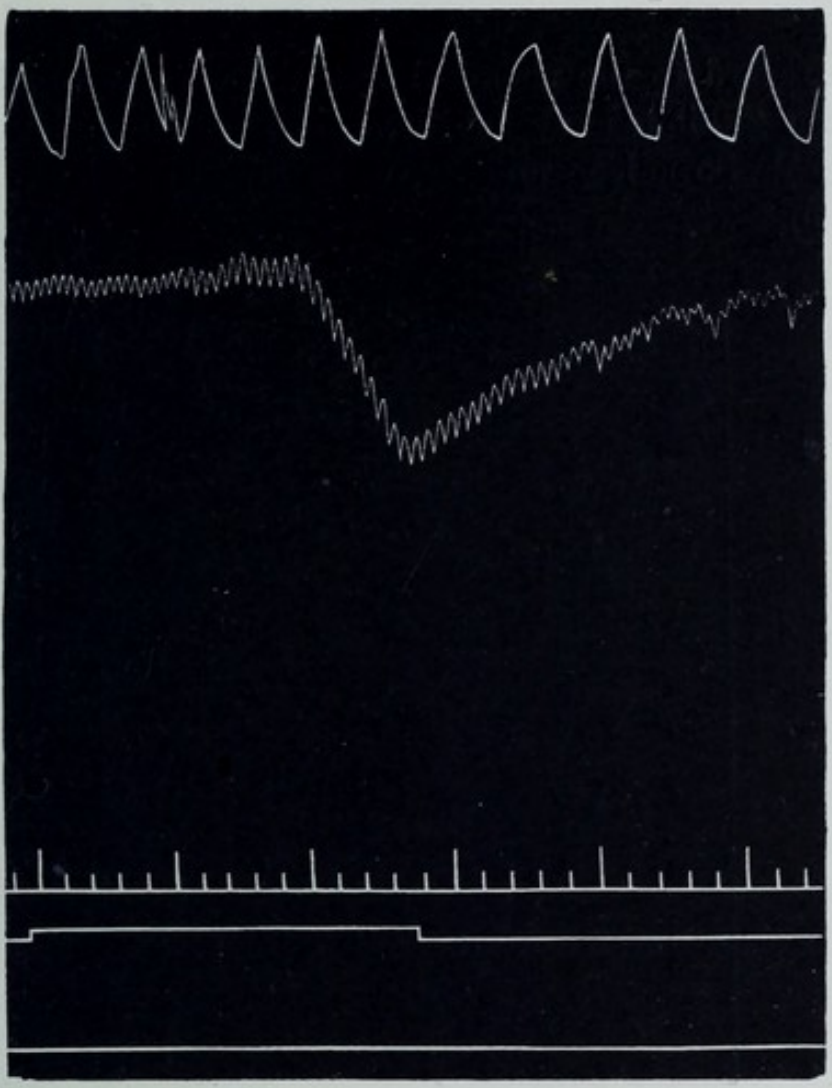

FIG. 16.—The uppermost line represents the respiration taken by the tambour method. The next line is the blood pressure from the carotid. The next is a time tracing in seconds; the next is the signal line, the raising of which indicates the period of the injection. The lowest line is the abscissa of the blood pressure. The various lines have the same meaning in all subsequent tracings. The respiration tracing is omitted in figures subsequent to fig. 17.

The injection produced no effect on respiration. This is true also for choline, and the similarity between these effects and those of choline on the blood pressure may be seen readily if our former tracings are consulted. All the tracings were taken from experiments on cats under A.C.E. mixture. All read from right to left. The actual volume of saline solution of the active material was in all cases 5 cc. This was injected into the external jugular vein. The numbers given with each tracing indicate the volume of the original blood to which this would correspond.

Fig. 16 represents the fall of arterial pressure produced by the injection of an amount equal to 10 cc. of the blood in the case of Beri-beri numbered 3 in the list on p. 68. This tracing and all that follow are of the original size.

cases ; the fall of pressure is either absent or replaced by a slight rise.

With regard to the chemical test, the various watch-glasses have been exhibited at meetings of the Physiological and Pathological Societies, and have admittedly borne out our contention.

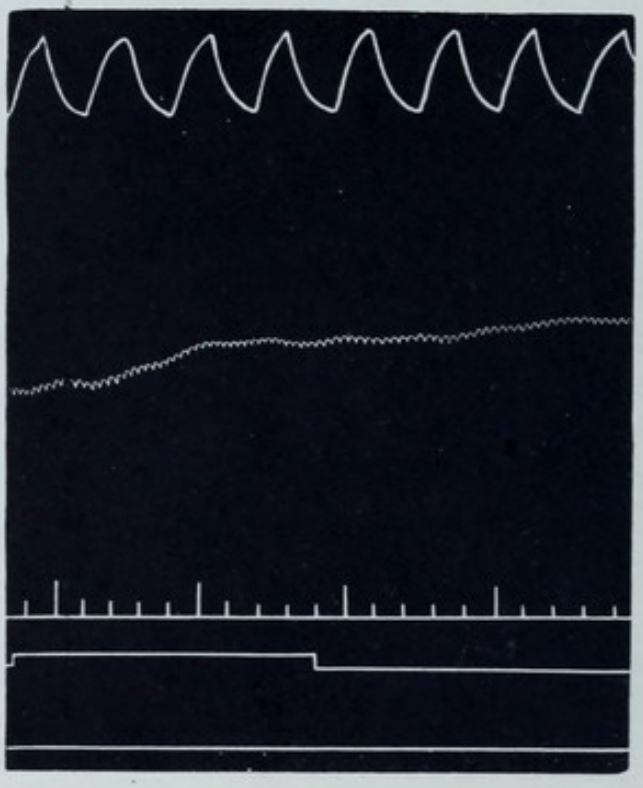

FIG. 17.—The result of injecting the same volume of the same solution in the same cat after atropine had been administered. There is now a rise of blood pressure.

These results are of some practical importance. A comparatively small quantity of blood will give the tests, and in cases where it is difficult to distinguish between serious cases of organic disease and cases of so-called functional neurosis, the performance of the tests described may come to the assistance of the practical physician in making his diagnosis. Choline does not pass into the urine,[1] so that the examination of that secretion would be insufficient in such cases.

[1] Mott and Halliburton, *loc. cit.*, p. 256.

(2) Experiments upon Animals.

In these experiments twenty cats were experimented upon. In the majority of cases both sciatic nerves were divided in the upper part of the thigh. During the operation the animal was anæsthetised with A.C.E. mixture, and antiseptic precautions rigorously adopted. Healing of the wound took place rapidly by the first intention in all cases but one. In this one case suppuration occurred, and the animal was killed three days after the operation. Another cat died suddenly a few hours after the operation. We have, therefore, only eighteen cats concerning which we have to record results.

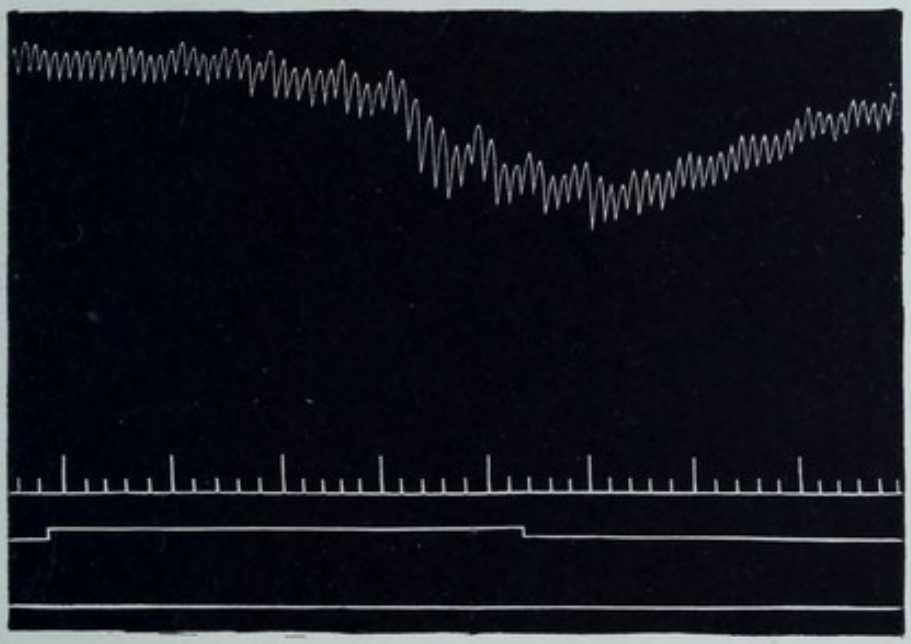

Fig. 18.—The result of injecting an amount equal to 30 cc. of blood from a case of alcoholic neuritis.

The animals suffered but little inconvenience; they were able to walk about in spite of the partial paralysis of the hind limbs; they walked on the heels of these limbs, and in consequence the fur was worn off here in those animals which were kept alive for sufficiently long periods. They were kept in warm comfortable hutches, were well fed, and soon became well nourished and sleek specimens of the feline tribe. Some wasting of the muscles of the hind limbs necessarily occurred in those kept alive for the longer periods.

The cut nerves were not sutured in the greater number of

cases, but union and regeneration took place in nearly all, provided the time which elapsed between the operation and the *post mortem* was long enough. In one or two cases the ends of the nerve of one side were sutured together with catgut. The details of the individual operations and main results are as follows :—

