PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Chemistry of Nerve-Degeneration

F. W. Mott and W. D. Halliburton

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VIII. *The Chemistry of Nerve-degeneration.*

By F. W. Mott, *M.D., F.R.S., and* W. D. Halliburton, *M.D., F.R.S.*

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[Plate 45.]

In a former paper* we have shown 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 cerebro-spinal fluid. We specially investigated one of these products, namely, choline, which is derived from the breakdown 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 we rely 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 cerebro-spinal 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 our disposal, and in order to obtain satisfactory evidence of choline, it is necessary to considerably concentrate the alcoholic extract. We have therefore been obliged to limit ourselves almost exclusively to these two tests; the two tests, however, appear to us 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

* 'Phil. Trans.,' Series B, vol. 191, pp. 211–267, 1899.

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.*

Although our previous paper dealt principally with General Paralysis, we indicated that the presence of choline in large quantities in the cerebro-spinal 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 cerebro-spinal fluid and blood.

Physiological saline solution will extract a small amount of choline from perfectly normal nervous tissue at the ordinary temperature; this is most apparent in 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.

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

* GUMPRECHT, 'Verhandl. des Congr. für innere Medicin,' Wiesbaden, 1900, p. 326.

† CLEGHORN, 'Journ. of the Boston Society of Medical Sciences,' vol. 4, p. 239, 1900; HUNT, 'Proc. American Physiol. Society, American Journ. of Physiol.,' vol. 3, p. xviii., 1900; OSBORNE and VINCENT, 'Journ. of Physiol.,' vol. 25, p. 283, 1900; W. D. HALLIBURTON, *ibid.*, p. vii., 1900; also vol. 26, p. 229, 1901.

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 allude to the composition of lecithin, and to the main features of the Marchi reaction.

Lecithin, the main 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.

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 not stained, 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, one of us (F. W. M.)* had made some preliminary experiments in this direction, which were continued in conjunction with Dr. BARRATT.† Spinal cords on one side of which degeneration had occurred, due to

* CLIFFORD ALLBUTT, 'System of Medicine,' vol. 1, "Pathology of Nutrition."

† Proc. Physiol. Soc., February 1899, in 'Journal of Physiol.,' vol. 24, p. iii.

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 direction is A. NOLL.* He showed that in Wallerian degeneration of peripheral nerves, the amount of protagon diminishes until there is at last none at all 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 one of us (F. W. M.). The experiments in which living animals were employed were carried out entirely by the other (W. D. H.) 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.

In our former paper, we mentioned the fact that we had examined the blood from several cases of General Paralysis. The blood was removed by venesection as a remedial measure during seizures, and gave both the chemical and physiological tests for choline which we have already described.

We have also described† 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 examination of the blood. Using the platinum test we only obtained some ill-formed

* 'Zeitsch. f. Physiol. Chem.,' vol. 27, p. 370, 1899.

† 'British Medical Journal,' July 29th, 1899.

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 two first 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 one of us (F. W. M.). 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 one of us (F. W. M.).

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 our results, it will be best to state rather more fully the methods we have 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 solution 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 cub. centims. 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 proteïd, 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 cub. centims. and the amount of the original blood to which this corresponded was noted. Concentration was usually carried out, so that the injection of 5 cub. centims. corresponded to 20 or 30 cub. centims. 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 cub. centim. 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 cub. centims. 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.

We found that choline was present in the cases enumerated. We further found that the two tests fitted together with great accuracy. If we performed the chemical test first, we could prophesy from the amount of crystals the result of the injection. If, on the other hand, we performed the physiological test first, we could prophesy from the fall of pressure whether or not we should get an abundant or a scanty crop of crystals.

The case where we obtained least result 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 we obtained the best result 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. 1), from that of Combined Sclerosis (fig. 5), from that of Disseminated Sclerosis (fig. 4), and from that of Alcoholic Neuritis (fig. 3). In the first case, also, the effect after the injection of atropine is shown (fig. 2). In all, the fall of pressure is either absent, or replaced by a slight rise.*

We are not able to represent graphically the results of our chemical test, but the

* The other tracings showing the effect after atropine are practically identical with this one (fig. 2), and have been omitted at the suggestion of the referees.

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

We venture to think that such 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 as we showed in our former paper,* so that the examination of that secretion would be insufficient in such cases.

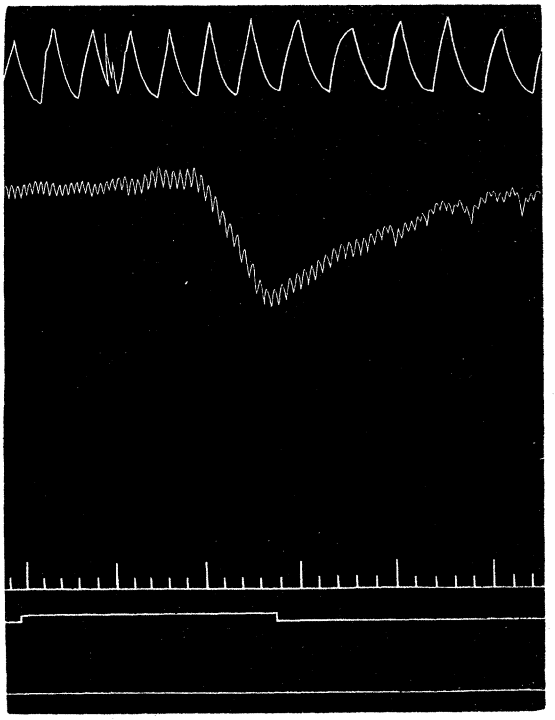


Fig. 1.

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 the signal line, the raising of which indicates the period of 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. 2.

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 paper is consulted. All the tracings were taken from experiments on cats under A.C.E. mixture. All read from left to right. The actual volume of saline solution of the active material was in all cases 5 cub. centims. 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. 1 represents the fall of arterial pressure produced by the injection of an amount equal to 10 cub. centims. of the blood in the case of Beri-beri numbered 3 in the list on p. 441.

* *Loc. cit.*, p. 256.

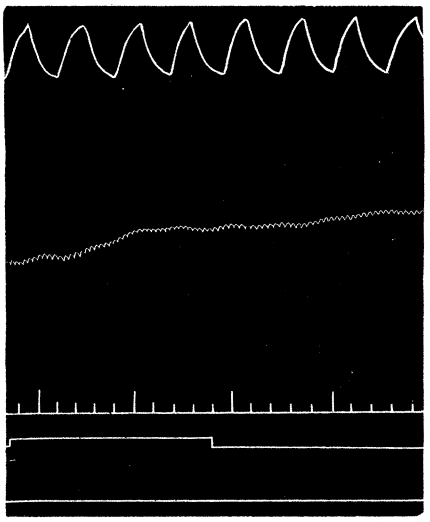


Fig. 2.

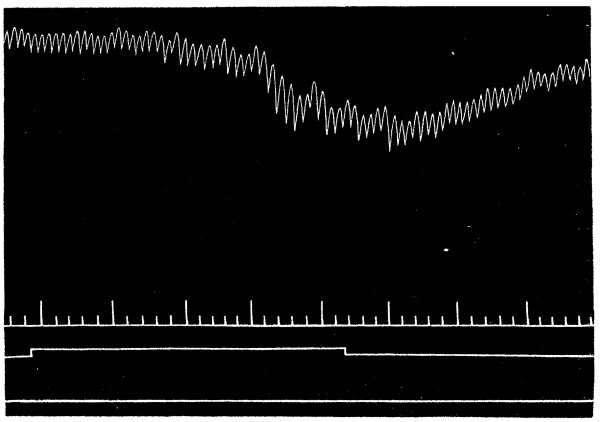


Fig. 3.

Fig. 2.—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.

Fig. 3.—The result of injecting an amount equal to 30 cub. centims. of blood from a case of alcoholic neuritis.

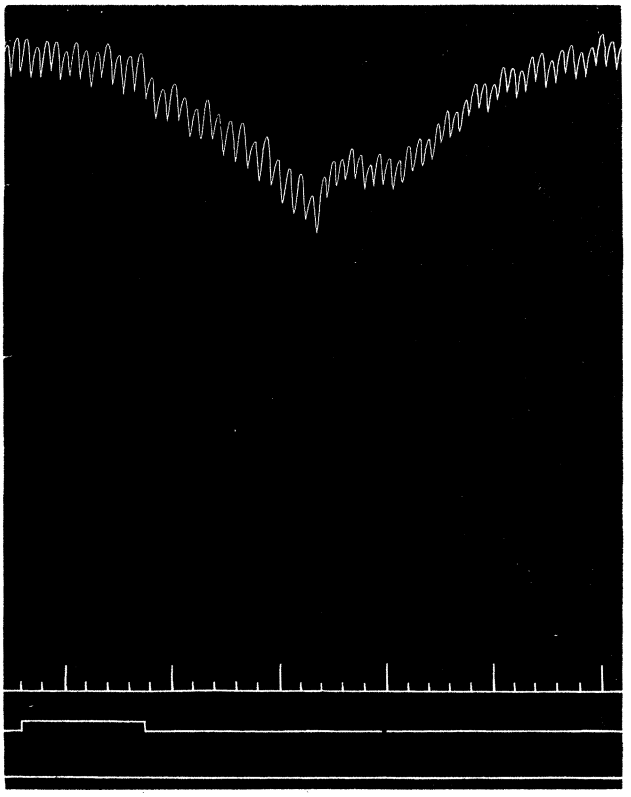


Fig. 4.

Result of injecting an amount equal to 20 cub. centims. of blood from a case of Disseminated Sclerosis (early stage of the disease).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

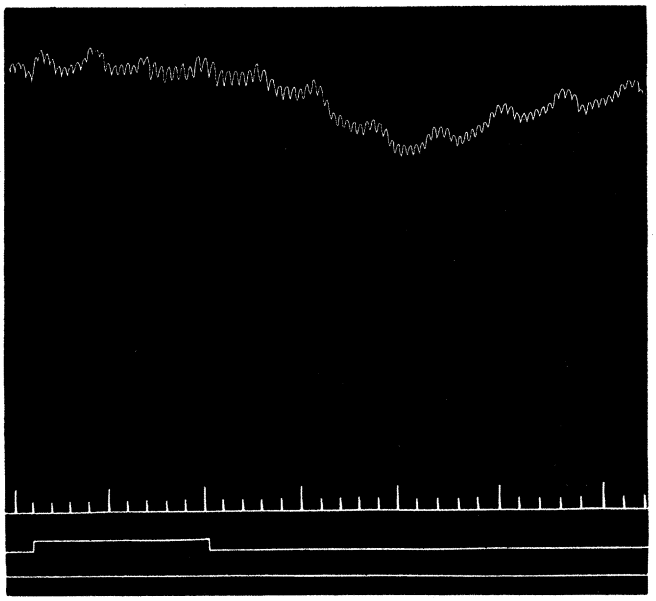


Fig. 5.