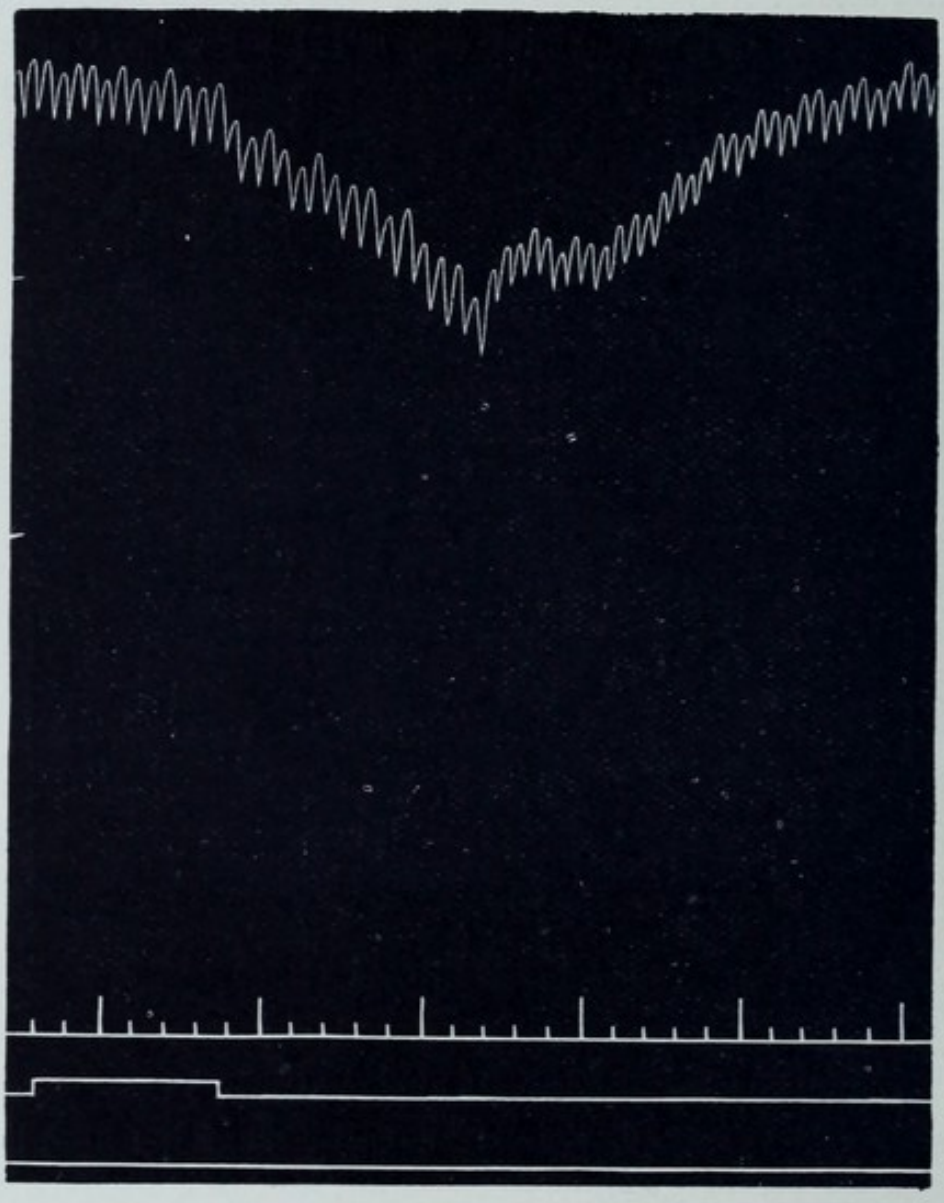

FIG. 19.--Result of injecting an amount equal to 20 cc. of blood from a case of disseminated sclerosis (early stage of the disease).

Cat K.—One sciatic nerve only divided. The animal was killed twenty-four hours later. The peripheral end of the cut nerve was stimulated mechanically and electrically ; well marked contraction followed. The divided nerve was compared with the

healthy nerve on the other side by means of faradisation, a du Bois Reymond coil with one cell being used. The weakest induction shock which produced a muscular response occurred with the secondary coil at 27 cm. on the healthy side and 25 in the case of the divided nerve. The divided nerve on subsequent examination, was found to be both histologically and chemically normal.

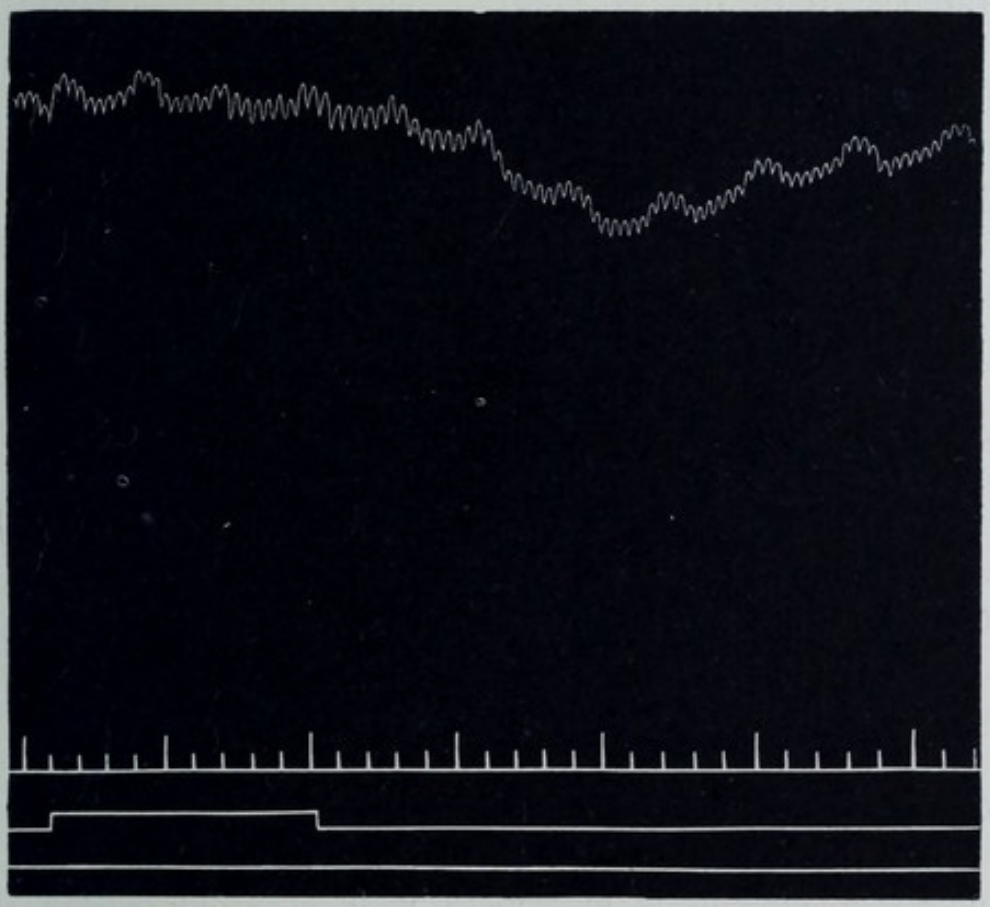

FIG. 20.—Result of injecting an amount equal to 30 cc. of blood from a case of combined sclerosis.

Cat A.—Both nerves divided. This animal was killed five days and two hours after the operation. Left nerve: muscles responded with the secondary coil at 20. Right nerve: muscles responded with the secondary coil at 25. The muscles on each side responded readily to mechanical excitation of the nerves. The nerves were practically healthy, both histologically and chemically.

Cat B.—Both nerves divided. This animal was killed three days after the operation. Left sciatic: muscles responded with

the secondary coil at 18. Right sciatic : muscles responded with the secondary coil at 22. The response to mechanical stimulation was slight. The nerves on chemical and histological examination show but little sign of degeneration.

Cat P.—Both nerves divided. This animal was killed three days and twenty hours after the operation. There was no muscular response on stimulating either nerve. Signs of commencing degeneration are evident.

Cat C.—Both nerves divided. This animal was killed four days and three hours after the operation. No response occurred on either side on stimulation. Signs of early stages of Wallerian degeneration are seen.

Cat E.—Both nerves divided. This animal was killed five days after the operation. In this animal no muscular response occurred on excitation of either nerve; and the same is true for the animals that were killed at later stages, until union and regeneration has taken place. Degenerative changes in the nerves are more marked.

Cat D.—Both nerves divided. This animal was killed six days after the operation. Degenerative changes in the nerves are about the same as in Cat E.

Cat R.—Both nerves divided. This animal was killed eight days after the operation.

Cat N.—Both nerves divided. This animal was killed ten days after the operation.

Cat Q.—Both nerves divided. This animal was killed thirteen days after the operation. In these three animals (R, N, Q) the degenerative change is extremely well seen by the Marchi method.

Cat J.—Both nerves divided. This animal was killed twenty-five days after the operation.

Cat O.—Both nerves divided. This animal was killed twenty-seven days after the operation.

Cat W.—Both nerves divided. The left nerve was sutured with catgut. This animal was killed twenty-nine days after the operation.

In these three cats (J, O, W) there was considerable wasting of the muscles supplied by the sciatics. Union of the divided nerves had occurred in all cases, but they were all irresponsive to stimulation. Little or no difference could be detected histologi-

cally or chemically in the two nerves of Cat W, one of which had been sutured. The Marchi reaction was still well marked, but there was evidence that absorption of the fatty products of degeneration was in progress. Phosphorised fat had almost, and in the case of Cat W entirely, disappeared.

Cat H.—Both nerves divided. This animal was killed forty-four days after the operation. Good union had occurred on both sides, but the nerves were irresponsive to stimulation. Degeneration had now advanced so far that there was almost complete removal of the products of degeneration. There are early signs of regeneration. Here also the wasting of muscles was very evident.

Cat T.—Both nerves divided; left sutured with catgut. This animal was killed forty-five days after the operation. In this animal for the few days preceding its death the paralysis was less marked on the sutured side.

The left (sutured) nerve responded to stimulation (secondary coil at 7·5 cm.); the right nerve did not respond. Examination of the left nerve showed early signs of regeneration; the right did not. We, however, found it difficult at this stage, or even in Cat S (sixty days after operation) to trace axis cylinders actually through the cicatricial tissue at the junction, although by the Stroebe method axis cylinders were found in the peripheral stump. A chemical examination of these nerves was unfortunately omitted.

Cat S.—Both nerves divided. This animal was killed sixty days after the operation. The right nerve had not joined up well; the left not at all. The right nerve responded to stimulation (secondary coil at 9); the left did not. The evidence of regeneration was not so manifest as in the last case, and the products of degeneration had not undergone so much absorption. This animal displayed much lethargy, and was ill nourished, as if it lacked vital reaction.

Cat I.—Both nerves divided. This animal was killed one hundred days after the operation.

Cat F.—Both nerves divided. This animal was killed one hundred and six days after the operation.

In these two cats (I and F) though the hind limbs were still thin, there was great recovery of function. The nerves responded well to both mechanical and electrical stimulation (secondary coil

at 16 to 18 cm.). The nerves, especially the sensory ones, showed well-marked regeneration, and chemically they had returned approximately to the normal state.

These results may be briefly summarised in the following way. Up to the third day the nerves remained excitable and approximately normal. Degenerative changes then set in and became well marked on the eighth day; from this time to the thirteenth day they were at their height. On the twenty-fifth day, and at periods later than this, union of the divided nerves had nearly always taken place. The twenty-ninth day marks the entire disappearance of phosphorus from the degenerated fat. Absorption and removal of the fat was nearly accomplished by the forty-fourth day. At the same period restoration of function began in the case of a sutured nerve; but where the nerves had not been sutured this was not seen until the sixtieth day. In the cats which were allowed to live one hundred days and longer, regeneration and restoration of function were well marked.

A point of some interest is the early date (44-45 days) at which the removal of degenerated products occurs in peripheral parts of the nervous system. In this there is a contrast to the central nervous system; there the Marchi reaction can be obtained in degenerated tracts many months after the lesion has occurred.

The further experiments performed in connection with these animals divide themselves into three sets.

(*a*) Experiments with the blood.

(*b*) Chemical examination of the nerves.

(*c*) Histological examination of the nerves.

(*a*) Experiments with the Blood of the Cats.

These may be very briefly stated. The methods adopted were the same as those already described in connection with human blood.

The blood of normal cats contains the merest traces of choline. A few crystals can generally be found by the platinum test. As stated at the commencement of this lecture, the most abundant yield from a normal animal was obtained from the blood of a young kitten. But the quantity was not sufficient to give, with the amount injected, the physiological test.

When signs of degeneration set in (fourth day) the quantity of choline in the blood increased. As with human blood, the chemical and physiological tests gave throughout corresponding results. The best yield of crystals, and the most marked fall of blood pressure we obtained was in the case of the eight-day cat. This fall was abolished by atropine. On the thirteenth day there

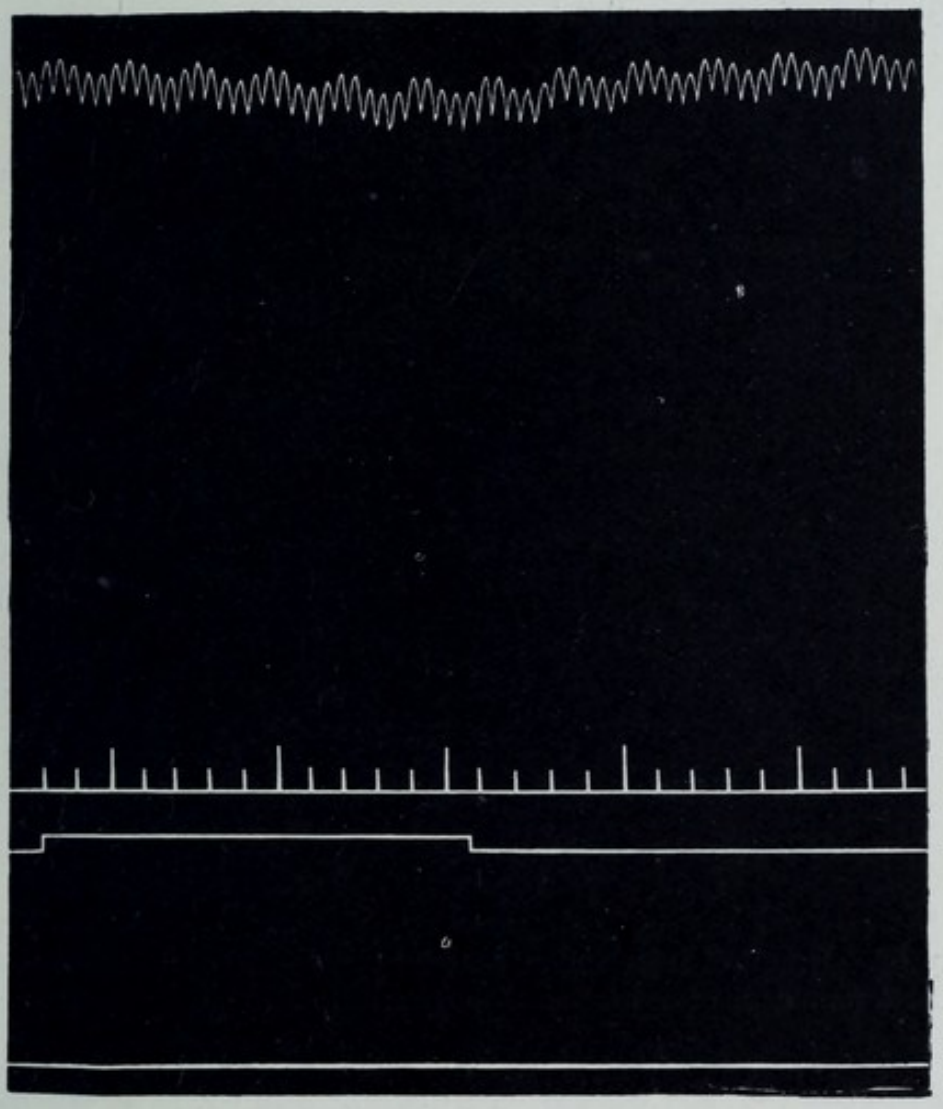

FIG. 21. Result of injecting an amount equal to 20 cc. of blood of normal kitten.

was still a marked positive result. From this time onwards the evidence of choline steadily diminished, until the normal was reached in the later stages of the degenerative process. The amount slightly increased when regeneration set in, and this is another piece of evidence in favour of the views already advanced concerning lecithin metabolism (see p. 26).

I select a few tracings to illustrate these facts.

It will be seen that the material from normal blood (fig. 21), from blood of an animal in which degeneration had not commenced (fig. 22), and from blood of an animal in which degeneration was complete (fig. 29), gave negative results.

In cases where degeneration had set in (blood of six-day cat, fig. 23), or had commenced to subside (twenty-five-day cat, fig. 28), the effect was slight.

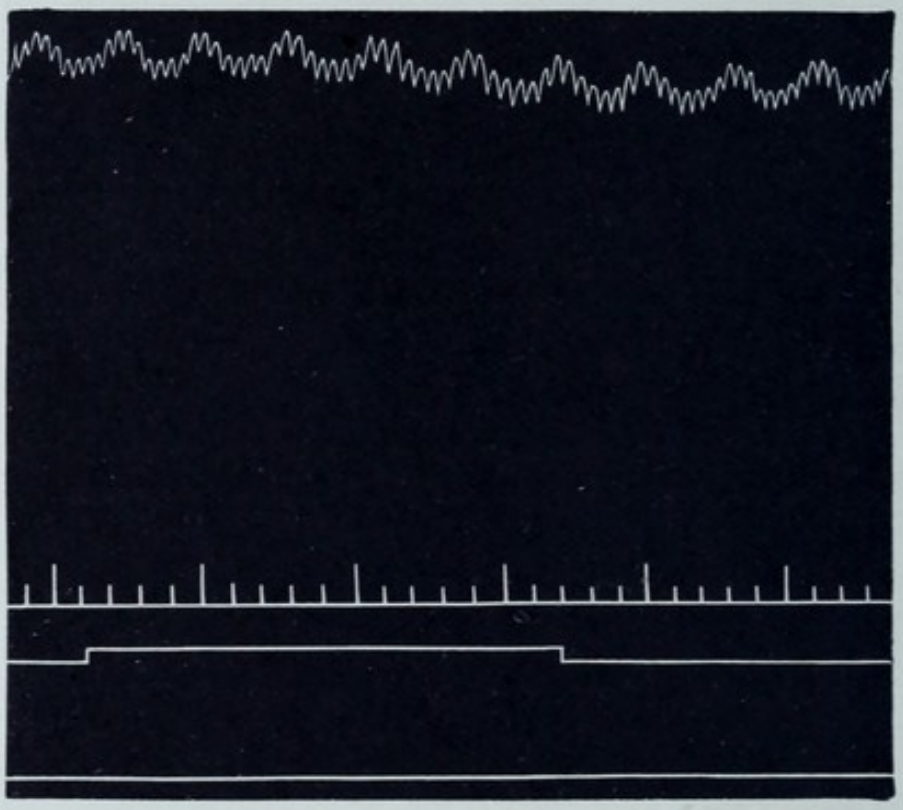

FIG. 22.—Result of injecting an amount equal to 20 cc. of blood obtained from Cat A (two days after section of both sciatic nerves).

In the case of the cats where degeneration was at its height, the physiological effect, fall of blood pressure, was very marked (figs. 24 and 26), and the fall was absent after atropinisation (figs. 25 and 27). This was true in all cases; wherever the material produced a fall of blood pressure before the subcutaneous injection of atropine, it failed to do so afterwards, or even produced a slight rise. This, in conjunction with the chemical test, conclusively proved the presence of choline. The slight effects obtained when regeneration set in were about equal to those seen in figs. 23 and 28.

As in the case of human blood, we attempted to make some quantitative estimation of the amount of choline present, by recrystallising the platinum salt and weighing it. We do not regard our figures as very trustworthy, for the following reasons :—

(1) Control experiments with pure choline in organic mixtures gave very variable results ; (2) the choline in our specimens was

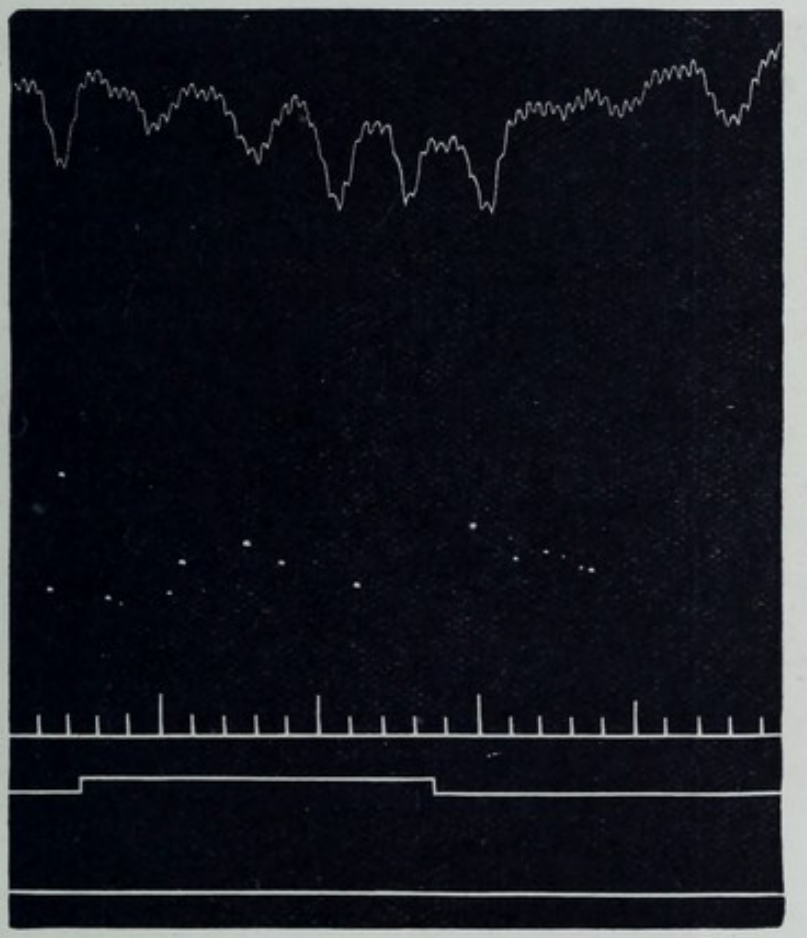

FIG. 23.—Result of injecting an amount equal to 20 cc. of blood obtained from Cat D (six days after section of both nerves).

mixed with small quantities of other organic substances ; (3) the numbers obtained are so small, that extremely minute errors (*i.e.*, legitimate errors of experiment) will make comparatively large variations. The amount of blood at our disposal for such experiments seldom exceeded 50 cc., and was usually less than this. For this reason, we have relied upon the rougher method of a microscopic survey of the watch-glasses as explained on p. 69. This method hardly appeals to those who read about it

like a table of quantitative estimations, but it is very convincing to those who actually see the preparations.

With this reservation, we may say that the method of quantitative analysis gave us, in the cats the blood of which contained most choline, numbers which corresponded to a percentage of from 0·0052 to 0·0078. In the animals in which the chemical and physiological tests indicated a low percentage of choline, the corresponding numbers varied from 0·0011 to 0·0037.