Result of injecting an amount equal to 30 cub. centims. of blood from a case of Combined Sclerosis.

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.

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 limb 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:—

Cat K.—One sciatic nerve only divided. The animal was killed 24 hours later. The peripheral end of the cut nerve was stimulated mechanically and electrically, and well-marked muscular 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 centims. 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.

Cat A.—Both nerves divided. This animal was killed 2 days and 5 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 3 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 3 days 20 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 4 days 3 hours after the operation. No response occurred on either side to stimulation. Signs of early stages of Wallerian degeneration are seen.

Cat E.—Both nerves divided. This animal was killed 5 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 6 days after operation. Degenerative changes in the nerves are about the same as in Cat E.

Cat R.—Both nerves divided. This animal was killed 8 days after the operation.

Cat N.—Both nerves divided. This animal was killed 10 days after the operation.

Cat Q.—Both nerves divided. This animal was killed 13 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 25 days after the operation.

Cat O.—Both nerves divided. This animal was killed 27 days after the operation.

Cat W.—Both nerves divided. The left nerve was sutured with catgut. This animal was killed 29 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 histologically 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 44 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 45 days after the operation. In this animal, for the few days preceding its death, the paralysis was less marked on the sutured side.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The left (sutured) nerve responded to stimulation (secondary coil at 7·5 centims.); 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 (60 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 60 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 100 days after the operation.

Cat F.—Both nerves divided. This animal was killed 106 days after the operation.

In these two last 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 centims.). The nerves, especially the sensory ones, showed well-marked regeneration, and chemically they had returned approximately to the normal state.

We may briefly summarise the results in the following way. Up to the 3rd day the nerves remained excitable and approximately normal. Degenerative changes then set in and became well marked on the 8th day; from this time to the 13th day they were at their height. On the 25th day, and at periods later than this, union of the divided nerves had nearly always taken place. The 29th day marks the entire disappearance of phosphorus from the degenerated fat. Absorption and removal of the fat was nearly accomplished by the 44th 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 60th day. In the cats which were allowed to live 100 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.

We have now to present the further experiments we have performed in connection with these animals; they divide themselves into three sets.

A. Experiments with the blood.

B. Chemical examination of the nerves.

C. Histological examination of the nerves.

We will take these three points in the order named.

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 in the introduction to this paper, 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 (4th 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 8-day cat. This fall was abolished by atropine. On the 13th day there 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 we have already advanced concerning lecithin metabolism (see p. 438).

We give illustrative tracings selected from the large number we have taken.

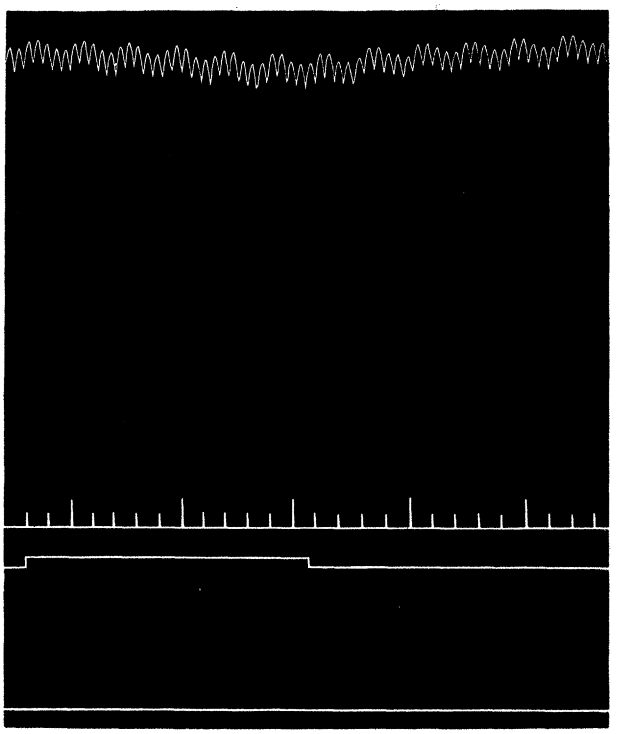


Fig. 6.

Result of injecting an amount equal to 20 cub. centims. of blood of normal kitten.

It will be seen that the material from normal blood (fig. 6), from blood of an animal in which degeneration had not commenced (fig. 7), and from blood of an animal in which degeneration was complete (fig. 14), gave negative results.

In cases where degeneration had set in (blood of 6-day cat, fig. 8), or had commenced to subside (25-day cat, fig 13), the effect was slight.

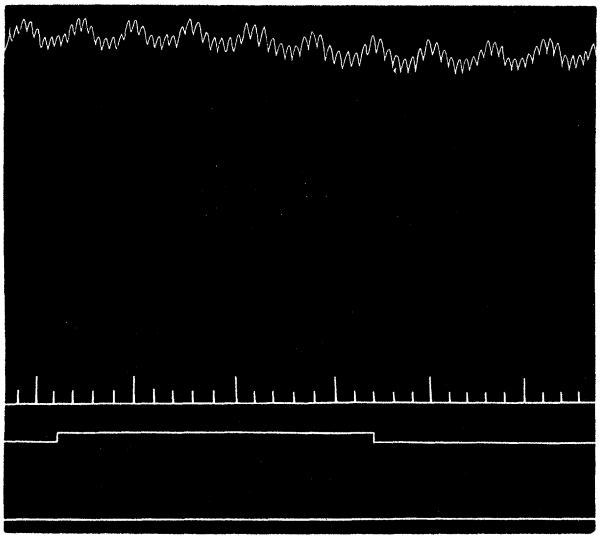


Fig. 7.

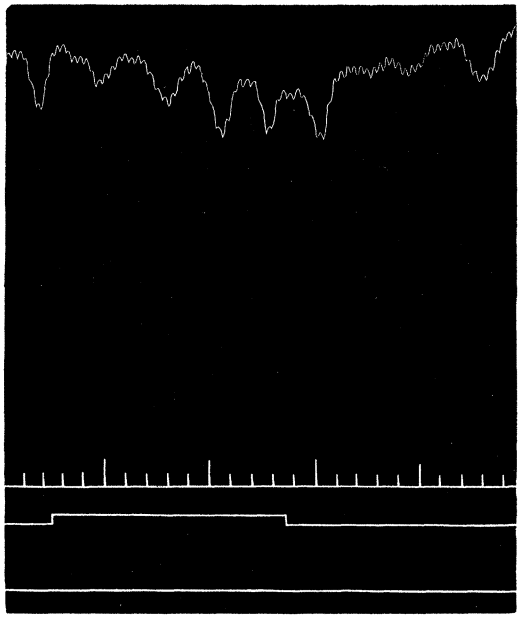


Fig. 8.

Fig. 7.—Result of injecting an amount equal to 20 cub. centims. of blood obtained from Cat A (two days after section of both sciatic nerves).

Fig. 8.—Result of injecting an amount equal to 20 cub. centims. of blood obtained from Cat D (6 days after section of both nerves).

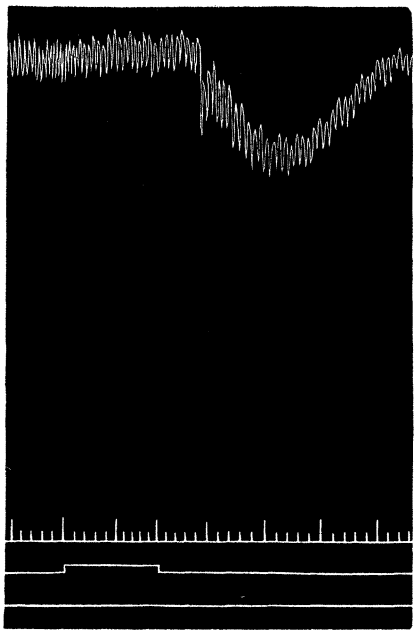


Fig. 9.

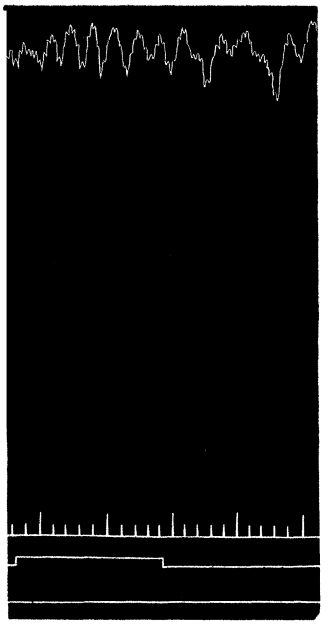


Fig. 10.

Fig. 9.—Result of injecting an amount equal to 30 cub. centims. of blood obtained from Cat R (8 days after section of both nerves).

Fig. 10.—Result of injection of the same amount after atropine.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In the case of the cats where degeneration was at its height, the physiological effect, fall of blood pressure, was very marked (figs. 9 and 11), and the fall was absent after atropinisation (figs. 10 and 12). 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. We have not presented tracings of the slight effects obtained when regeneration set in; they were about equal to those seen in figs. 8 and 13.

As in the case of human blood, we attempted to make some quantitative estimations 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 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 cub. centims., 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. 441. 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.

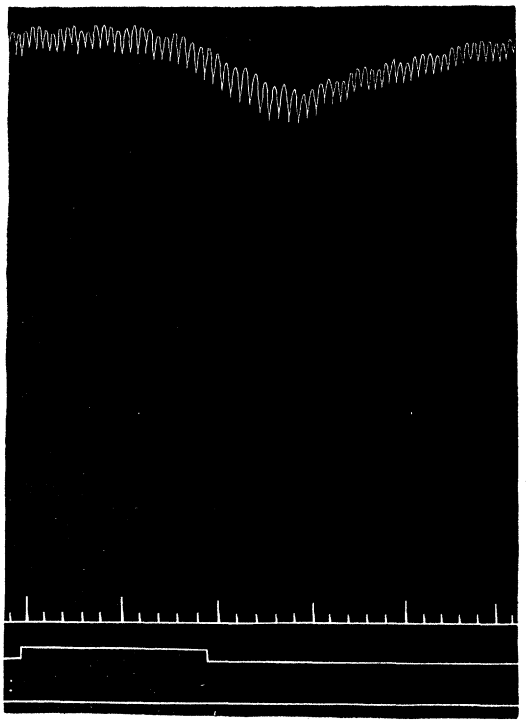


Fig. 11.