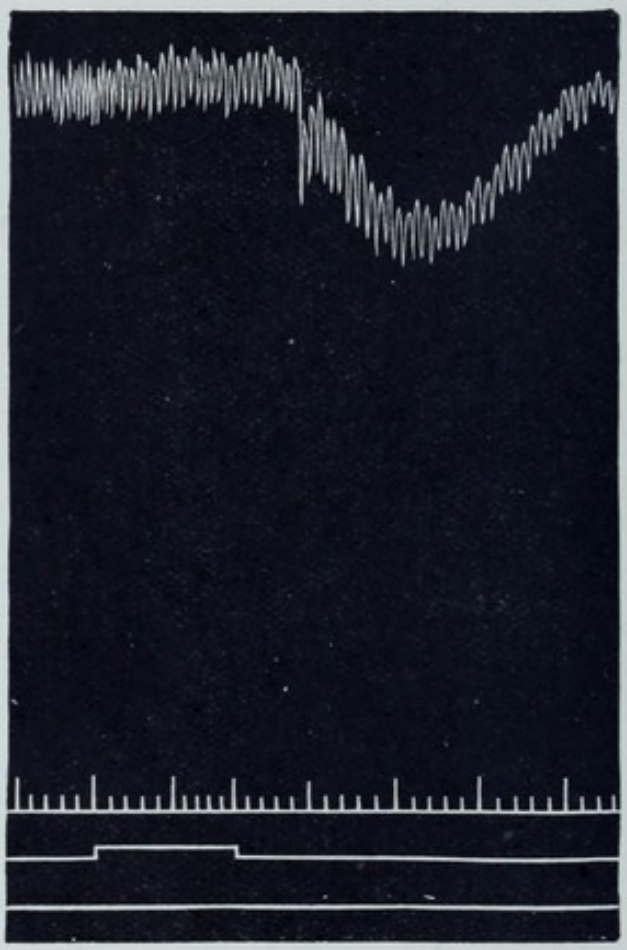

FIG. 24.—Result of injecting an amount equal to 30 cc. of blood obtained from Cat R (eight days after section of both nerves).

(*b*)—CHEMICAL EXAMINATION OF THE CATS' NERVES.

The nerves were carefully dissected out, weighed, dried to constant weight at 110° C., and again weighed. The dry residue was used for the determination of phosphorus. Considering the small weight of the nerves, we judged this would give more accurate results than any attempt to isolate the fatty material, and determine either the phosphorus or protagon in that.

The dried nerve was soaked in 5 per cent. hydrochloric acid

for three weeks in order to get rid of inorganic phosphates. Such treatment apparently gets rid of the phosphorus combined as nuclein or nucleo-proteid; for in many of the nerves of later date there was considerable nuclear proliferation seen microscopically, and yet little or no phosphorus was obtained from the nerves after treatment in this way with hydrochloric acid. The

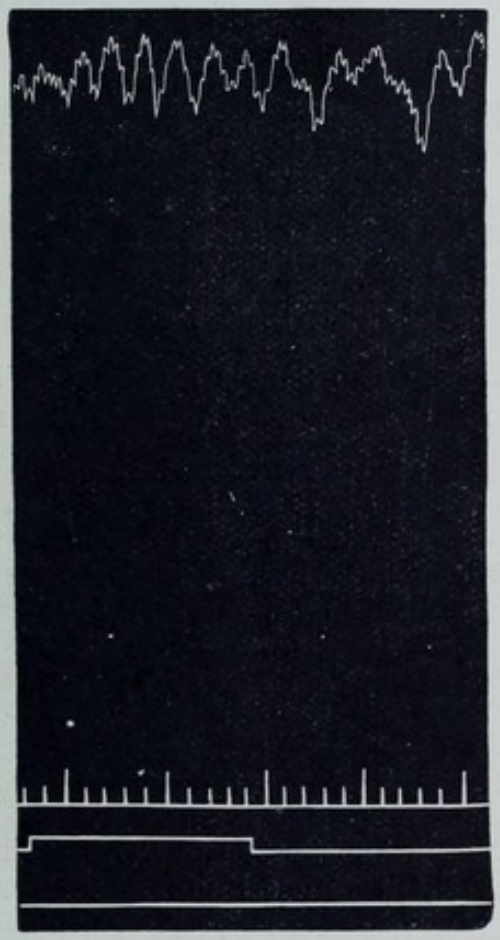

FIG. 25.—Result of injection of the same amount after atropine.

phosphorus we did obtain came, therefore, either wholly or chiefly from the phosphorised fat (lecithin or protagon). The nerves were then dissolved on the water bath at 100° C. in fuming nitric and sulphuric acids, to which an occasional pinch of potassium chlorate was added. The heating with acid was continued for many hours. The phosphate so formed was precipitated by ammonium nitro-molybdate. The yellow precipitate so obtained was washed, and dissolved in dilute ammonia, then precipitated by magnesia mixture; this precipitate was incinerated and weighed as magnesium pyrophosphate.[8]

[8] A full description of this method of phosphorus estimation is given in the *Journ. of Physiology*, xiii., pp. 814, 821, 1892.

The following tables give the results:—

TABLE X.

PROPORTION OF WATER AND SOLIDS IN SCIATIC NERVES OF NORMAL CATS.

	Percentages	
	Water	Solids
Cat 1	66·479	33·521
,, 2	65·803	34·197
,, 3	66·554	33·446
,, 4	62·559	37·441
Average	65·349	34·651

TABLE XI.

PROPORTION OF WATER AND SOLIDS IN SCIATIC NERVES FROM CATS OPERATED ON, IN WHICH THE NERVES WERE STILL EXCITABLE.

	Percentages	
	Water	Solids
Cat K. 1 day after operation	64·199	35·801
,, A. 2 days, 5 hours after operation ,, B. 3 days after operation	64·618	35·185
Average	64·507	35·493

TABLE XII.

PROPORTION OF WATER AND SOLIDS IN SCIATIC NERVES FROM CATS OPERATED ON, IN WHICH DEGENERATION OCCURRED.

	Percentages.	
	Water	Solids
Cat P. 3 days, 20 hours after operation	73·941	26·059
,, C. 4 days after operation .. .. ,, E. 5 ,, ,, ...	68·018	31·982
,, D. 6 ,, ,, ...	69·325	30·675
,, R. 8 ,, ,, ...	68·231	31·769
,, N. 10 ,, ,, ...	70·718	29·282
,, Q. 13 ,, ,, ...	71·257	28·743
,, J. 25 ,, ,, ...	67·886	32·114
,, O. 27 ,, ,, ...	72·141	27·819
,, W. 29 ,, ,, right nerve (joined spontaneously)	72·505	27·495
left nerve (sutured)	72·601	27·399
,, H. 44 ,, ,, ...	72·641	27·359
,, S. 60 ,, ,, left nerve (not united well)	72·646	27·354

TABLE XIII.

PROPORTION OF WATER AND SOLIDS IN SCIATIC NERVES FROM CATS OPERATED ON, IN WHICH REGENERATION HAD COMMENCED.

		Percentages.	
		Water	Solids
Cat S.	60 days after operation. Right nerve. Early stage of regeneration	72·645	27·354
,, I.	100 days after operation. Regeneration well marked	63·853	36·147
,, F.	106 days after operation. Regeneration well marked	68·531	31·469
	Average of I. and F.	66·192	34·308

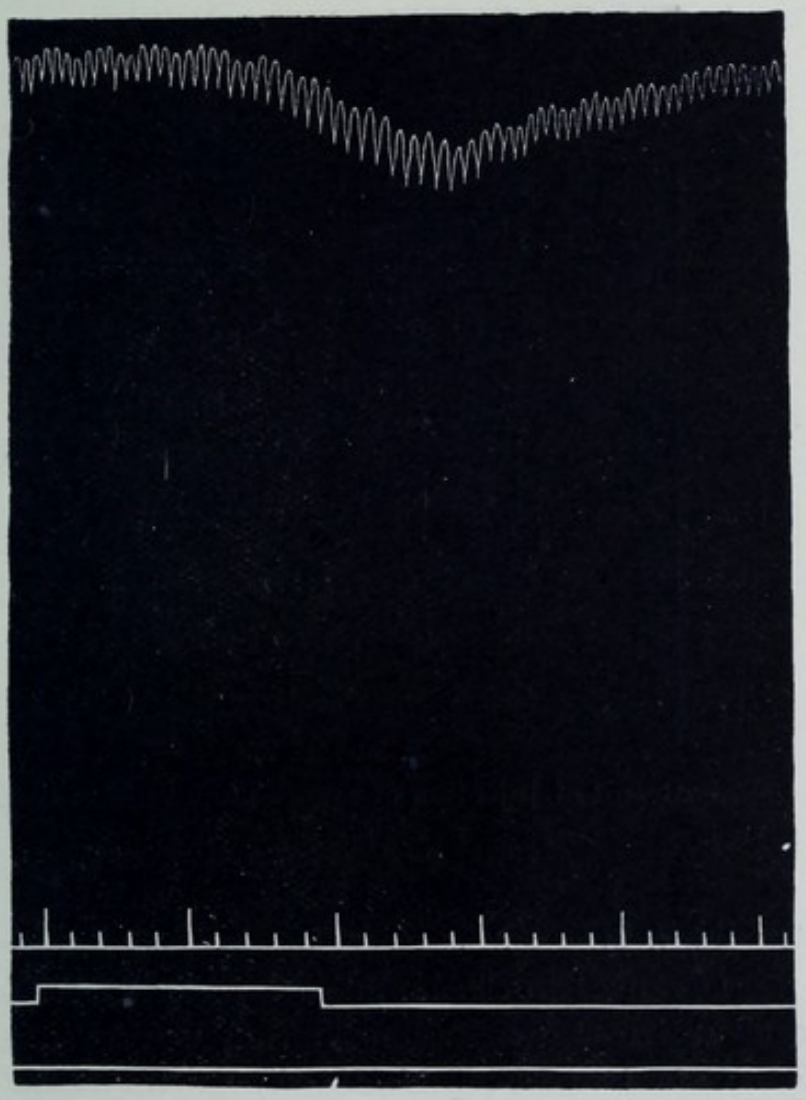

FIG. 26.—Result of injecting an amount equal to 25 cc. of blood obtained from Cat N (ten days after section of both nerves).

From these four tables we see that, as far as water and solids are concerned, before degeneration sets in there is no marked change in the proportion; the slight difference between Tables X. and XI. comes well within the limits of individual variation in normal cats. If we compare these with the cats

FIG. 27.—Result of injection of same amount after atropine.

killed later there is a marked rise in the percentage of water, and this rise advances in the main *pari passu* with the progress of the degenerative state (Table XII.). But after restoration of function (Cats I and F, Table XIII.) the proportion of water to solids returns approximately to normal.

We now come to the phosphorus estimations.

TABLE XIV.

CATS' SCIATIC NERVES.