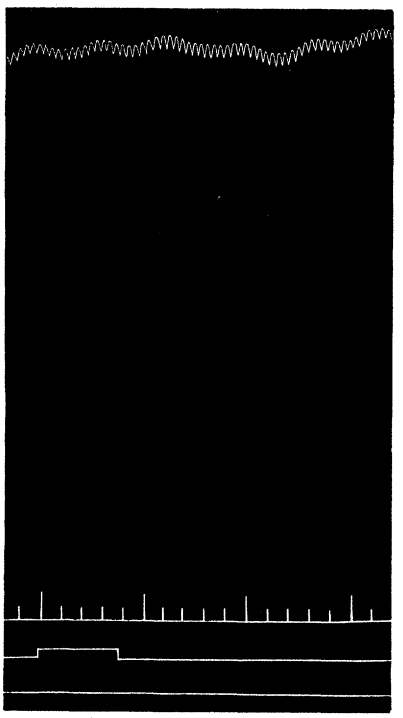


Fig. 12.

Fig. 11.—Result of injecting an amount equal to 25 cub. centims. of blood obtained from Cat N (10 days after section of both nerves).

Fig. 12.—Result of injection of the same amount after atropine.

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.

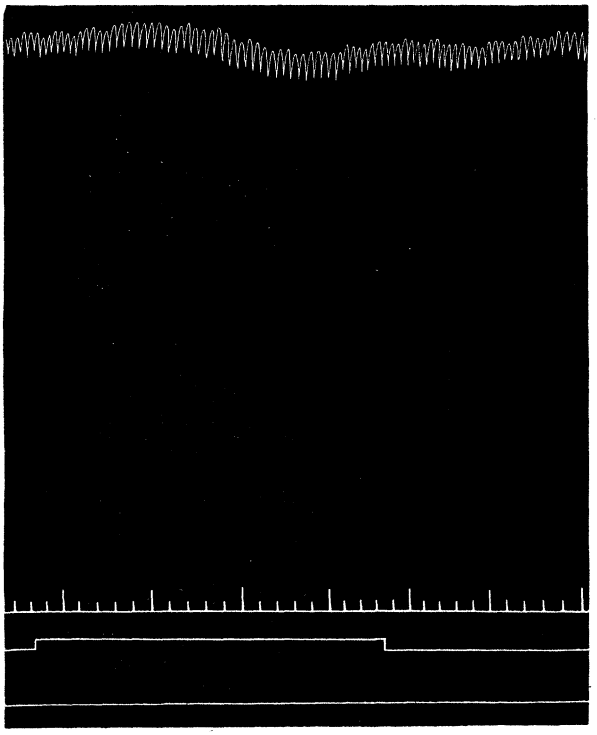


Fig. 13.

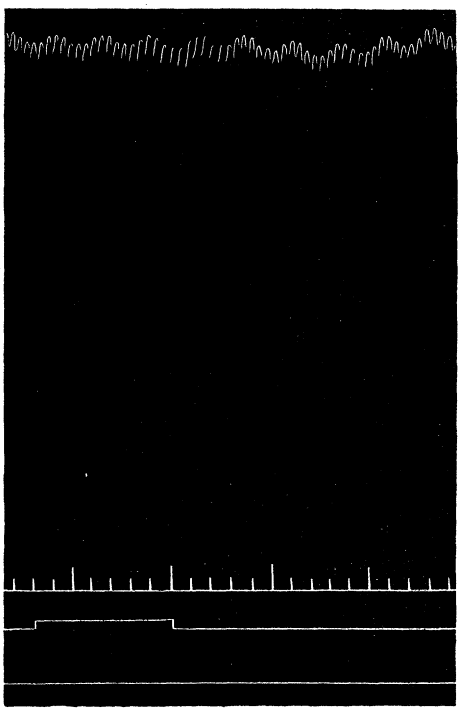


Fig. 14.

Fig. 13.—Result of injecting an amount equal to 25 cub. centims. of blood from Cat J (25 days after section of both nerves.

Fig. 14.—Result of injecting an amount equal to 25 cub. centims. of blood from Cat H (44 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 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 *

The following table gives our results:—

TABLE I.—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 II.—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 . .	64·618	35·185
,, B.	3 days after operation		
	Average	64·507	35·493

* A full description of this method of phosphorus estimation is given by one of us (W. D. H.) in the 'Journal of Physiology,' vol. 13, pp. 814, 821, 1892.

TABLE III.—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	68·018	31·982
,, E. 5 ,, ,, ...		
,, 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 IV.—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	72·646	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

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 I. and II. comes well within the limits of individual variations in normal cats. If we compare these with the cats 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 III.). But after restoration of function (Cats I and F, Table IV.) the proportion of water to solids returns approximately to normal.

We now come to the phosphorus estimations.

TABLE V.—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	0·347	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	0·025	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 8th 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 25th, and absolutely by the 29th 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.*

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.

* 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 majority of his experiments he estimated protagon, not phosphorus as we have done. He puts the date of disappearance of the phosphorised fat at 28 days. This date and many other of his facts fit in very well with our work.

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 microscopic 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.

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 10 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 specimens 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
1. 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 drawings appended. A. represents the condition of the nerve in transverse section, which was obtained from a cat 2 days after the operation of cutting the nerve. The fibres are healthy, the dark greyish-green colour of the medullary sheath is seen, and the axis cylinder with its tubular fibrils can also be distinguished. B. represents the staining obtained from a cat 92 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 C. (10 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. (See Plate 45.)

We have had these drawings made in colour, because the 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.

We obviously cannot attempt to describe all the microscopic specimens we have made. The main results of the examination have been stated already on pp. 446, 447. 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 53 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. 15) the tubular character of the fibrils of the axis cylinder* is shown.

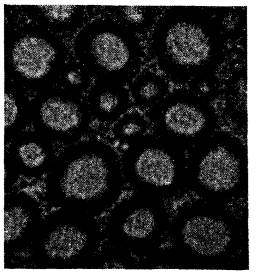


Fig. 15.

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.

* See SCHÄFER, 'Quain's Anatomy.' Tenth edition, 1891, vol. 1, part 2, p. 311.

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 plate, fig. A).

Cat B.—Nerves removed 72 hours after the operation. The accompanying figures (figs. 16 and 17) 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 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.

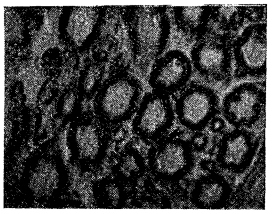


Fig. 16.

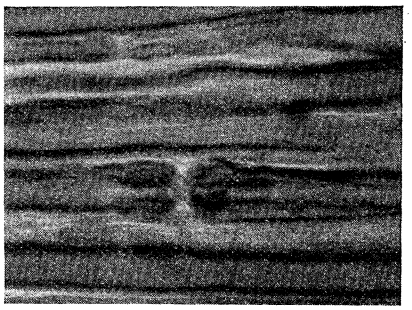


Fig. 17.

Fig. 16.—Transverse section of nerve, 3 days after operation (Cat B). Same method. 500 diameters.
Fig. 17.—Same nerve in longitudinal section. Same method. 600 diameters.

Cat P.—Nerves removed 92 hours after operation. Fig. 18 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

Fig. 18.

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

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 III.) 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 (B in coloured plate), 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 4 days 3 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. 19).

Cats E and D.—Nerves removed 5 and 6 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 (6 days) than in Cat E (5 days).

Cat R.—Nerves removed 8 days after operation. Here there is a very marked increase in the degenerative change. The transverse and longitudinal sections (figs. 20 and 21) 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 V. 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. 9).

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. 22).

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 10 days after the operation. The degenerative change is still better marked. The accompanying photo-micrograph (fig. 23) speaks for itself. A transverse section of this nerve is shown in fig. C, coloured plate.

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

Cat Q.—Nerves removed 13 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 27 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. 24).

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. 13). But a new point is here also 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. 25. 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 44 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 photo-micrograph (see fig. 26).

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. 27. 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 (26 and 27) 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 photo-micrographs were all taken from a sensory branch, for here regeneration had advanced further than in the motor fibres.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

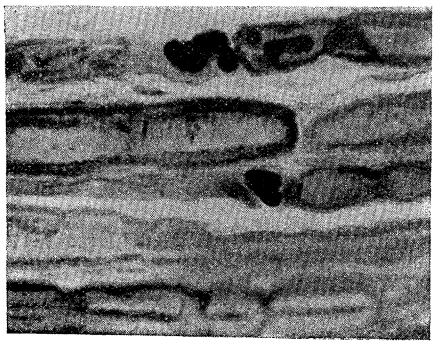


Fig. 19.

Longitudinal section of nerve, 99 hours after operation (Cat C). Same method. 600 diameters.

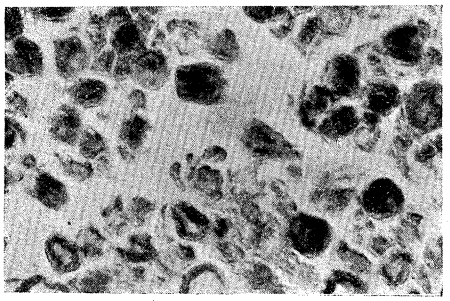


Fig. 20.

Fig. 21.

Fig. 20.—Transverse section of nerve, 8 days after operation (Cat R). MÜLLER'S then FLEMMING'S solution. 700 diameters.

Fig. 21.—Same nerve in longitudinal section. Same method. 450 diameters.

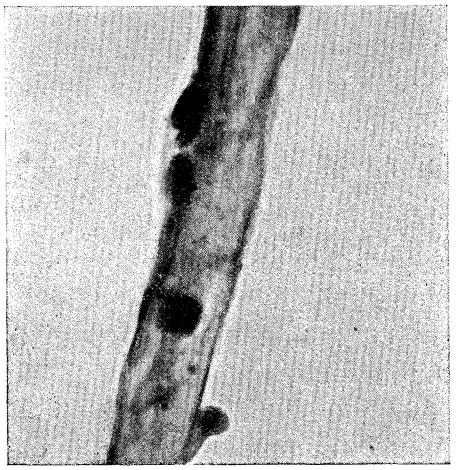


Fig. 22.

Single fibre from nerve of same animal, to show multiplication of nuclei of the primitive sheath. Teased specimen; after hardening in MÜLLER'S fluid it was washed, stained with logwood, and mounted in FARRANT'S solution. 870 diameters.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

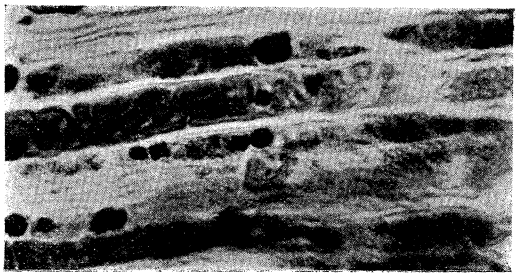


Fig. 23.

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

Fig. 24.

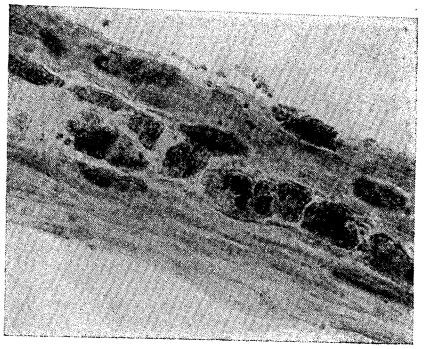


Fig. 25.

Fig. 24.—Longitudinal section of nerve, 27 days after operation (Cat O). Same method. 500 diameters.

Fig. 25.—Longitudinal section of nerve from same cat. Method—MARCHI'S fluid direct, then logwood. 450 diameters.

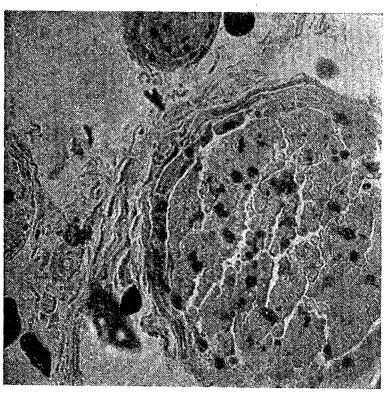


Fig. 26.

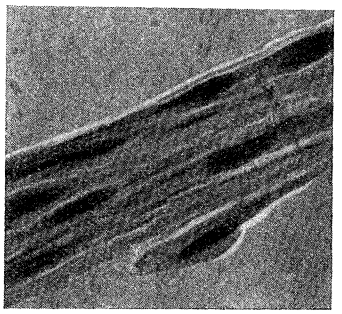


Fig. 27.

Fig. 26.—Transverse section of nerve, 44 days after operation (Cat H). Method—MARCHI'S fluid direct. 200 diameters.

Fig. 27.—Nerve from same cat. Teased preparation, stained with logwood and mounted in FARRANT. 500 diameters.

The new fibres are small but myelinated. Figs 28 and 29 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. 15, the amount of magnification (700 diameters) being the same in both cases. Fig. 30 is from a section stained by STROEBE's method to show the 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. 31 and 32) present some puzzling features. The new axis cylinder is well seen in fig. 32, but it will be noticed that its contour is uneven, some parts being distinctly thicker than others. In fig. 31 it almost appears as though the elongated spindle-shaped nuclei of the primitive sheath (previously seen in an earlier stage in fig. 27) 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 distalwards, 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. 31, may be situated outside the axis cylinder altogether, and conceal it underneath or within them. The transverse section (fig. 30) would support this idea. Again in fig. 32, 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 cerebro-spinal 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. Our evidence on this branch of the question is described in the first part of the present paper. We had 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 second part of this paper, 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 1 to 106 days after the operation.

The nerves remained excitable up to the 3rd 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 3rd 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 8th day the Marchi reaction became strongly marked. This date is coincident with the first 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 13th 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 27th day all the phosphorus had nearly, and by the 29th 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 44th day the removal of the fat was all but complete, and little remained

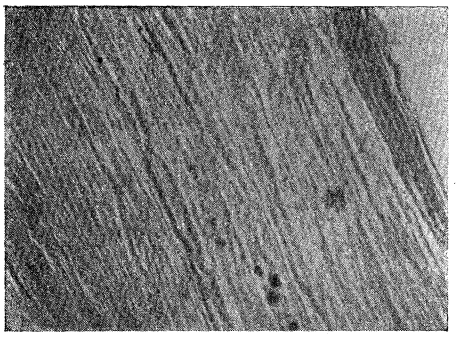


Fig. 28.

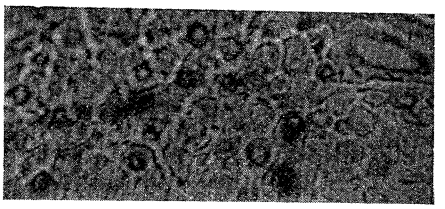


Fig. 29.

Fig. 28.—Longitudinal section of sensory nerve, 106 days after operation (Cat F). Method—MARCHI'S fluid direct. 700 diameters.

Fig. 29.—Transverse section of the same. Same method. 700 diameters.

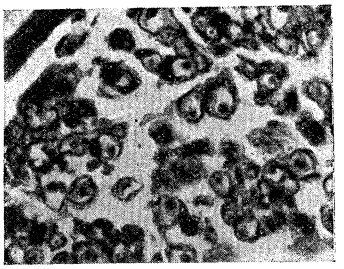


Fig. 30.

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

Fig. 31.

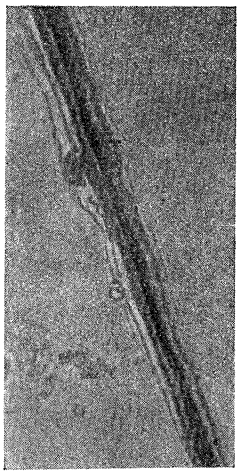


Fig. 32.

Fig. 31.—Teased preparation of the same. Stained with logwood; mounted in FARRANT. 600 diameters. The apparent excrescences in the fibre are air bubbles accidentally entangled in the FARRANT'S solution.

Fig. 32.—Teased preparation of the same. Stained with logwood; mounted in FARRANT. 600 diameters.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

except shrunken empty nerve tubules. But examination of the nerves 60 days after operation shows that 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 60th 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 60th, 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. . . .	72·6	27·4	0·0	Choline almost disappeared	Absorption of fat practically complete.
100–106. .	66·2	33·8	0·9	„ „ „	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 8th 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.

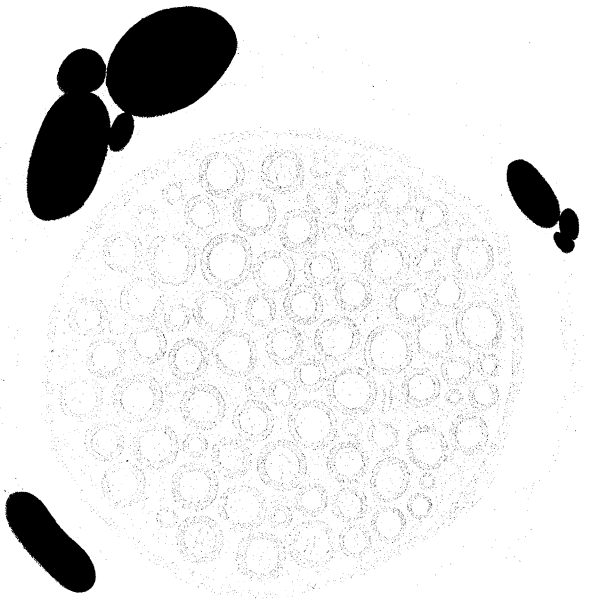
These, after all, are but side issues from our main point, which has been the elucidation of the relations between the histological and chemical characters of the degeneration process.

Description of Coloured Plate. (Plate 45.)

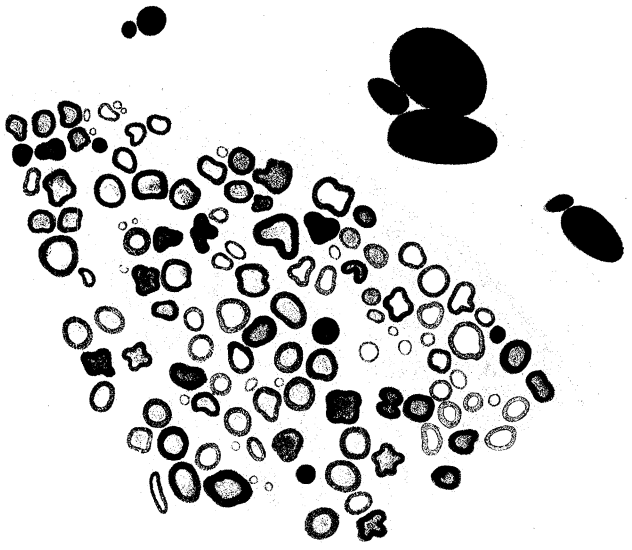
A. Transverse section of nerve, 2 days after operation (Cat A). Direct Marchi method. 425 diameters.

B. Transverse section of nerve, 92 hours after operation (Cat P). Direct Marchi method. 425 diameters.

C. Transverse section of nerve, 10 days after operation (Cat N). Direct Marchi method. 425 diameters.



A.



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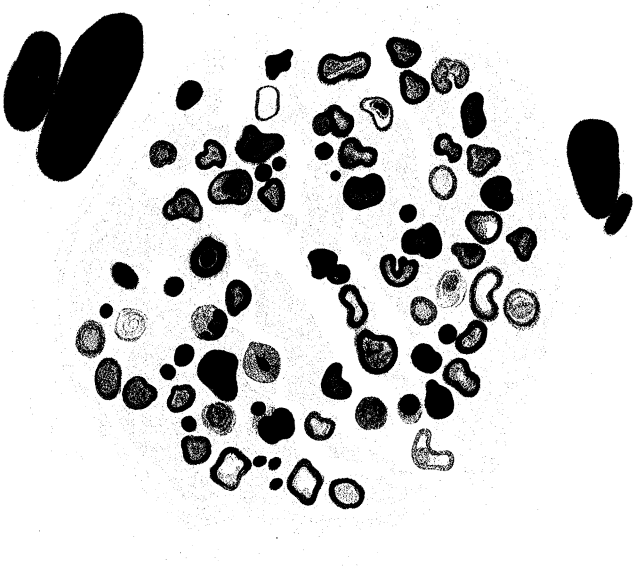

C.