Animal	Days after section	Weight in grammes of nerves taken		Yield in grammes of $Mg_2P_2O_7$	Phosphorus per cent. in dry tissue
		Fresh	Dry		
Cat 1	Normal	1·793	0·620	0·026	1·16
Cat 2	,, ...				
A.	2	3·297	1·007	0·035	0·97
B.	3				
P.	3 days, 20 hours				
C.	4	3·594	1·1247	0·039	0·97
E.	5				
D.	6				
R.	8	1·193	0·379	0·0078	0·57
N.	10	1·185	0·348	0·004	0·32
Q.	13	1·79	0·5145	0·005	0·27
J.	25	1·655	0·5315	traces	
O.	27	1·215	0·338	traces	
W.	29 Right nerve joined spontaneously	0·478	0·1314	0	0
	Left nerve sutured ..	0·502	0·1375	0	0
H.	44	1·272	0·348	0	0
S.	60 Left nerve not joined ..	0·505	0·139	0	0
	Right nerve, regeneration beginning	0·393	0·1075	0·0003	0·08
I.	100	2·161	0·741	0·045	0·93
F	106				

This table shows that in the early stages of degeneration the amount of phosphorus is not far removed from the normal. It is on the eighth day, when the Marchi reaction becomes well marked, that the first great drop in the percentage of phosphorus occurs. It continues to diminish and has practically disappeared by the twenty-fifth, and absolutely by the twenty-ninth day. With signs of commencing restoration of function it reappears, and is near the normal in the last two cats where regeneration had occurred.

We have confined our observations to the peripheral parts of the nerves.[9]

[9] Noll (*loc. cit.*) in some of his experiments examined the central stump of the divided nerve. He was able to perform some of his work on large animals (horses), and so could obtain sufficient material from the central end for analysis. Corresponding to what is termed "disuse atrophy" he found some diminution in the amount of protagon in this region, but the lessening was not so marked as in the peripheral end of the nerve. In the

We can now pass on to the histological side of the subject, after which we shall be better able to correlate our facts and draw general conclusions.

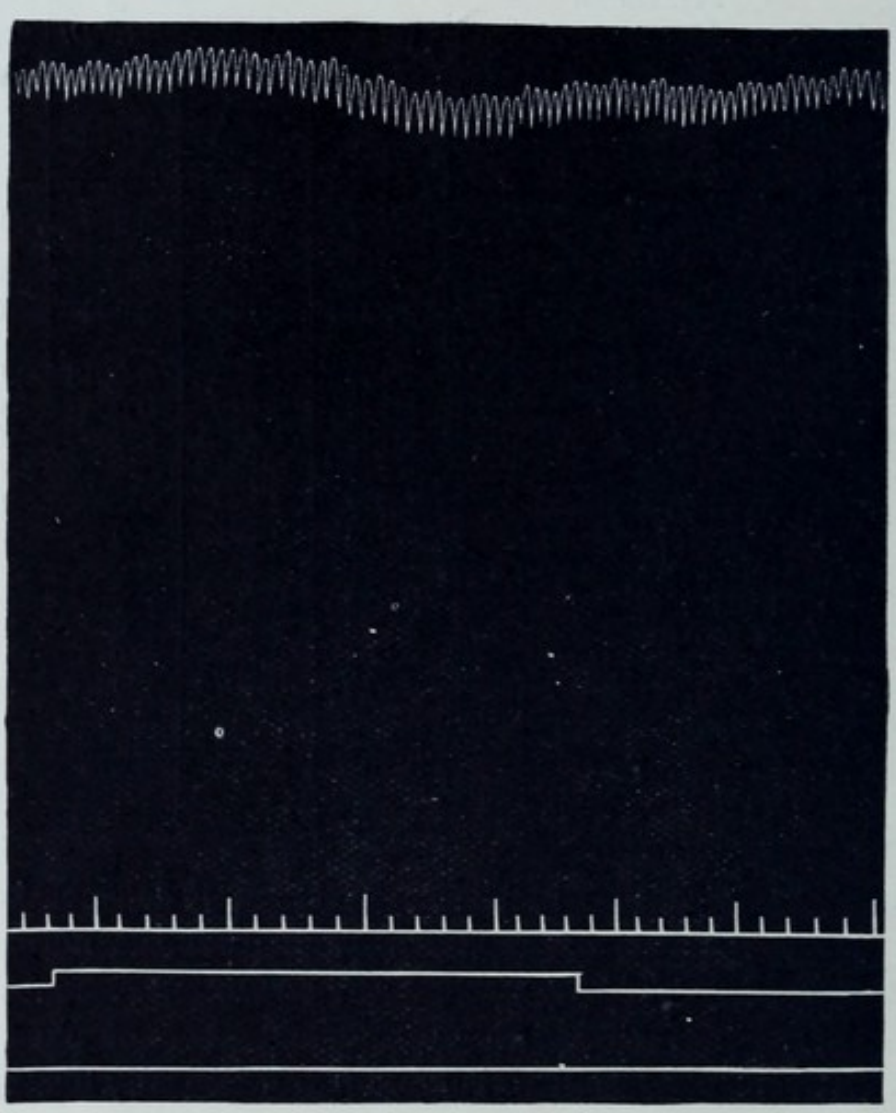

FIG. 28.—Result of injecting an amount equal to 25 cc. of blood from Cat J (twenty-five days after section of both nerves).

(*c*) HISTOLOGICAL EXAMINATION OF THE CATS' NERVES.

Portions of motor and sensory branches of the peripheral portion of the cut nerves were in each case taken for micro-

---

majority of his experiments he estimated protagon, not phosphorus as we have done. He puts the date of disappearance of the phosphorised fat at twenty-eight days. This date and many other of his facts fit in very well with our work.

scopic investigation. In the cases where union had occurred, the junction was also reserved for the same purpose.

Each piece of nerve was pinned out across a cork frame, so that the tissue was entirely surrounded by fluid.

They were placed (*a*) in Marchi's fluid; (*b*) in Müller's fluid.

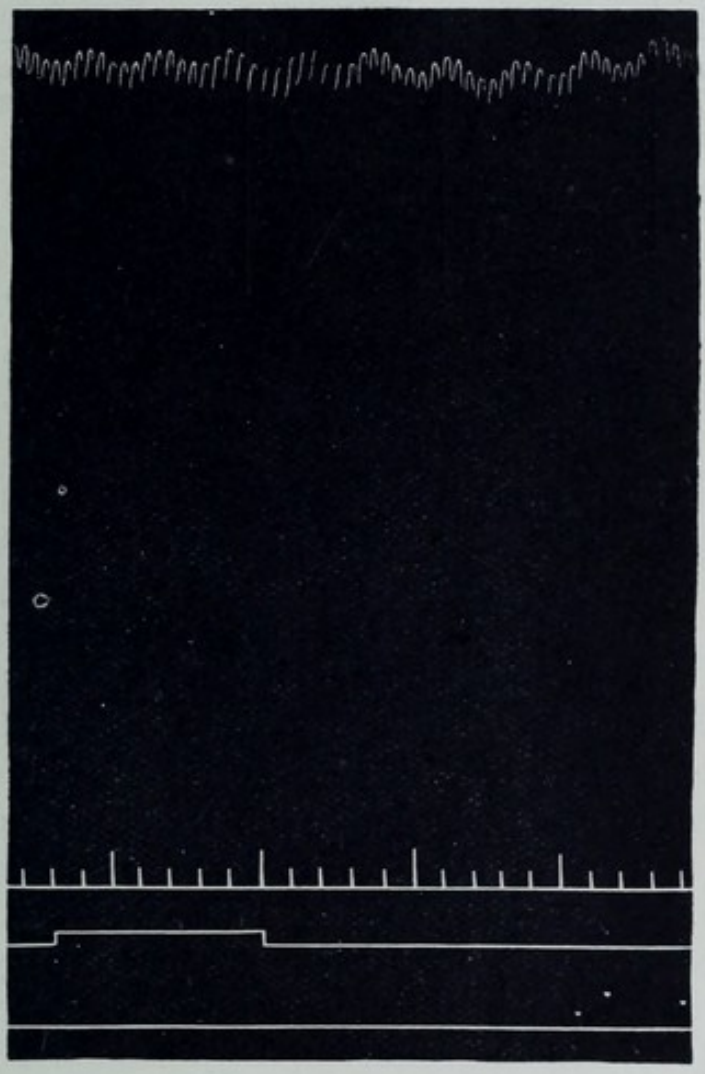

FIG. 29.—Result of injecting an amount equal to 25 cc. of blood from Cat H (forty-four days after section of both nerves).

Portions of the former were used for teasing, and for sections, transverse and longitudinal. Portions of the latter were used for teasing and for sections; portions also were, after ten days, placed in Marchi's fluid, but the results obtained by this method differed in no essential way from those obtained by placing the nerve into Marchi's fluid direct. This simple method of placing the nerve directly into Marchi's fluid enables one to obtain speci-

mens within a week, but it is not applicable to the central nervous system.

The result, comparing the two methods, may be stated in tabular form as follows:—

	Nerve-fibres		White matter of central nervous system	
	Healthy	Degenerated	Healthy	Degenerated
1. MARCHI direct	Dark greyish green	Black; adipose tissue takes the same colour	Black on surface; the fluid does not penetrate well to the interior	Black
2. MARCHI after MÜLLER	Greyish green, but not quite so dark	Black	Greenish all through	Black

In order to use the Marchi reaction for the central nervous system, it is essential to harden first in Müller's fluid, for the healthy nerve-fibres, unprotected as they are by a primitive sheath, would stain black if placed directly into Marchi's fluid.

The actual colour obtained by the direct Marchi method is seen in the coloured plates appended. Plate II. represents the condition of the nerve in transverse section, which was obtained from a cat two days after the operation of cutting the nerve. The fibres were healthy, the greyish green colour of the medullary sheath is seen, and the axis cylinder with its tubular fibrils can also be distinguished. Plate III. represents the staining obtained from a cat's nerve ninety-two hours after the operation; mixed with numerous fibres which still take on the normal appearance, are others in which the myelin ring is black; a few are black all through; evidently here the axis cylinder has ruptured and retracted, leaving the nerve tube filled with a lump of degenerated fat. The individual fibrillæ of the axis cylinder can no longer be made out in any fibre. In Plate IV. (nerve from an animal ten days after the operation) the degenerative condition is much more marked. In this case the phosphorus obtained on analysis had sunk to about a quarter of the normal. In the drawings, some fat cells of the surrounding adipose tissue are included; they take a deep black colour in all cases, exactly similar to the colour of the thoroughly degenerated fibres.