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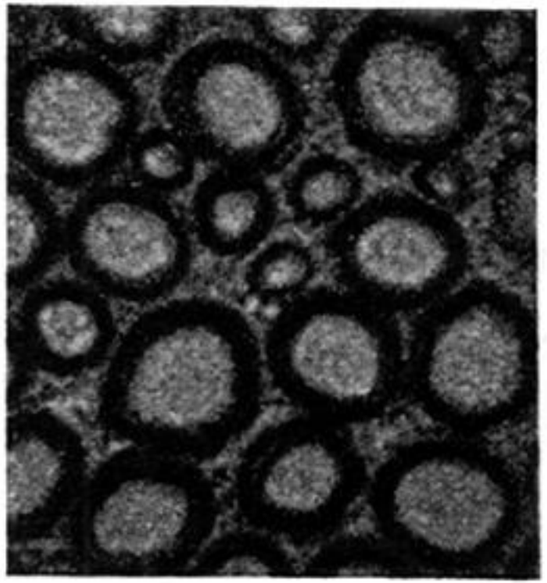


Fig. 15.

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

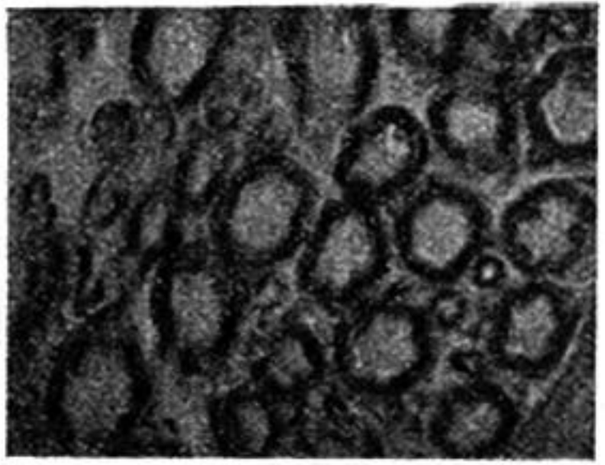


Fig. 16.

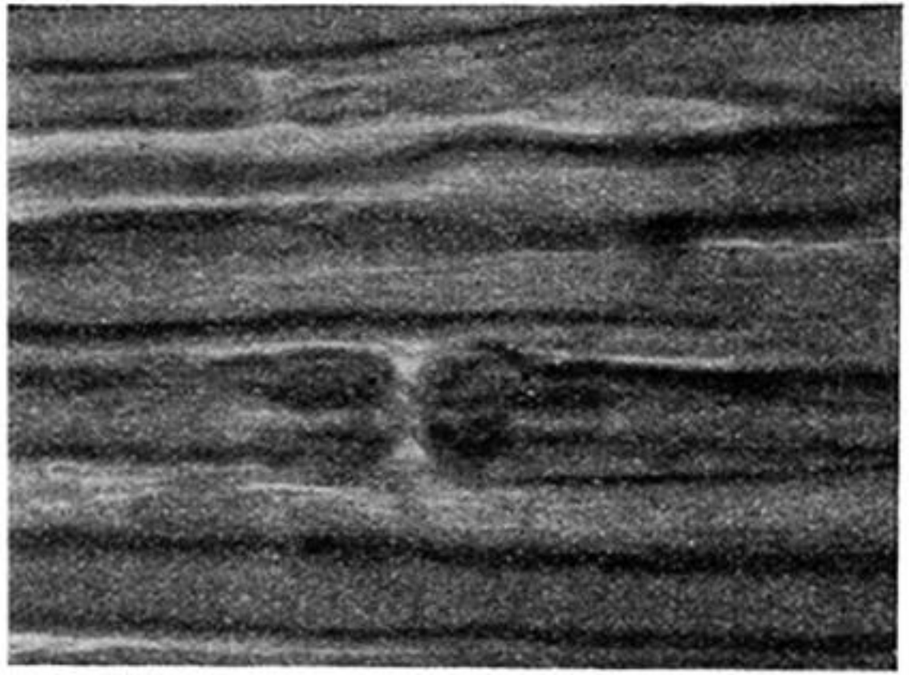


Fig. 17.

Fig. 16.—Transverse section of nerve, 3 days after operation (Cat B). Same method. 500 diameters.

Fig. 17.—Same nerve in longitudinal section. Same method. 600 diameters.

Fig. 18.

ongitudinal section of nerve, 92 hours after operation (Cat P). Same method. 700 diameters.

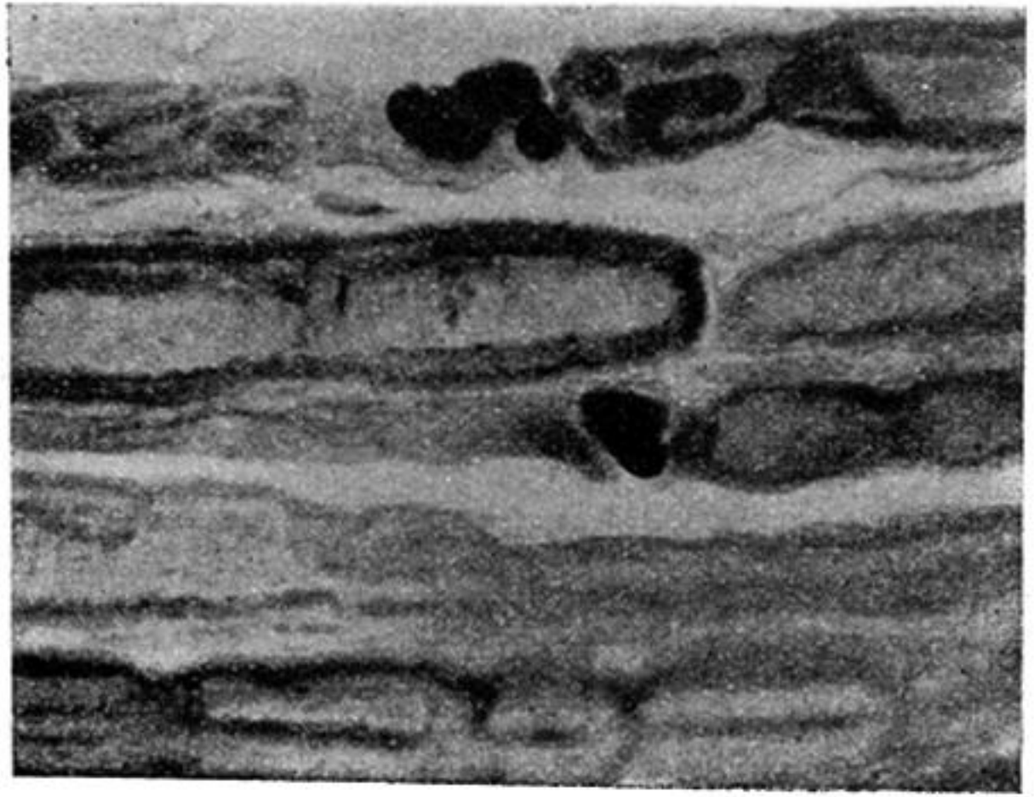


Fig. 19.

ongitudinal section of nerve, 99 hours after operation (Cat C). Same method. 600 diameters.

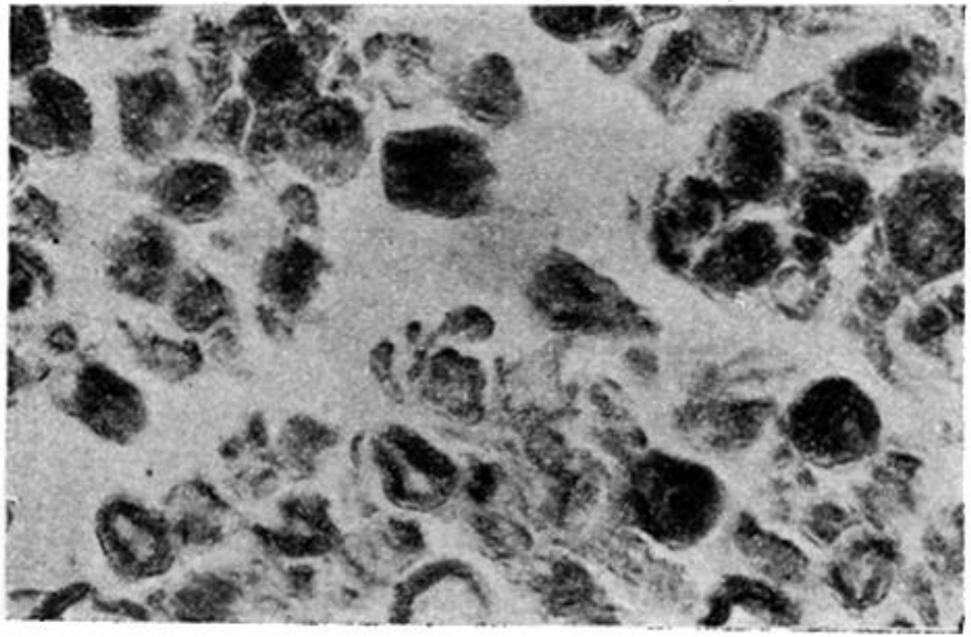


Fig. 20.

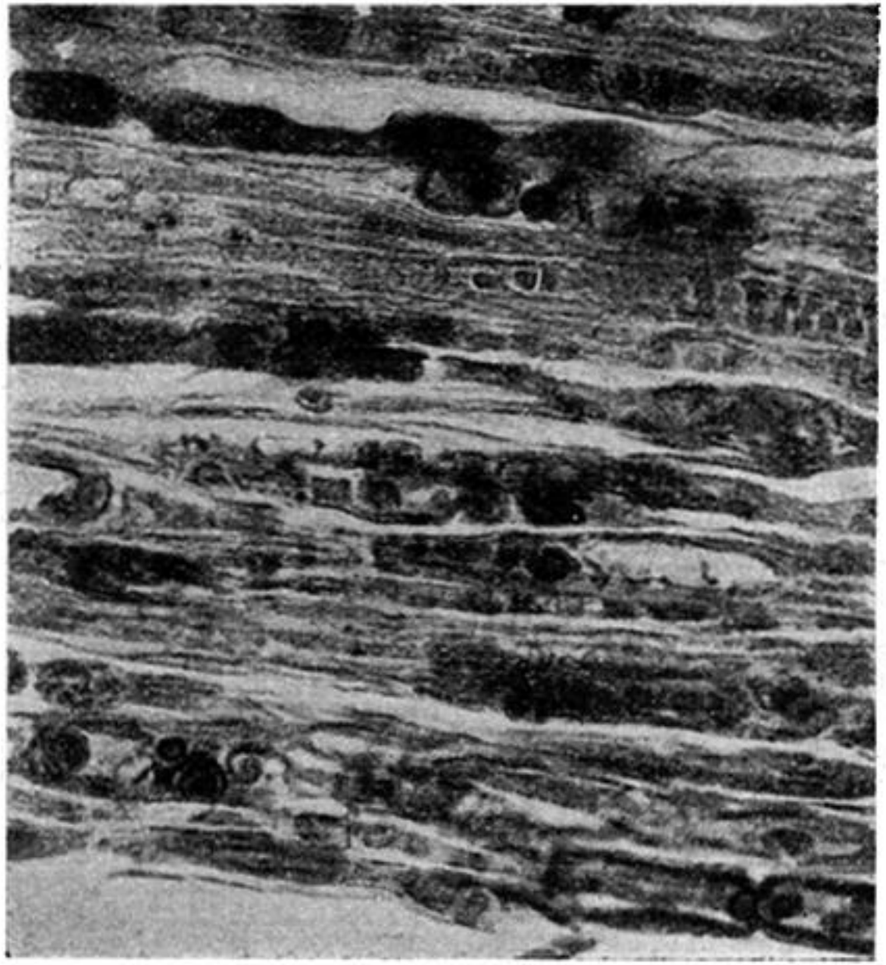


Fig. 21.

Fig. 20.—Transverse section of nerve, 8 days after operation (Cat R). MÜLLER'S then FLEMMING'S lution. 700 diameters.

Fig. 21.—Same nerve in longitudinal section. Same method. 450 diameters.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

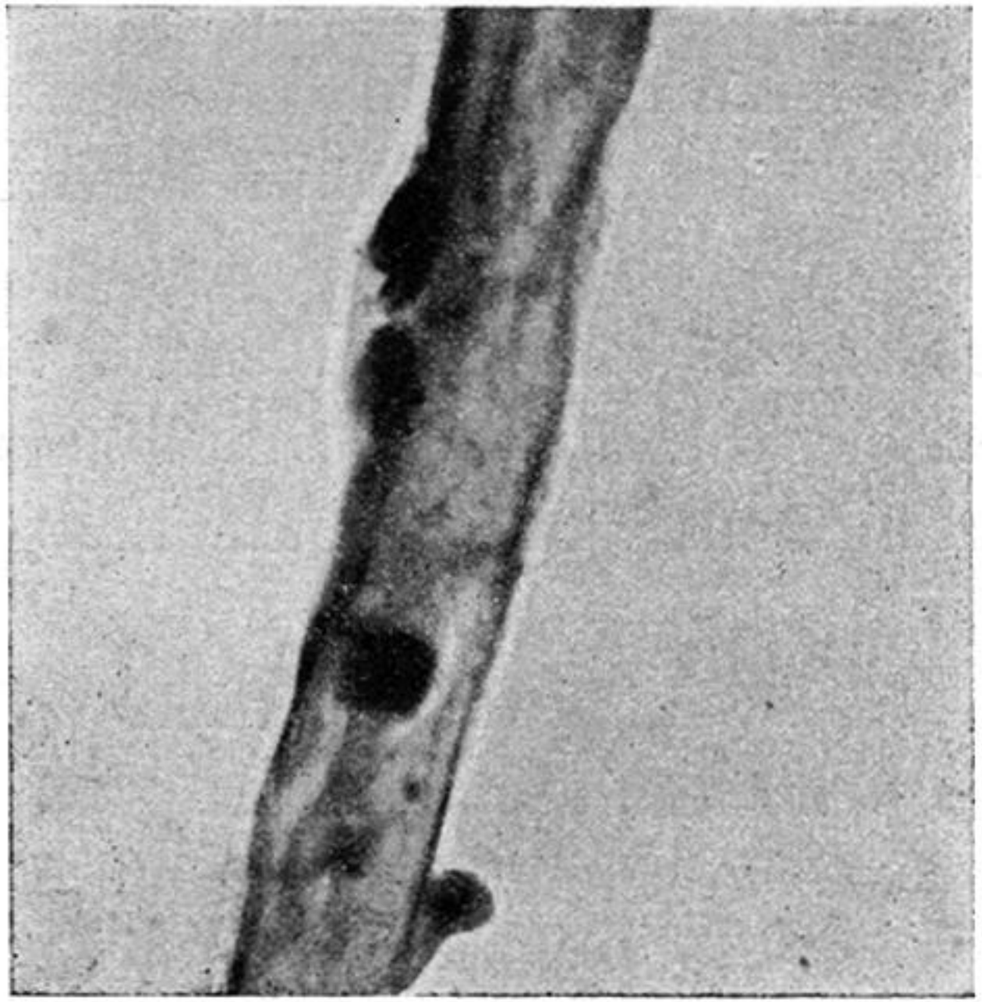


Fig. 22.

Single fibre from nerve of same animal, to show multiplication of nuclei of the primitive sheath. eased specimen; after hardening in MÜLLER'S fluid it was washed, stained with logwood, and mounted in ARRANT'S solution. 870 diameters.

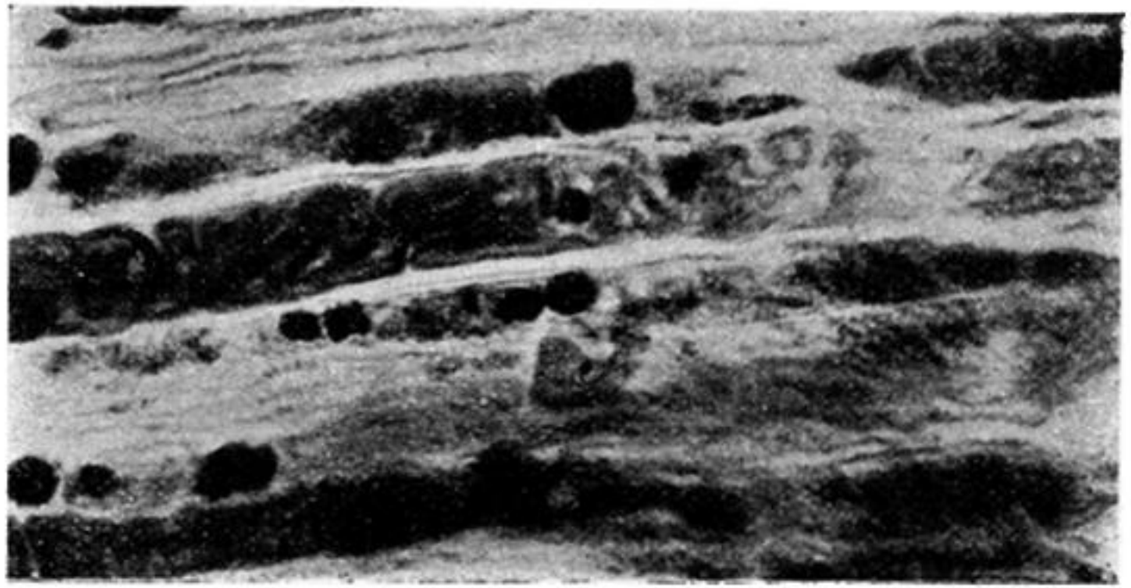


Fig. 23.

Longitudinal section of nerve, 10 days after operation (Cat N). Method—MARCHI's fluid direct. 00 diameters.

Fig. 24.

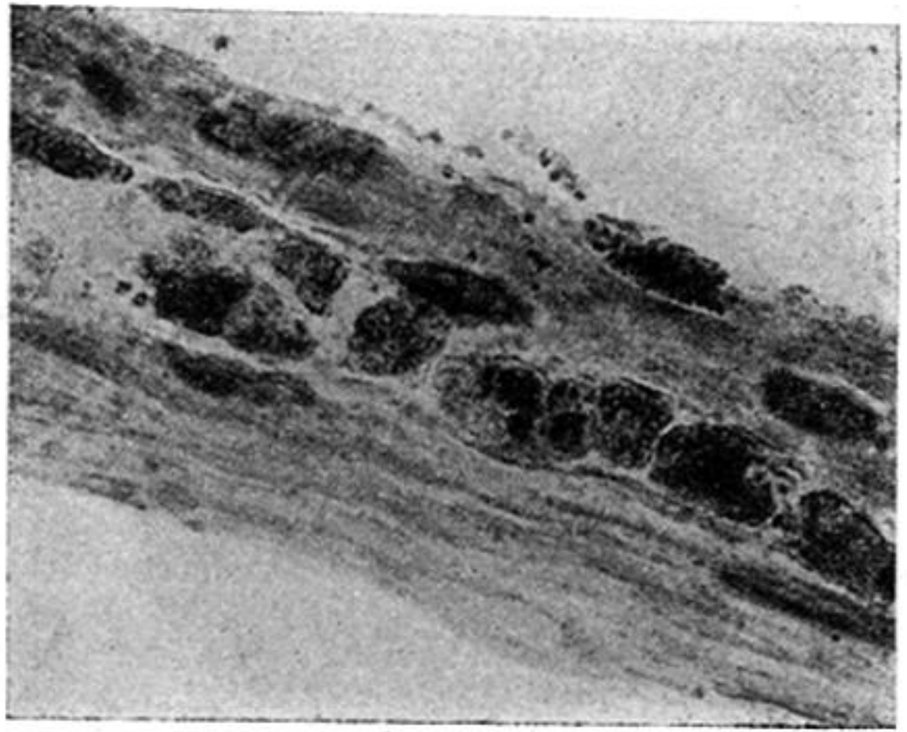


Fig. 25.

Fig. 24.—Longitudinal section of nerve, 27 days after operation (Cat O). Same method. 500 ameters.