These drawings have been made in colour, because the

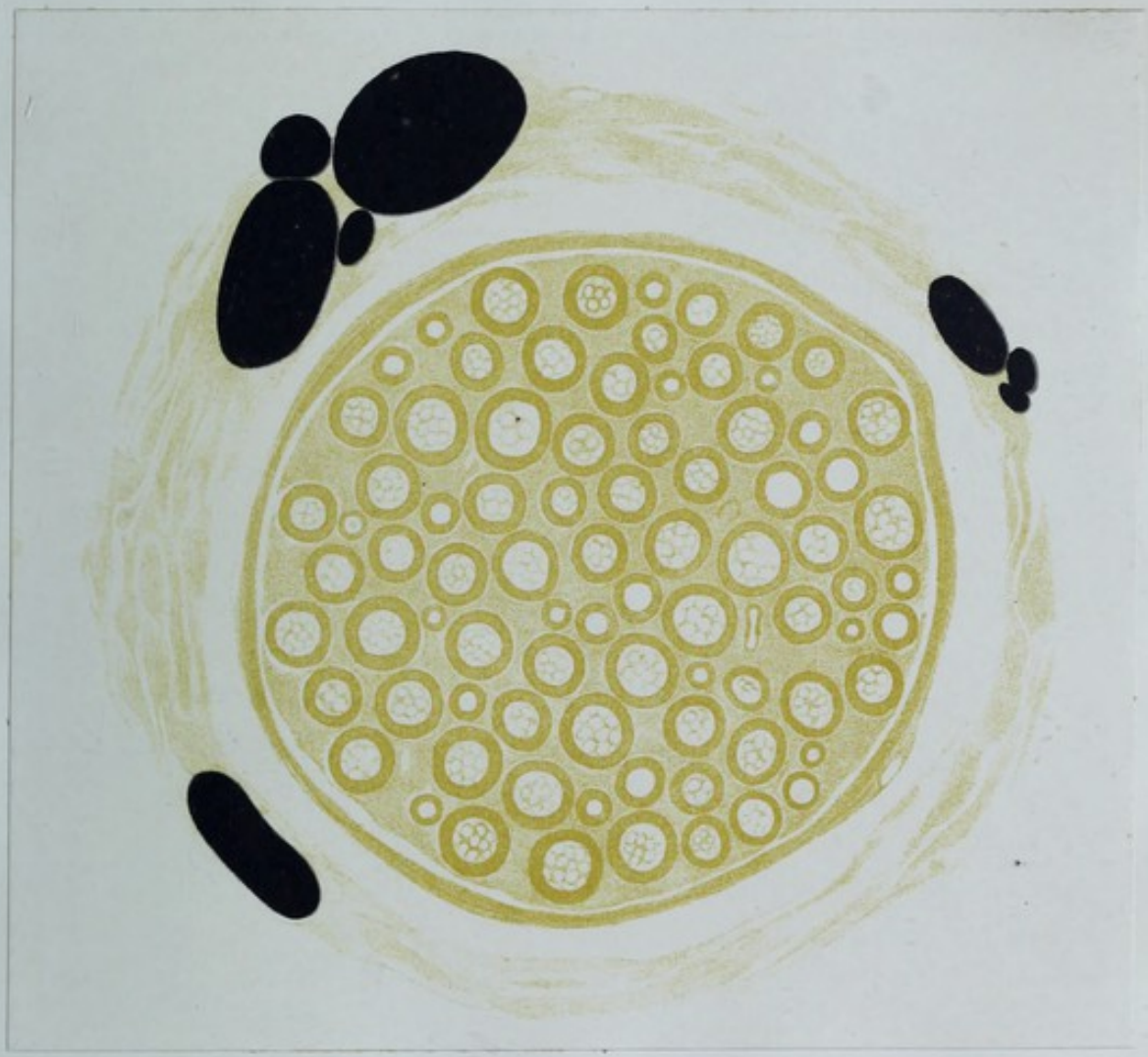

PLATE II.—Transverse section of nerve, two days after operation (Cat A). Direct MARCHI method. 425 diameters.

PLATE III.—Transverse section of nerve, 92 hours after operation (Cat P). Direct MARCHI method. 425 diameters.

PLATE IV.—Transverse section of nerve, 10 days after operation (Cat N). Direct MARCHI method. 425 diameters.

PLATE V.

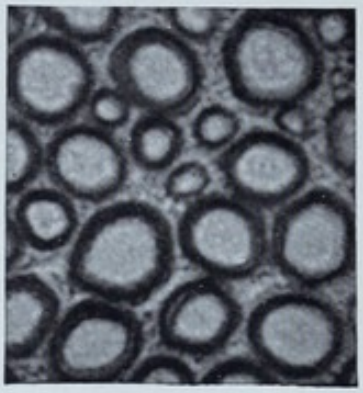

FIG. 30.

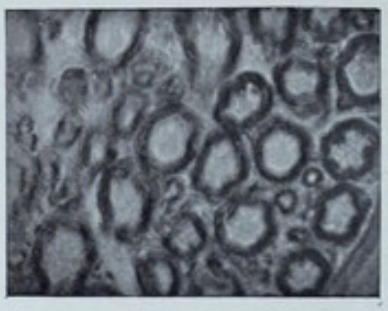

FIG. 31.

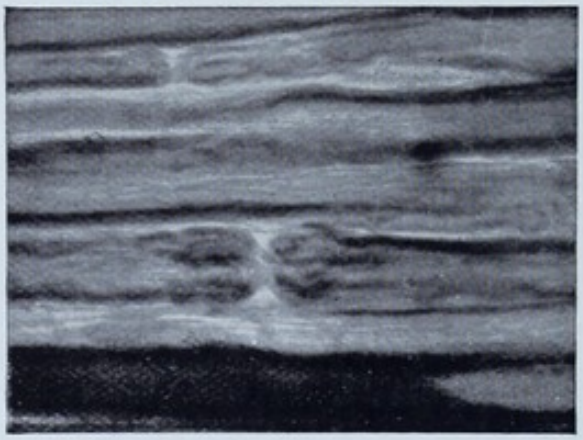

FIG. 32.

FIG. 33.

FIG. 30.—Transverse section of motor nerve, 53 hours after operation (Cat A). Method—MARCHI's fluid direct. The photograph is printed darkly to show tubular structure of the fibrils of the axis cylinder. 700 diameters.

FIG. 31.—Transverse section of nerve, 3 days after operation (Cat B). Same method. 500 diameters.

FIG. 32.—Same nerve in longitudinal section. Same method. 600 diameters.

FIG. 33.—Longitudinal section of nerve, 92 hours after operation (Cat P). Same method. 700 diameters.

photo-micrographs by which the remainder of the paper is illustrated, hardly show the difference of tint in healthy and degenerated tissues; much here depends on the depth of the printing; still, in marked cases the difference is seen even here.

Teased specimens from the nerves hardened by both methods were stained with logwood and eosin and mounted in Farrant's solution.

The sections were cut after imbedding in paraffin. The sections were 10 μ in thickness. Both transverse and longitudinal sections were mounted in series. Some of these were stained by the Stroebe method, others with logwood and eosin, and others by the Marchi-Pal method The great majority of the observations, however, were made on the sections obtained after hardening in Marchi's fluid direct, without extraneous staining.

It is obviously impossible to describe all the microscopic specimens we have made. The main results of the examination have been stated already on pp. 74-77. We have made photo-micrographs of our most typical specimens ; some of these are here reproduced, and our object will be attained if we devote our description of results mainly to these. They show the different stages in the degenerative process.

Cat A.—Nerves removed fifty-three hours after the operation. The nerves in either transverse or longitudinal section show no departure from the normal. With Marchi's fluid the medullary sheath takes on the greyish-green colour before alluded to. In transverse section (fig. 30, Plate V.) the tubular character of the fibrils of the axis cylinder[10] is shown.

This photo-micrograph is darkly printed to show this point: the myelin sheaths are not really so dark as the print would indicate (*see* coloured drawing, Plate II.).

Cat B.—Nerves removed seventy-two hours after the operation. The accompanying figures (figs. 31 and 32, Plate V.) represent respectively transverse and longitudinal sections of a motor nerve. It will be remembered that this nerve was still excitable, and its percentage of phosphorus approximately normal. In longitudinal section no change is observable ; in transverse section the myelin rings are seen to be for the most

[10] *See* Schäfer, " Quain's Anatomy," Tenth Edition, 1891, vol. i., part 2, p. 311.

part crinkled in outline, but still stain greyish-green; the well-defined tubular character of the fibrillæ of the unstained axial core is no longer visible.

Cat P.—Nerves removed ninety-two hours after the operation. Fig. 33, Plate V., represents a longitudinal section of a motor bundle. There is here breaking up of the myelin sheath into short segments of irregular length; this sheath is stained a blackish colour, and in some cases the whole fibre appears either completely or partially filled with the same material. The axis cylinder can be seen here and there broken across and retracted; this allows the myelin droplet to fill the tube in these situations. The degenerated fatty matter, where the change is most intense, stains just like the fat cells of the surrounding adipose tissue. This is obviously the beginning of the chemical change, though the loss of phosphorus could not be detected chemically, partly because of a considerable admixture with nerve-fibres whose sheath still takes the greyish-green colour of the normal state; these appear lighter in the print. All shades between this and the deep black are also seen. It is also possible that though the dissociation of the phosphorised constituent of the fat has taken place in the degenerated fibres, the removal of the phosphoric acid does not occur immediately after dissociation.

The nerves in this animal were not excitable.

On referring to the table of analyses (Table XII., p. 84) it will be noted that the increase of water was in this case a very marked feature. The explanation of this is well seen in the transverse section (Plate III.), the separation of fibres from one another by fluid, and the increased size of the lymph channels and spaces, are very well shown.

Cat C.—Nerves removed four days, three hours after operation. The changes are very similar to those just described; the breaking up of the medullary sheath is somewhat more pronounced in certain fibres, whereas in others it is not (*see* longitudinal section, fig. 34, Plate VI.).

Cats E and D.—Nerves removed five and six days respectively after operation. The nerves are but little different from those of Cat C. Comparative results in different animals with different resisting power are obviously difficult to make, but if anything the sections examined show less degenerative change in Cat D (six days) than in Cat E (five days).

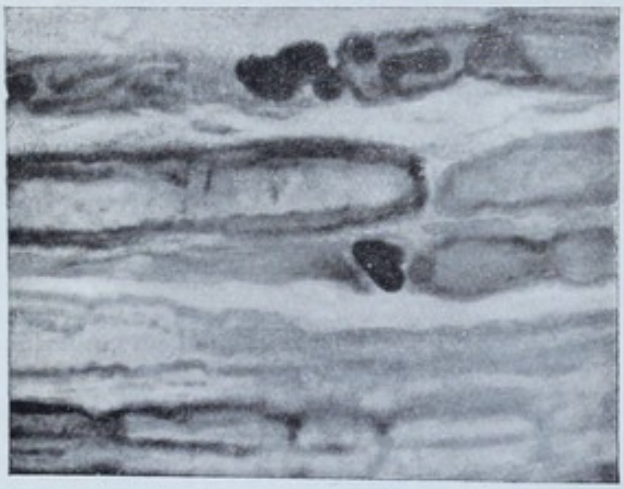

FIG. 34.

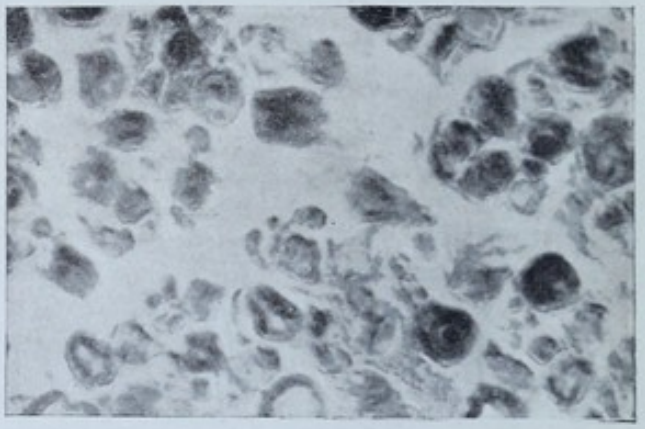

FIG. 35.

FIG. 36.

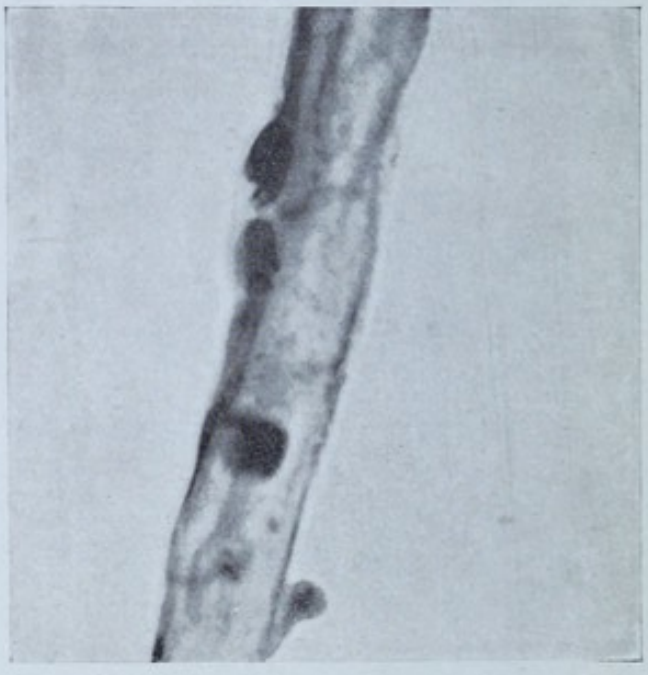

FIG. 37.

FIG. 34.—Longitudinal section of nerve, 99 hours after operation (Cat C). Method—MARCHI's fluid direct. 600 diameters.

FIG. 35.—Transverse section of nerve, 8 days after operation (Cat R). MÜLLER's then FLEMMING's solution. 700 diameters.

FIG. 36.—Same nerve in longitudinal section. Same method. 450 diameters.

FIG. 37.—Single fibre from nerve of same animal, to show multiplication of nuclei of the primitive sheath. Teased specimen; hardened in MÜLLER's fluid, washed, stained with logwood, mounted in FARRANT's solution. 870 diameters.

PLATE VII.

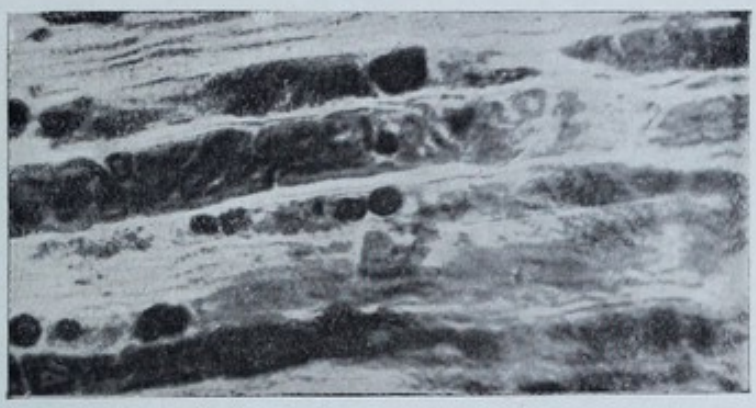

FIG. 38.

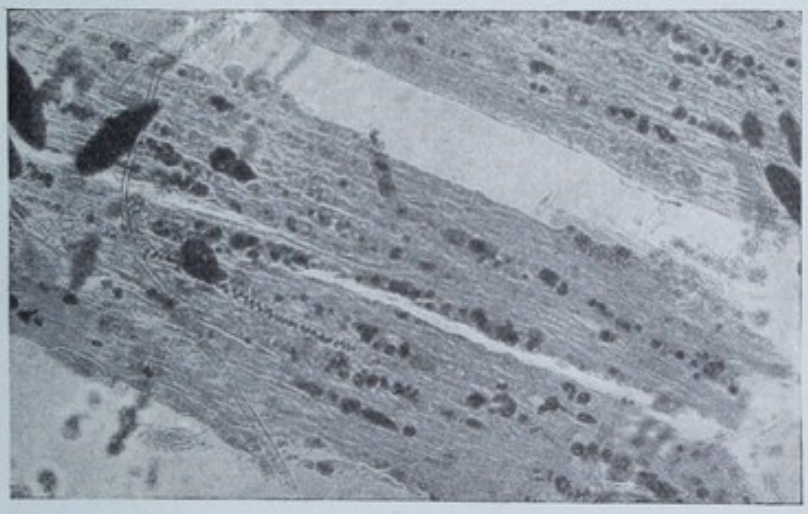

FIG. 39.

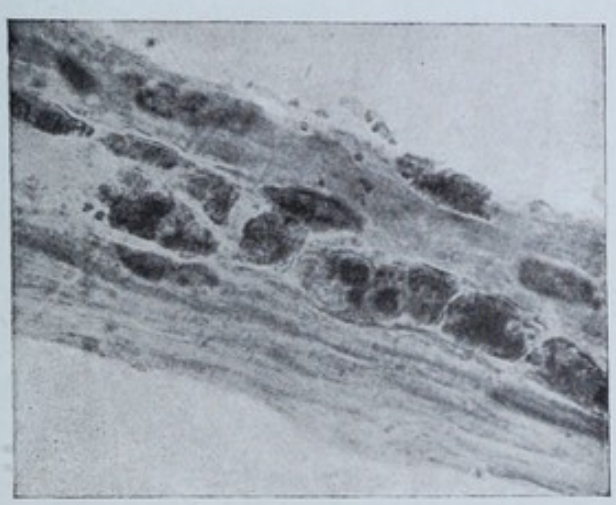

FIG 40.

FIG. 38.—Longitudinal section of nerve, 10 days after operation (Cat N). Method—MARCHI's fluid direct. 600 diameters.

FIG. 39.—Longitudinal section of nerve, 27 days after operation (Cat O). Same method. 500 diameters.

FIG. 40.—Longitudinal section of nerve from same cat. Method—MARCHI's fluid direct, then logwood. 450 diameters.

Cat R.—Nerves removed eight days after operation. Here there is a very marked increase in the degenerative change. The transverse and longitudinal sections (figs. 35 and 36, Plate VI.) show hardly a fibre which in some part of its course does not take on the black stain. The enlargement of the lymphatic channels is also marked. On referring to Table XIV. (p. 87) it will be seen that at this stage occurred the first great fall in the percentage of phosphorus. It had sunk to half the normal. Further evidence that not only dissociation of the lecithin molecule into its constituent parts, but also removal of the products of such change had begun to occur, is derived from the fact that at this date most choline was found in the blood (*see* tracing, fig. 24). Up to this point increase in the nuclei of the neurilemma had been looked for without success. It was now seen very well (*see* fig. 37, Plate VI.). It rather looks as though this increase in the nuclei was the result of the irritation of the presence of degenerative products.

Cat N.—Nerves removed ten days after the operation. The degenerative change is still better marked. The accompanying photo-micrograph (fig. 38, Plate VII.) speaks for itself. A transverse section of this nerve is shown in the coloured drawing (Plate IV.).

The percentage of phosphorus has here sunk to 0·32, or about a quarter of the normal.

Cat Q.—Nerves removed thirteen days after the operation. No noteworthy change has occurred in the microscopic appearances. The percentage of phosphorus has sunk to 0·27.

Cat O.—Nerves removed twenty-seven days after the operation. Here there is still considerable evidence of the Marchi reaction; the black staining of the degenerated myelin is precisely the same as that of the cells of adipose tissue which also appear in the photo-micrograph (fig. 39, Plate VII.).

The products of degeneration have, however, been either wholly or in part removed by absorption, for practically no phosphorus could be obtained on analysis, and the blood was by this date almost free from choline (*see* tracing, fig. 28). But a new point is here seen, for the fat is also beginning to be absorbed, and the part played in its removal by the phagocytic action of certain cells is illustrated in fig. 40, Plate VII. Whether the cells which are seen there crowded with the fat particles are

in part phagocytes we are unable positively to state, but we are inclined, from a careful study of our specimens, to take the view that they are mainly neurilemmal in origin.

Cat H.—Nerves removed forty-four days after the operation. Here the process of absorption is all but complete. The whole nerve bundle has shrunken, and little but the sheaths, either empty or filled with undifferentiated material, is to be seen. A few black-staining fat droplets are still visible, and in some bundles are even fewer than in the one represented in the photomicrograph (*see* fig. 41, Plate VIII.).

Some of the enlarged cells of the primitive sheath still contain fat granules (varying from 1 to 0·7 μ in diameter), but the majority are free from these, and present the elongated appearance shown in fig. 42, Plate VIII. The possibility that they may form an important factor in regeneration will be discussed later. At this date additional evidence of the completeness of the removal of the degenerated products is derived from the chemical examination of the nerves, which contained no phosphorus whatever.

The figures here given (41 and 42) are from a motor branch of the nerve; the sensory branches have practically the same appearances, but in transverse sections stained with Van Giessen's fluid many very minute tubes can be seen with central axis cylinders.

Cat F.—Nerves removed 106 days after the operation. We take this as an instance of a case where regeneration of structure and return of function had occurred. At this date chemical examination revealed the return approximately to the normal condition, the percentage of water has fallen, and that of phosphorus has now risen to 0·93. The accompanying photomicrographs were all taken from a sensory branch, for here regeneration had advanced further than in the motor fibres.

The new fibres are small but myelinated. Figs. 43 (Plate VIII.) and 44 (Plate IX.) show them in longitudinal and transverse section respectively. The myelin takes on the greenish-grey colour of normal fibres, but the small size of the fibres (1 to 5 μ in diameter) will be appreciated by comparing the figures with those in fig. 30 (Plate V.), the amount of magnification (700 diameters) being the same in both cases. Fig. 45 (Plate IX.) is from a section stained by Stroebe's method to show the

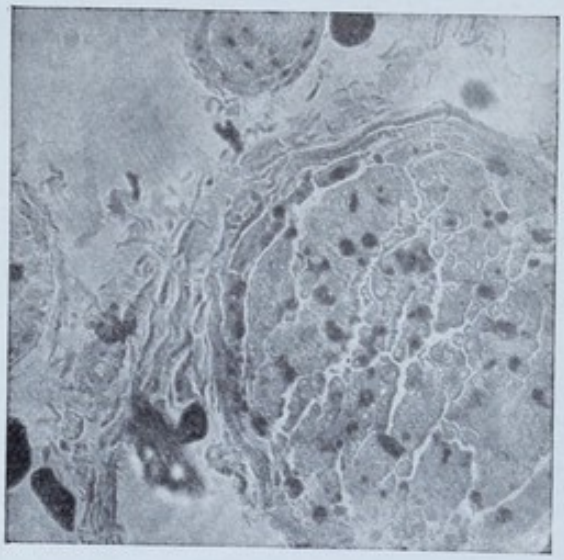

FIG. 41.

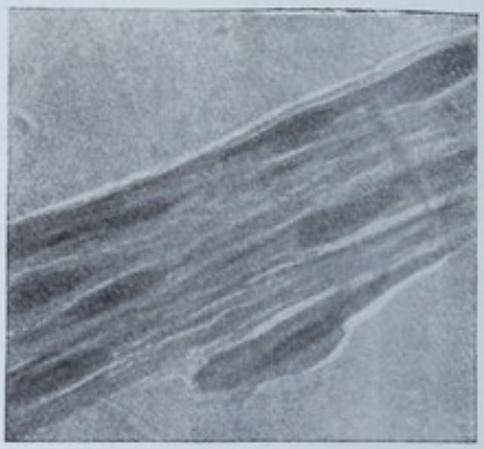

FIG. 42.

FIG. 43.

FIG. 41.—Transverse section of nerve, 44 days after operation (Cat H). Method—MARCHI'S fluid direct. 200 diameters.

FIG. 42.—Nerve from same cat. Teased preparation, stained with logwood and mounted in FARRANT. 500 diameters.

FIG. 43.—Longitudinal section of sensory nerve, 106 days after operation (Cat F). Method—MARCHI'S fluid direct. 700 diameters

FIG. 44.

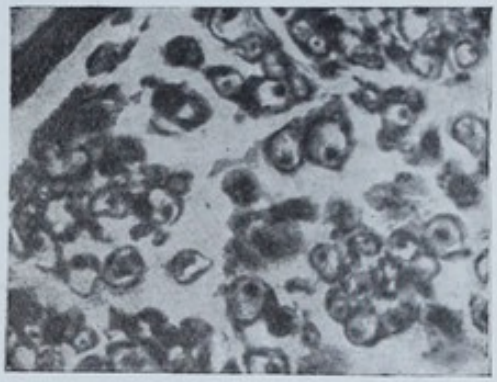

FIG. 45.

FIG. 46.

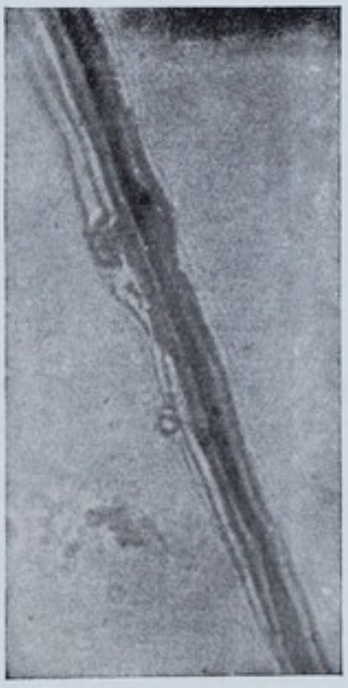

FIG. 47.

FIG. 44.—Transverse section of the same. Same method. 700 diameters.

FIG. 45.—Transverse section of the same, stained by STROEBE'S method. 700 diameters.

FIG. 46.—Teased preparation of the same. Stained with logwood; mounted in FARRANT. 600 diameters. The apparent excrescences on the fibre are air bubbles accidentally entangled in the FARRANT'S solution.

FIG. 47.—Teased preparation of the same. Stained with logwood; mounted in FARRANT. 600 diameters.

axis cylinders. Here the outer sheath is seen to be frequently thickened on one side; this is no doubt due to the section having gone through a nucleus of an internodal cell of the neurilemma.

The isolated nerve-fibres in teased preparations (figs. 46 and 47, Plate IX.), present some puzzling features. The new axis cylinder is well seen in fig. 47, but it will be noticed that its contour is uneven, some parts being distinctly thicker than others. In fig. 46 it almost appears as though the elongated, spindle-shaped nuclei of the primitive sheath (previously seen in an earlier stage in fig. 42 (Plate VIII.) were joining up to form the basis of the new axis cylinder. We do not by any means commit ourselves to the view that this is what really occurs. Such a view would upset previous work, showing that the axis cylinder is essentially the branch of a nerve cell growing distal-wards, and it would require much more evidence to prove the contrary than our preparations afford. The manner of regeneration is, after all, only a side issue of the main purpose of our present work, which has been to correlate the histological with the chemical features of the degeneration process. At some future time we may return to this other equally important problem.

We will be content with saying that we think our preparations prove that the manifest activity of the neurilemmal cells is related in some degree (perhaps nutritional if not formative) to the process of regeneration; we may also recollect that in situations where no neurilemma exists regeneration does not occur, namely, in the central nervous system.

We can also see that the appearances to which we have just called attention are capable of another interpretation; for the elongating and apparently contiguous nuclei, as seen in fig. 46, may be situated outside the axis cylinder altogether, and conceal it underneath or within them. The transverse section (fig. 45) would support this idea. Again in fig. 47, where the central strand is evidently the axis cylinder, the varicose condition observed may be a natural condition of the axis cylinder, and accords with the description of earlier writers, who have drawn attention to enlargements on the course of an axis cylinder, and to varicosities of its constituent fibrillæ.

(3) General Conclusions.

Our previous work had shown us that in degenerative diseases of the central nervous system, evidence of the breakdown of the

nervous tissue can be obtained by the discovery of certain products in the cerebrospinal fluid and blood of the patients. Of these products choline, which can be readily identified by chemical and physiological tests, was the one to which we particularly directed our attention. We now show that choline is also discoverable in various diseases of the central and peripheral nervous system, other than the one (General Paralysis) which was the special subject of our earlier investigation. The evidence on this branch of the question is described in the first part of the present lecture. We have also directed our attention to the micro-chemical reaction of Marchi, which is the histological test most often resorted to for the detection of degenerated nerve-fibres. We had noted that the same black colour is given by ordinary fat, and it seemed possible that the explanation of the Marchi reaction was that in the disintegration of lecithin, which results in the liberation of choline, there is also a liberation of the phosphorised portion of the molecule; this would leave the fatty portion of the molecule, which would give the Marchi reaction. Preliminary experiments on human spinal cords, one side of which showed degeneration, supported this view, for the amount of phosphorised fat was much diminished on the degenerated side.

Still, in order to place the matter on a satisfactory basis, it appeared essential to undertake experiments on animals; for in these the steps of the process can be more accurately studied, and the histological and chemical changes considered side by side and correlated. The details of the experiments on cats to work out this idea are given in the later part of this lecture, and the results have fully confirmed our expectations. Wallerian degeneration was produced in a series of eighteen cats by section of both sciatic nerves; the animals were killed at intervals of from one day to one hundred and six days after the operation.

The nerves remained excitable up to the third day; these nerves were practically healthy both to the microscope and in chemical composition; the amount of water and of phosphorus were the chemical data which were worked out.

Beyond the third day, early signs of degeneration set in; the amount of phosphorus in the nerves slightly dropped, and the amount of choline in the blood slightly increased.

On the eighth day the Marchi reaction became strongly

marked. This date is coincident with a great drop in the amount of phosphorus in the nerves, and with the appearance of a large quantity of choline in the blood.

The Marchi reaction remained at its acme up to the thirteenth day, and the amount of phosphorus became less and less. The amount of choline became somewhat less in the blood; it therefore appears that of the disintegration products of lecithin the choline is earliest removed, the phosphorus probably in the form of phosphoric acid next, leaving the fatty material to give the Marchi reaction, and to be absorbed last.

By the twenty-seventh day all the phosphorus had nearly, and by the twenty-ninth day entirely, disappeared. The removal of the fat had also commenced, and we have drawn attention to the phagocytic action of certain cells (in addition to leucocytes), probably the multiplied neurilemmal cells in this process of fat removal.

At the forty-fourth day the removal of the fat was all but complete, and little remained except shrunken, empty nerve tubules. But examination of the nerves sixty days after operation shows this date is variable with the vital reaction of different animals; at any rate in comparison with the central nervous system the date is an early one.

Regeneration appears to begin about the same date. This is about the sixtieth day in nerves which had united spontaneously, though somewhat earlier in cases where the loose ends of the nerves had been sutured together.

By the 100th to 106th day regeneration was well marked, especially in sensory fibres, and the nerves were once more excitable. By this date the fibres were seen to be fine and medullated; they took stains normally. Their chemical condition had practically returned to the normal also. The first sign of the return of the phosphorus was seen with the commencement of myelinisation on the sixtieth, but it was well marked on the 106th day. We found the percentage of phosphorus in normal nerves to be 1·16. In the regenerated nerves it was 0·93. Whether all the phosphorus in the regenerated fibres was in the medullary sheath, or partly in the comparatively large axis cylinder, we cannot say.

With regard to the amount of water in the nerves, the tables of analyses we present show that the amount of water increases

with the degeneration, and continues high while absorption is occurring. It sinks to the normal when regeneration has set in. The following tabular summary gives our main results :—

Days after section	Cats' sciatic nerves			Condition of blood	Condition of nerves
	Water	Solids	Percentage of phosphorus in solids		
Normal ..	65·1	34·9	1·1	Minimal traces of choline present	Nerves irritable and histologically healthy
1—3 ..	64·5	35·5	0·9		
4—6 ..	69·3	30·7	0·9	Choline more abundant	Irritability lost; degeneration beginning
8 ..	68·2	31·8	0·5	Choline abundant	Degeneration well shown by Marchi reaction
10 ..	70·7	29·3	0·3		
13 ..	71·3	28·7	0·2		
25—27..	72·1	27·9	traces	Choline much less	Marchi reaction still seen, but absorption of degenerated fat has set in
29 ..	72·5	27·5	0·0		
44—60 ..	72·6	27·4	0·0	Choline almost disappeared	Absorption of fat practically complete
100—106	66·2	33·8	0·9	Choline almost disappeared	Return of function; nerves regenerated

We have paid some attention to the multiplication of the cells of the primitive sheath. This first becomes a marked phenomenon on the eighth day, and is possibly the result of irritation by the degenerated products. The phagocytic action of these cells at a later stage has been already mentioned. Later still, the cells become spindle-shaped and united end to end ; it appears to us that these cells are in their activity related nutritionally to the regeneration process. We have discussed the question whether or not they may take any part in the actual formation of the new axis cylinder, but at present we have insufficient evidence to show that they do so.

Another question, which has not been alluded to in the foregoing pages, but which has inevitably been considered by us, is the mode of origin of the medullary sheath. The activity of the neurilemmal cells is a point in favour of the origin of the medullary sheath from them. On the other hand, the association of the complete return of function with the appearance of the

medullary sheath, would rather be in favour of the other theory, that it originates from the axis cylinder. This is supported by the fact that in the central nervous system the primitive sheath is absent.

The axis cylinder and its sheaths must necessarily, for descriptive purposes, be considered separately. There is little doubt in our own minds that, functionally, all three parts of a nerve-fibre must be considered to act as an organic whole, with intimate inter-relations of a nutritional or metabolic nature.

These, after all, are but side issues from our main point, which has been the elucidation of the relations between the histological and chemical characters of the degeneration process.