Fig. 25.—Longitudinal section of nerve from same cat. Method—MARCHI's fluid direct, then logwood. 50 diameters.

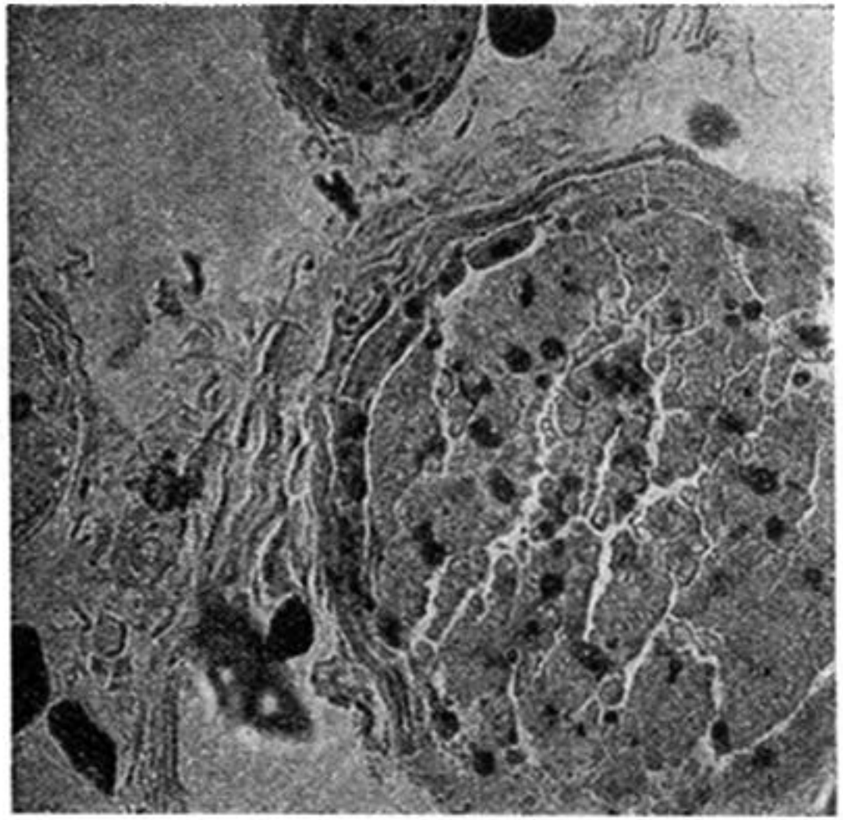


Fig. 26.

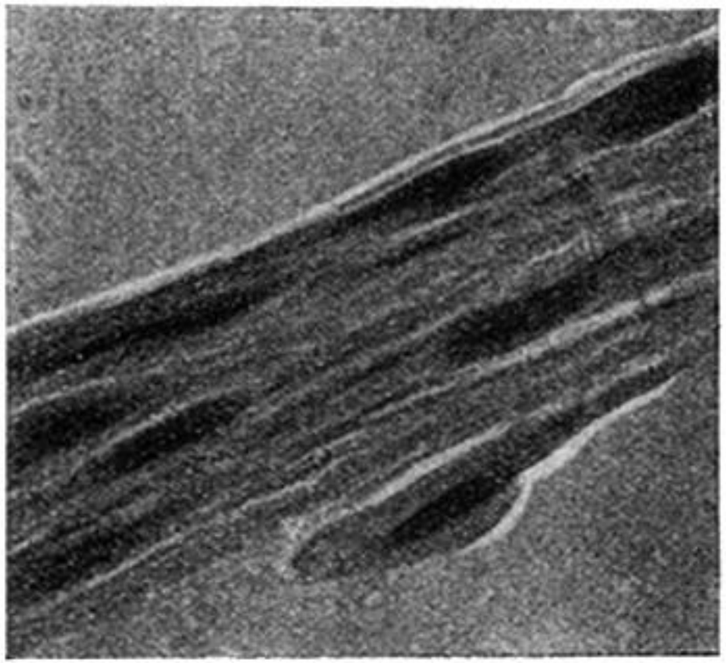


Fig. 27.

Fig. 26.—Transverse section of nerve, 44 days after operation (Cat H). Method—MARCHI's fluid rect. 200 diameters.

Fig. 27.—Nerve from same cat. Teased preparation, stained with logwood and mounted in FARRANT. 00 diameters.

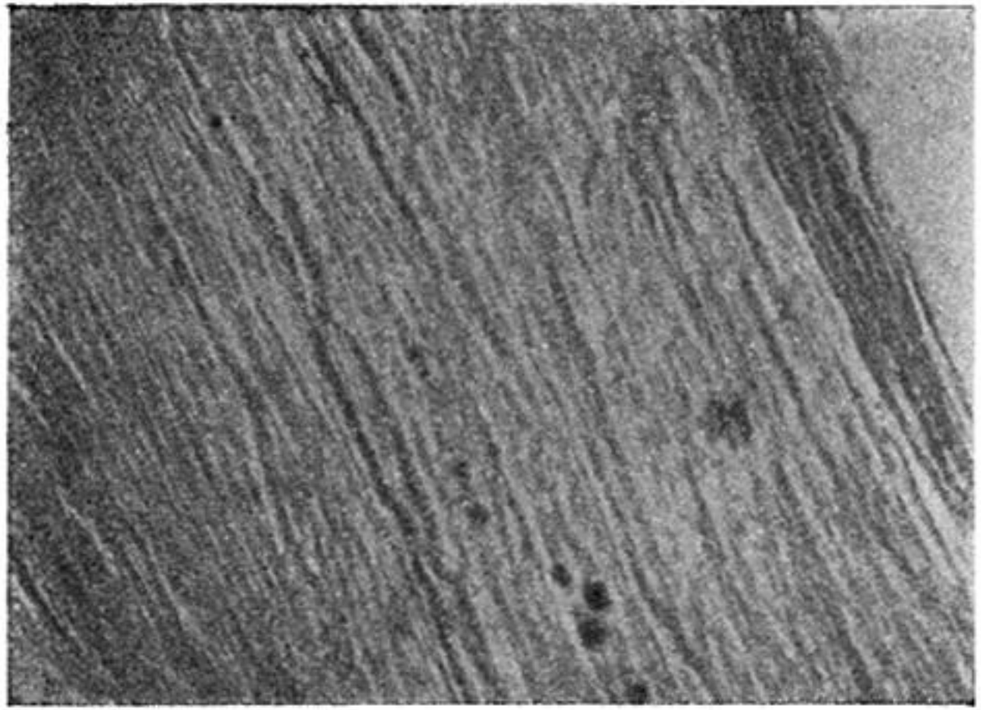


Fig. 28.

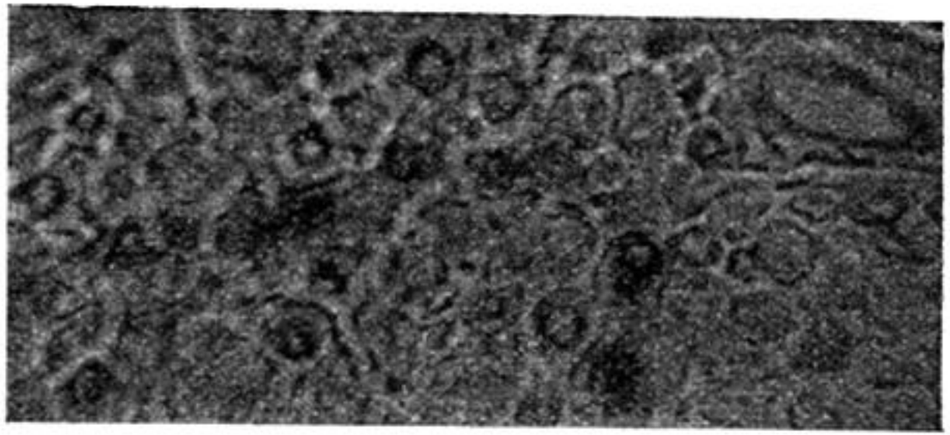


Fig. 29.

Fig. 28.—Longitudinal section of sensory nerve, 106 days after operation (Cat F). Method—MARCHI's
uid direct. 700 diameters.

Fig. 29.—Transverse section of the same. Same method. 700 diameters.

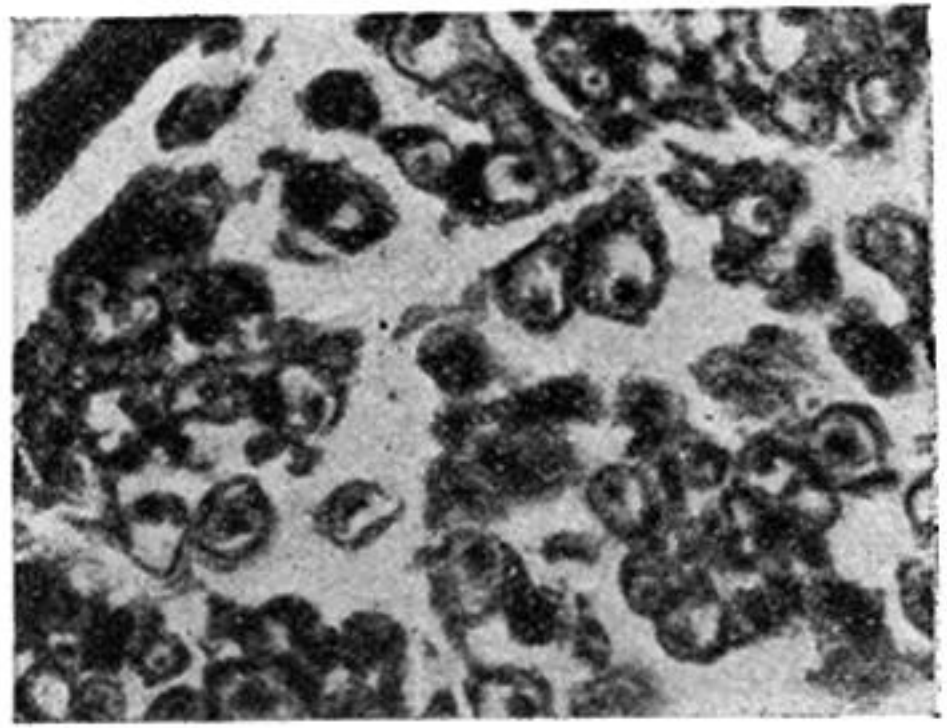


Fig. 30.

ransverse section of the same, stained by STROEBE'S method. 700 diameters.

Fig. 31.

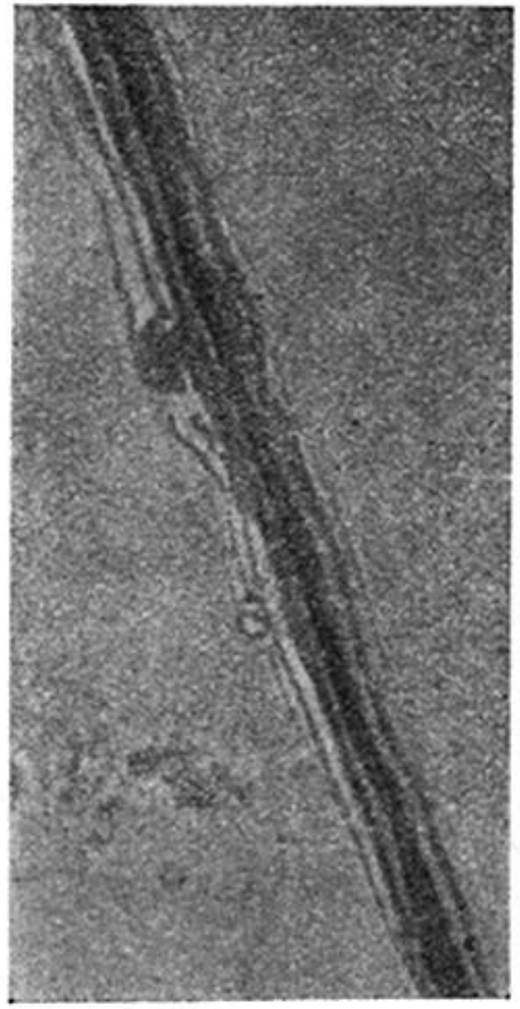
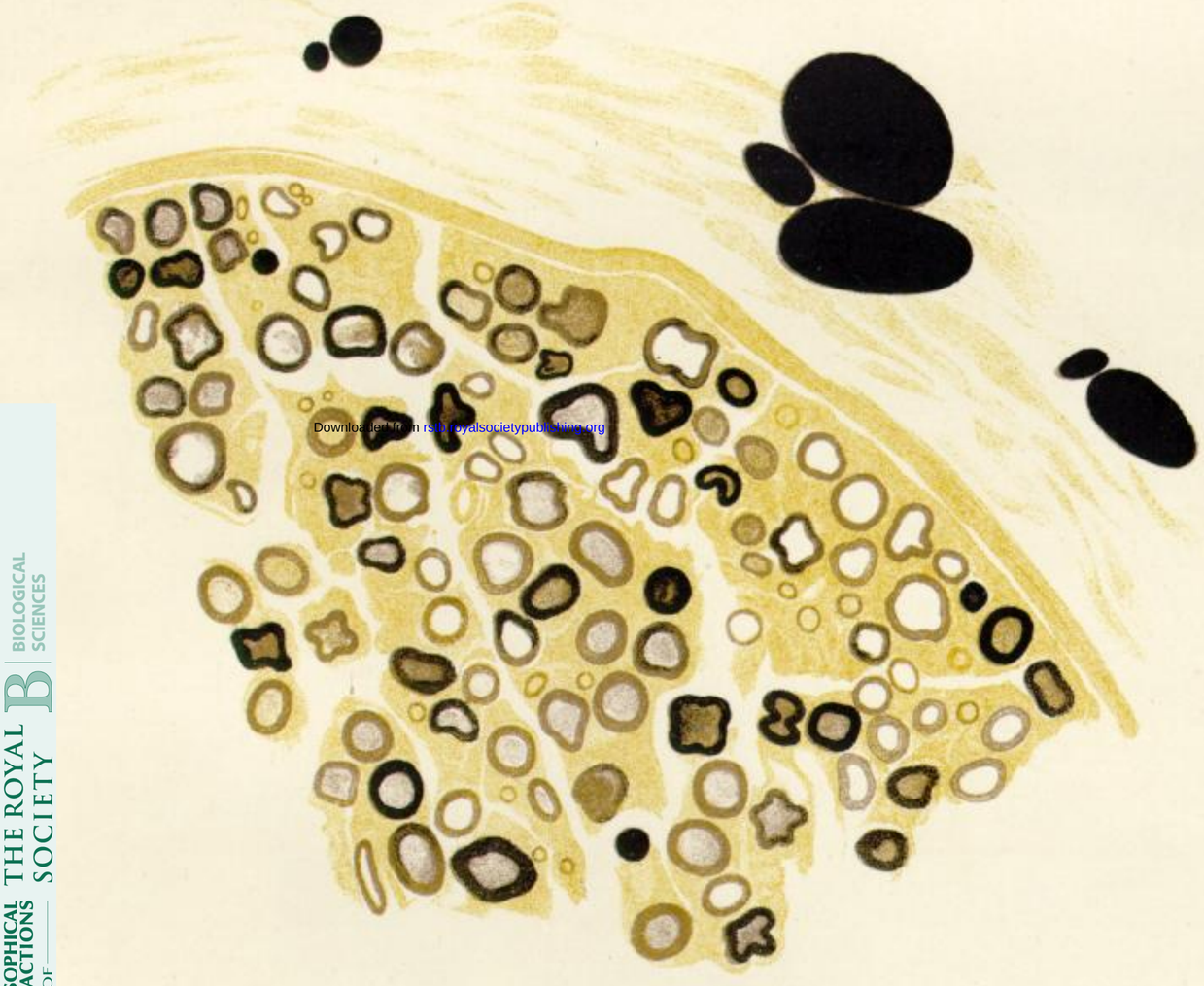


Fig. 32.

Fig. 31.—Teased preparation of the same. Stained with logwood; mounted in FARRANT. 600 diameters. The apparent excrescences in the fibre are air bubbles accidentally entangled in the FARRANT'S solution.

Fig. 32.—Teased preparation of the same. Stained with logwood; mounted in FARRANT. 600 diameters.

A.



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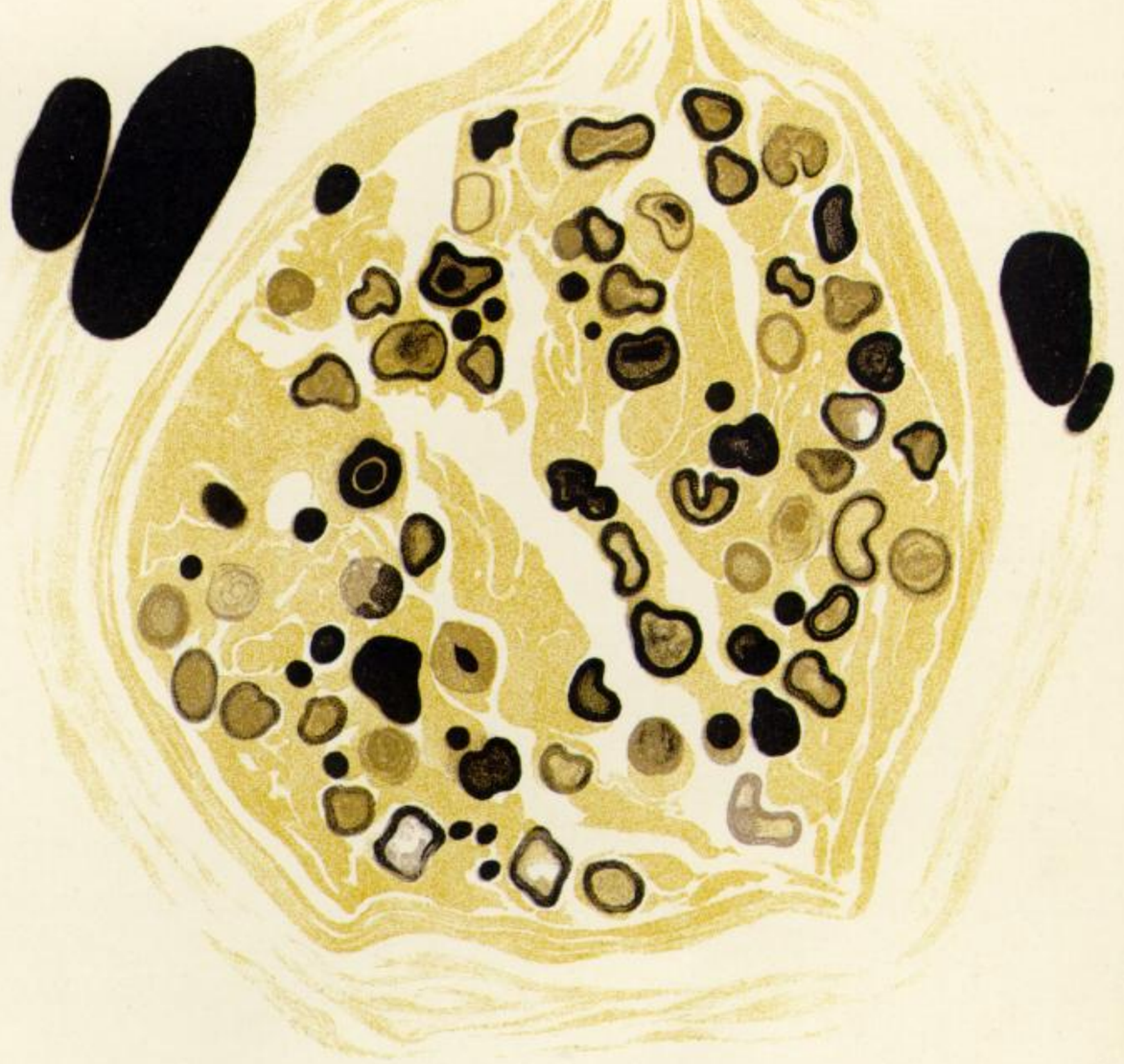

C.

Description of Coloured Plate. (Plate 45.)

A. Transverse section of nerve, 2 days after operation (Cat A). Direct Marchi method. 425 diameters.

B. Transverse section of nerve, 92 hours after operation (Cat P). Direct Marchi method. 425 diameters.

C. Transverse section of nerve, 10 days after operation (Cat N). Direct Marchi method. 425 diameters.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES