Regeneration of medullated nerves in the absence of embryonic nerve fibers, following experimental non-traumatic degeneration ... / by Elbert Howard Clark.

The University of Chicago

REGENERATION OF MEDULLATED NERVES IN THE ABSENCE OF EMBRYONIC NERVE FIBERS, FOLLOWING EXPERIMENTAL NON-TRAUMATIC DEGENERATION

A DISSERTATION
SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

BY

ELBERT HOWARD CLARK

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ELBERT CLARK

From The Anatomical Laboratory, University of the Philippines

THIRTY-TWO FIGURES

INTRODUCTION

The present study is based upon experiments in which degeneration and regeneration of medullated nerve fibers were brought about under new experimental conditions. The results obtained relate, for the most part, to phases of the subject upon which the evidence has heretofore been incomplete. In this investigation, an experimental obstacle which has been responsible for the strikingly contrary observations between the supporters of auto-regeneration on the one hand and the advocates of an outgrowth of the axis cylinder on the other, has been entirely avoided. I refer to an ingrowth of foreign nerve fibers through the scar tissue into a regenerating medullated nerve. This obstacle was avoided by inducing degeneration in the peripheral medullated nerves of the domestic fowl by a prolonged exclusive feeding of polished rice, and subsequent regeneration by a return to an adequate nutritive diet.

In 1897 Eijkman first described 'polyneuritis' in fowls which had been kept for three or four weeks on an exclusive diet of polished rice. This has since been confirmed by numerous other investigators and Frazer and Stanton ('11) have noted and illustrated 'Wallerian degeneration' in the nervus ischiadicus of the domestic fowl which developed paralysis on a polished rice[1] diet.

[1] White rice, polished rice or decorticated rice is the clear white table rice of commerce. It is rice, which, after having the husk taken off, is further sub-

In another place[2] I have described more in detail the changes occurring in the nervous system of such fowls Here it was also pointed out, in agreement with Frazer and Stanton and others, that "The neuritis produced in fowls by a prolonged diet of polished rice is, so far as the best evidence indicates, a neuritis due to a deficiency of some food constituent or constituents necessary for the maintenance of the metabolic and functional activity of the nervous system."[3]

In the paralysis of fowls brought about by an exclusive diet of polished rice the medullated fibers of the sciatic undergo a rapid degeneration. This degeneration, however, is much slower than that produced as a result of transection of the nerve. Moreover, for the rice-fed fowls, the following conditions obtain: In

jected to a process of 'milling' or polishing. "In this process the fruit wall or pericarp, the layers subjacent to it (the subpericarpal layers) as well as the embryo are removed," Frazer and Stanton ('11). These authors give the following as the average composition of polished and unpolished rice:

	PROTEIN	FAT	CARBO-HYDRATE	ASH	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Polished rice	7.7	0.25	77.23	0.25	14.3
Unpolished rice	9.0	1.65	75.52	1.08	12.75

Unpolished rice or red rice is rice which has not been subjected to the polishing process, and which as a consequence still has the pericarp, subpericarpal layers and the embryo. Fowls fed exclusively upon unpolished rice for long periods never develop neuritis as when fed exclusively upon polished rice. Further, neuritis in fowls as the result of an exclusive rice diet can most frequently be cured by placing the fowl on an exclusive diet of unpolished or red rice. There are several qualities of white rice, which, aside from the quality of the grain, are denoted by the amount of polishing to which the rice has been subjected. As might be expected the most highly polished grade is the most effective in producing paralysis in the fowl.

[2] Edward B. Vedder and Elbert Clark. A study of polyneuritis gallinarum. Philippine Journal of Science, vol. 7, no. 5, Sec. B, p. 423.

[3] Richard P. Strong and B. C. Crowell have produced experimentally in man a similar neuritis by the prolonged feeding of a diet of which polished rice formed by far the main constituent. In one case which resulted fatally, the peripheral nerves showed marked degeneration (The etiology of beri-beri. Phil. Jour. Science, B, vol. 7, p. 271). John M. Little has also described beri-beri in man resulting from an almost exclusive diet of white bread (Beri-beri caused by fine white flour. Jour. Amer. Med. Assoc., vol. 58, p. 2029).

degeneration the fibers are intact and all traumatic and inflammatory effect produced by cutting the tissues and the nerve or of tying the latter are obviated; the process of degeneration can be stopped at almost any stage or greatly prolonged, and several stages of degeneration are to be observed in different fibers of the same nerve. In regeneration, the possibility of an ingrowth of fibers from other nerves into the regenerating nerve under observation is obviated and recovery of the animal can be accomplished after any stage of degeneration of the peripheral nerves. And lastly, the slowness of the cycle of degeneration and recovery, makes it possible to draw a sharper distinction between the process of degeneration and regeneration in medullated nerve fibers. At the present time these experimental conditions are especially desirable.

PARALYSIS IN FOWLS RESULTING FROM AN EXCLUSIVE DIET OF POLISHED RICE

A typical case of a fowl which has become unable to walk after a diet of polished rice is found in that of No. 54 whose history is as follows: No. 54, brown hen, fed polished rice since February 7, 1912; 25 days later, on March 3, the first definite signs of unsteadiness in the legs were noted. March 4, the bird was found balanced on its 'haunches,' was scarcely able to rise and could not take more than two or three haphazard steps without tumbling over in a heap. The diet was changed to a 'regenerative' diet consisting of whole grain, meat scraps, bread, grass, etc. March 14, could stand up very unsteadily for a few seconds but was scarcely able to take a step. March 20, same —never stood up nor attempted to walk unless forced and assisted in this. April 3, good general appearance but was scarcely able to walk. April 10, improved, but walked with much difficulty. May 3, apparently entirely recovered within the last few days.

It should be noted that many fowls on the polished rice diet lose complete control of the lower part of the legs. Others show wing drop, droopiness of the head and inability to swallow.

Still others show complete collapse.[4] The greatest variety of symptoms are manifested by various birds, but loss of control of the legs is the most frequent. Fowls showing the latter symptom, with otherwise fair to good general condition, were the ones selected for this study; nerves of these show a more pronounced degeneration, and recovery in this class of fowls is more easily accomplished. Twenty to thirty days on the white rice diet is the usual length of time before symptoms of neuritis are manifested. Some birds resist for 35 or 40 days, and two fowls[5] that were receiving a small quantity of calcium lactate with the rice did not 'come down' till the fifty-first and sixtieth day respectively. Nitrogenous and fatty foodstuffs in very small amounts added to the rice also greatly defer the development of the neuritis. For more complete data on this interesting affection and for feeding experiments, reference should be made to the recent article by Vedder and Clark ('12).

DEGENERATION

A few remarks should be made at this point concerning the nature and extent of the degeneration in the medullated fibers of the sciatic nerve in fowls of the class under consideration. In the nerves of 60 chickens, which had been fed 20 days or more on an exclusive diet of polished rice, degeneration in the fibers of the sciatic nerve was observed by the aid of the Marchi method in every case regardless of what symptoms were manifested by the fowl before death. Many of these were confirmed by the Weigert method for staining the myelin sheath. Several fowls fed as long as 35 to 40 days showed no signs of weakness in the legs but well marked nerve degeneration. The nerves from each of twelve fowls fed from 7 to 22 days consecutively

[4] Several workers have observed that fowls occasionally do not lose weight on the polished rice diet. Frazer and Stanton ('11) who have kept very complete records report several fowls which kept their weight up for as long as 35 days. Other fowls even showed a gain in weight.

[5] Courtesy of Dr. R. B. Gibson of the Department of Physiology; from experiments being conducted by him to study the influence of an addition of various salt mixtures to the white rice on the production of this affection, to be reported shortly.

with no leg weakness showed, by the same methods, myelin degeneration in their fibers. It was a constant observation that different fibers of a given nerve present the greatest variation in the degree of their degeneration. In two fowls killed after feeding 7 days on white rice, small areas of blackening after treatment by the Marchi method were observed in approximately one-third of the fibers of the sciatic. These areas very seldom involved the entire diameter of the individual fiber at any one point and the great majority ranged from 1 to 8 microns in diameter (fig. 32).

In the sciatic nerve of those fowls fed for a longer time and which developed a typical paralysis in the legs, every fiber showed larger areas of blackening. Advanced degeneration was found in from 10 per cent to 20 per cent of the fibers. The change shown by these latter fibers presents an identical picture of degeneration with that in medullated fibers of a mammalian nerve 10 to 14 days after section, but for the nuclei of the neurilemma sheath. The nuclei of the neurilemma sheath have undergone little or no multiplication. This will be again referred to and more fully discussed in the consideration of the "embryonic nerve fiber." By both the Marchi method and the Weigert method for the medullary sheath, the change of the medullary sheath substance into fatty globules and droplets appears complete in some fibers. As I have pointed out in a previous paper ('12),

> Fibers showing advanced degeneration are marked by accumulation of degenerated myelin in large globules and droplets, a swelling and bulging of the nerve sheath at these points and a disintegration of the axis cylinder. The largest globules usually appear vesicular and in their center, segments or fragments of the axis cylinder are frequently to be seen. In these larger, and in some of the smaller globules also, the stainable material is found at the periphery and appears laminated. This laminated appearance is very characteristic in Weigert preparations and is the rule in the larger globules. Usually 3 distinct layers are clearly visible, of which the outer is the thickest. Other incomplete layers and fragments are seen centrally.

But for an increase in the number of nuclei of the neurilemma sheath, this description applies with equal exactness to fibers of similar preparations of the peripheral segment of the sciatic nerve of a fowl 7 days after section. Figures 2, 3, 4 and 5

illustrate the marked degeneration described, and figure 6 shows segments of axis cylinders enclosed within large globules.

Howell and Huber ('92), Bethe ('07), Mott, Halliburton and Edmonds ('04), Cajal ('05), Ranson ('12), and others have observed that after section not all medullated fibers degenerate with equal rapidity. As noted, this is particularly true of degeneration in the fibers of the sciatic in fowls on a white rice diet. In the nerves of these fowls, a fiber showing the first indication of degeneration may be found side by side with one in which the neuraxis and medullary sheath are completely broken up (fig. 7).

These few remarks, with the accompanying figures 2 to 7, make it clear that paralysis in the rice-fed fowls presents an experimental condition resulting in degeneration in which almost every stage of the process is to be observed in the same nerve at one and the same time, in which the continuity of the fibers is not disturbed, where reaction to trauma is obviated and where degeneration, though proceeding rather rapidly, is much slower than after section of the nerve.

THE EMBRYONIC NERVE FIBER

Rapid multiplication of the nuclei of the sheath of Schwann, coincident with the change in the medullary sheath and axis cylinder, has been a constant finding with all those observers who have studied degeneration of nerves after section. It was a little surprising to find it not to obtain in the present case which in every other respect resembles Wallerian degeneration and in which the process of degeneration is also rapid. A careful search of both teased and sectioned preparations of nerves, taken at time of paralysis and in which the nuclei were well stained, failed to reveal any marked or definite increase in the number of nuclei of the neurilemma sheath or any structure which could be definitely identified as an embryonic nerve fiber in the degenerated fibers of a single fowl.

An explanation of this marked variation was of course required, and inasmuch as fowls frequently recovered after the most marked paralysis of the legs, it became necessary to determine if regeneration occurs in that 10 to 20 per cent of fibers which show

such marked degeneration after a prolonged diet of white rice. This being the case, it would then be desirable to know whether regeneration could take place in these fibers without going through that stage termed 'embryonic nerve fiber,' 'Bandfiber' or 'protoplasmic band.' For a more complete understanding of the significance of the multiplication of the nuclei of the neurilemma sheath and the embryonic nerve fiber, I sought to determine if degeneration in medullated nerve fibers without multiplication of the nuclei of the neurilemma sheath could be brought about by any other experimental means; and if so, could regeneration be accomplished in these without the embryonic nerve fiber stage? Further, could it be shown that the increase in the number of nuclei usually observed is due to trauma or inflammatory influences or to an infiltration of phagocytes? And lastly, does the embryonic nerve fiber represent a stage of regeneration or degeneration? An answer to these questions would manifestly throw additional light upon the significance of the increase in number of nuclei of the sheath of Schwann and of the embryonic nerve fiber. This proved to be a most attractive phase of the investigation.

Atrophic degeneration without multiplication of the nuclei of the sheath of Schwann has been frequently described in certain chronic pathologic conditions lasting many weeks or months. In the present case, however, where globulation and breaking up of the axis cylinder have been observed as early as the nineteenth day of the white rice diet and where the first evidences of change noted was on the seventh day, the degeneration (taking place in 12 days) can not be said to have anything in common with atrophy.

It soon became evident on histologic examination that regeneration does occur in those fibers in which the neuraxis and medullary sheath have broken up. Having failed to find in the degenerated fibers a single embryonic nerve fiber or any marked or definite increase in the number of nuclei of the neurilemma sheath, I next examined nerves from fowls at various periods during regeneration. With this end in view, fowls were killed after having been kept on a regeneration diet for 4, 11, 13, 14,

16, 19, 21 and 30 days respectively. The fowl which had been kept for 30 days on the nutritive diet showed marked improvement. In addition to nerves from this series, segments of the sciatic, cut out from fowls 48, 56, 59 and 60 days in regeneration respectively, were examined for embryonic nerve fibers and multiplication of the nuclei of the neurilemma sheath. These four fowls had just become able to walk again. In neither teased nor sectioned preparations of the nerves of all these fowls were embryonic nerve fibers to be found. Nor was there observed a more definite indication of multiplication of the nuclei of the neurilemma sheath than was seen in the nerves of those fowls killed at the time paralysis developed. This lack of nuclear multiplication also obtained in nerves of fowls 108, 125, 171 and 275 days in regeneration.

Attempts to produce by other means a degeneration in the medullated fibers without a multiplication of the nuclei of the sheath of Schwann were not successful. Freezing a small portion of the sciatic with carbon dioxide snow, treating a portion with chloroform vapor, and injecting a drop or two of chloroform into the nerve, when successful in producing degeneration, resulted also in the typical and rapid increase in the nuclei of the neurilemma sheath as has been constantly described after section or ligature of the nerve. Less violent means were then adopted in that rubber bands, tight enough to cut off the circulation but not tight enough to do mechanical injury to the nerve were placed around the thigh of fowls. Loss of control of the leg resulted after 3 or 4 days, both when the bandage was allowed to remain for 24 hours at a time and when it was on and off every few hours over a course of 2 or 3 days. Although by this method the result sought for was not accomplished, yet another observation of equal importance was made. Fowl No. 78, on which the bandage was allowed to remain for 24 hours, experienced considerable œdema of the bandaged leg; this condition progressed, without infection, to dry gangrene of nearly all of that portion peripheral to the bandage by the twenty-eighth day, or approximately 24 days after loss of control of the leg. This, then, represents a nerve in which only retrogressive changes

could have taken place as gangrene early manifested itself—by the twelfth day of the loss of control. Fibers from that portion of the leg affected by gangrene presented an appearance from which they were readily recognized as embryonic nerve fibers (fig. 8). For the most part these were very slender with long nuclei, an appearance which is readily accounted for by the partial desiccation. Many still contained droplets of degenerated myelin. It is difficult to see how the least regenerative reaction could have taken place in this nerve.

As stated above, I have not observed a single instance in which there was a marked increase in the number of nuclei of the sheath of Schwann in those degenerated fibers of fowls which showed paralysis of the legs after 20 to 30 days on polished rice. In fibers showing the most advanced degeneration, that is, marked globulation of myelin and breaking up of axis cylinder, measurements were made between neighboring nuclei of the sheath of Schwann. Among these measurements, there were observed such distances as these between successive nuclei: 368, 386, 477 and 379 microns respectively. The distance between 2 nodes of Ranvier is variously estimated as from 80μ to 900μ according to the diameter of the fiber, and "in the higher vertebrates a single nucleus is found midway between each two nodes"—Huber. Mitosis has not been observed.

Some few fibers, however, were seen in which the nuclei of the sheath of Schwann occurred at intervals frequent enough to be suggestive of a slight increase in their number. This was suggestive enough to make it desirable to examine, for an increase of the number of nuclei of the sheath of Schwann, nerves of fowls with which the onset of paralysis had been deferred by the addition of very small amounts of other foodstuffs to the rice. At this period I was fortunate in securing from Dr. R. B. Gibson of the Department of Physiology, a fowl, No. 17, *G*, which suddenly developed paralysis of the legs after 60 days on a diet of white rice and calcium lactate. This fowl developed a typical case of leg paralysis two days before death. Marchi preparations of the sciatic showed very extensive and advanced myelin degeneration.

Teased preparations from the sciatic of this fowl stained to bring out the nuclei revealed a few fibers of the embryonic nerve fiber type. These were very slender fibers with nuclei at frequent intervals. Protoplasm was scant even around the nuclei, though the structures stained well (fig. 9). Many resembled very closely non-medullated nerves in degeneration described by Ranson ('12). Others were found, however, which contained a larger amount of protoplasm and in which small droplets of degenerated myelin could occasionally be found (fig. 10). This observation clearly shows these to have been derived from medullated nerve fibers. No axis cylinder could be demonstrated by special stains. Another fowl, No. 9, *G*, on a similar diet and with a somewhat similar history developed paralysis of the legs after 51 days. Teased preparations from the sciatic of this fowl, when stained to bring out the nuclei, also showed embryonic nerve fibers. These were a little more numerous than in No. 17, *G*, were larger and contained more protoplasm. Protoplasmic granules were seen in the immediate neighborhood of the nucleus and droplets of degenerated myelin were of rather frequent occurrence. Many of the fibers bore an exact resemblance to the protoplasmic bands to be seen in medullated nerves after section. Figure 11, which is from a teased preparation of the sciatic nerve of No. 9, *G*, illustrates this resemblance and shows beyond question their identity with the embryonic nerve fibers of a sectioned nerve.

Fowl No. 57, which came down with severe paralysis and prostration after 23 days on a diet of polished rice and which made a most difficult recovery, was killed 1 year and 14 days after being placed on a regeneration diet and approximately 10 months after all symptoms of paralysis had disappeared. Teased preparations, stained as above, showed among several thousand fibers one very frail fiber with nuclei at frequent intervals. The fiber appeared scarcely more than a strand of connective tissue with well staining spindle-shaped nuclei along its course. This was probably an embryonic nerve fiber. Previous observations had convinced me that regeneration of the axis cylinder fails to take place in a very small percentage of those fibers which

have shown degenerative changes. This slender fiber with frequent nuclei, then, might easily represent a later stage or atrophy of a fiber which failed of regeneration.

To summarize briefly: Neither embryonic nerve fibers nor a marked multiplication of the nuclei of the neurilemma sheath have been found in degenerating fibers of fowls showing paralysis after 20 to 30 days on polished rice: they have not been observed (with one possible exception) in regenerating fibers of fowls recovering from this paralysis: embryonic nerve fibers containing droplets of degenerated myelin were found in the nerves of fowls fed for 51 and 60 days on a polished rice diet, and embryonic nerve fibers entirely replaced the nervous elements in the sciatic of a fowl whose leg was undergoing progressive necrosis.

This chain of evidence seems complete and can leave little doubt as to the significance of the so-called embryonic nerve fiber, Band-faser or protoplasmic band. In each instance except the last we have the embryonic nerve fiber occurring in a nerve which is undergoing progressive retrogression. In fowl No. 78 the degeneration was due to mechanical causes. In No. 17, *G*, and No. 9, *G*, those few fibers which always showed marked degeneration by the thirtieth day, regardless of whether symptoms are manifested or not, had had time for a more advanced degeneration than those fibers of fowls killed at an earlier date. In either case, it was impossible for regeneration to be taking place.

Thus the conclusion that the embryonic nerve fiber represents a late stage of degeneration is a logical one. Degeneration as used above is meant to imply a retrogressive change in the myelin sheath and axis cylinder. It is to be noted that multiplication of the nuclei of the neurilemma sheath and the resulting embryonic nerve fiber appear only after degeneration in the axis cylinder and medullary sheath has advanced to a late stage. As Van Gehuchten points out, the sequence of events in the formation of the embryonic nerve fiber with cytoplasm and cytoplasmic granules around the nuclei must be considered evidence of protoplasmic activity and in themselves bear a close resemblance to regenerative processes. This, however, does not necessarily imply an attempt on the part of the structure at formation of a new

medullary sheath or axis cylinder. Indeed, Mott, Halliburton and Edmonds ('04) in regeneration in nerves after section, find that the new medullary sheath, as well as the axis cylinder, progresses from the point of union of central and peripheral stumps. I will suggest that the multiplication of the nuclei of the neurilemma sheath and the formation of the embryonic nerve fiber in the rice-fed fowls is comparable to the proliferation of connective tissue in organs undergoing atrophy or degeneration from other causes. In the fowls degeneration of the nerve fibers is slow and it is probable that the stimulus is not sufficient to bring about a multiplication of the neurilemma nuclei until very late. On the other hand, the trauma occasioned by transsection of the nerve introduces a violent reaction and multiplication of the nuclei of the neurilemma sheath rapidly ensues.

INFILTRATION OF PHAGOCYTES AND ABSORPTION OF THE DEGENERATED MYELIN

Stroebe ('93), Mott, Halliburton and Edmonds ('04), Nageotte ('11) and other have described an infiltration of phagocytic wandering cells into medullated fibers undergoing myelin degeneration. Nageotte ('11) claims it as a constant occurrence and sees in their presence a means of removal of the degenerated myelin. According to Nageotte ('11), these foreign elements

> constitute the agents of greatest activity in the resorption of the degenerated myelin. This does not signify that the syncytium of Schwann remains inert; it can, indeed, resorb the myelin, and it is probable that, in the fine fibers, it accomplishes the work of phagocyotosis of the mylein without the aid of the foreign elements. In the large fibers, at the end of the third day, one sees in the perinuclear protoplasmic mass special granulations which result from the disintegration of the myelin; but, in these fibers the larger part of the myelin becomes the prey of the leucocytes. It is probable that the leucocytes emigrate once their work is accomplished.

In nerves of fowls with marked leg paralysis after about 30 days on polished rice, I have sometimes seen fibers in degeneration in which the appearance of infiltration by wandering cells is very striking. These cells were all very small and, after Müller fixation, scarcely anything but the nucleus was to be seen in

hematoxylin and carmine preparations. In an occasional fiber they were quite numerous, though uniformly absent from those fibers showing the larger globules of degenerated myelin with enclosed segments of the axis cylinder. Figure 12 shows the most marked instance of infiltration observed in this series. Here it is clearly evident that not more than one nucleus belongs to the sheath of Schwann. These nuclei have not been observed in fibers from fowls which developed paralysis after 51 days or more on polished rice. Their presence in some fibers, however, is sufficient to show that trauma is not alone responsible for their presence.

I have not been able to determine the significance of these wandering cells or their fate. That their participation in the removal of the degenerated myelin is improbable and at most can be only extremely slight, is clearly manifested by the persistence of degenerated myelin seen in fibers of Marchi preparations from fowls which had been kept a long time on a regeneration diet and for several months after all symptoms of paralysis had disappeared.

In nerves from these fowls, both small droplets and large globules of degenerated myelin still obtained, and indeed in some fibers the picture resembled very closely that of degeneration in medullated nerves of fowls but recently affected by paralysis. There seemed to have been some little absorption of degenerated myelin in the nerves of those birds kept for 4 months in regeneration, yet globulation was just as marked and the globules were equally large. The globules of degenerated myelin in the fibers of fowls dead just after paralysis appeared dense, while in the fibers of fowls 4 months in regeneration the globules frequently appeared honey-combed or somewhat reticulated. The droplets of degenerated myelin were uniformally smaller in the latter. Fowl No. 38 (leg paralysis after 22 days on polished rice, recovery apparently complete after 59 days on regeneration diet) was killed on the 108th day of regeneration or 49 days after all symptoms of neuritis had disappeared. Every fiber of the sciatic nerve of this fowl contained droplets or globules of myelin and many of the larger globules just described. Even

the swelling of the fiber and out-bulging of the sheath at the large globules obtained and the larger globules were still vesicular. Figure 13 is a photomicrograph of a Marchi preparation of the sciatic nerve from this fowl. Droplets of degenerated myelin are clearly visible in every fiber and the larger globules occur at frequent intervals. Figure 14 is a similar picture of the sciatic of No. 54, 171 days in regeneration. The droplets and globules of degenerated myelin are equally definite here.

Degenerated myelin was found to persist in the sciatic nerves of a fowl 10 months after all signs of paralysis had disappeared and 1 year and 14 days after regeneration diet was started. Figure 15 is taken from a Marchi preparation of the sciatic nerve of No. 57, whose recovery history was as is just indicated. The largest globules have completely disappeared or more probably have considerably decreased in size. The smaller droplets are also decidedly less numerous and the total amount of degenerated myelin is comparatively small.

In view of the finding that the sensory nerves usually show an apparent recovery before the motor, it was thought worth while to compare the sensory and motor portions of the sciatic nerve of No. 57 (1 year and 14 days in regeneration). Accordingly the motor and sensory portions of one of the larger roots were separated for some distance peripheral to the dorsal root ganglion, each stained by the Marchi method and teased. No difference whatever could be distinguished between the two. It was quite impossible to tell one from the other (compare fig. 15 and fig. 16).

It would thus seem that, in the present case at least, phagocytes are in no way concerned in the removal of degenerated myelin. Phagocytosis is clearly excluded inasmuch as the disappearance of myelin is so extremely slow and since wandering cells are as a rule not found in degenerating nerve fibers of the fowls under consideration.

Just why degenerated myelin should persist so long in nerve fibers of these fowls and disappear so quickly from those nerves which have been sectioned or ligated or even from No. 78 of my series, whose leg had been bandaged for 24 hours, is not clear. The above observations would seem to exclude removal

by wandering cells which are common to both. An explanation might be sought in the multiplication of the nuclei of the sheath of Schwann and the resulting embryonic nerve fiber, which are such marked characteristics of degeneration in the latter, while being uniformally absent from the nerves of fowls showing an early paralysis after a white rice diet. This is rendered still more probable when it is remembered that in those fowls in which paralysis was deferred—No. 17, *G*, and No. 9, *G*—typical embryonic nerve fibers were present and within these fibers degenerated myelin occurred in only very small amounts or was entirely absent. These two fowls differ from those showing paralysis at an earlier date in that in the former degeneration in a certain proportion of the fibers had progressed to a later stage than with those coming down at an early period. Were it possible to keep the fowl alive on the white rice diet till all fibers were given opportunity to undergo advanced degeneration, I have no doubt but that after regeneration in the same animal the medullated nerve fibers would be quite devoid of even droplets of degenerated myelin.

In those fowls that have recovered from paralysis after 20 to 30 days on polished rice, the whole chain of evidence convinces me that here degeneration is interrupted in the middle, as it were; and regeneration is accomplished or superimposed without passing through the later stages of degeneration. This being the case, we must attribute the rapid removal of degenerated myelin in sectioned nerves to the activity of the new nuclei of the sheath of Schwann and the embryonic nerve fiber. This assumption is further borne out by the well-known observations that in medullated fibers of the central nervous system in degeneration the globules of degenerated myelin persist for a very long time. Halliburton ('07), in speaking of this point, says: "In situations like the central nervous system where the neurilemma is non-existent, not only is the removal of degenerated myelin a very slow process, but as is well known, regeneration does not occur." Schröder ('08) points out, in speaking of degeneration in medullated fibers of the cord: "Noch nach einem Jahr sind grobe Schollen sowie namentlich feine Tropfchen zu

finden." This is a constant finding in fibers of the cord unless special precautions are taken to produce marked inflammation of the affected area, such as by infection. Then the degenerated myelin is removed somewhat more rapidly, though even here not so rapidly as in the peripheral nerves after section. With pronounced inflammation other factors are introduced which would readily account for the more rapid removal of the degenerated myelin; as they have no bearing on the question they need not be considered here. Now, medullated fibers of the cord differ histologically from medullated fibers of the peripheral nerves in that the former do not possess a neurilemma. After section Wallerian degeneration of the one differs from that of the other only in that the proliferation of the nuclei of the neurilemma sheath and the resulting embryonic nerve fiber are lacking in the fibers of the cord. Infiltrating phagocytes are found in degeneration in both cases.

It is clear then that the rapid multiplication of the nuclei of the neurilemma sheath introduces a factor which is responsible for the rapid removal of the degenerated myelin. It is probable that the protoplasmic activity, represented by the multiplying nuclei and the accumulation of protoplasm around these, is directly concerned with the rapid resorption of the degenerated myelin.

I have no evidence suggestive of a further activity of the embryonic nerve fiber and its nuclei and this phase of the subject was not taken up. A definite zone rich in protoplasm was, however, observed around the nuclei on the embryonic nerve fibers of the sciatic of fowl No. 9, *G*. In this protoplasm, discrete granules were to be observed in preparations stained with Mallory's phosphomolybdic acid hematoxylin for axis cylinders. These granules have been noted by Reich and others. According to Stroebe ('93), Nageotte ('11) and others they bespeak an activity of the nuclei concerned in the development of a new medullary sheath.

This view in one form or another has been frequently advocated, both as an hypothesis and as an interpretation of the fact that, as pointed out by Stroebe ('93), the protoplasm which

increases in amount with the multiplication of the nuclei and the disappearance of the degenerated myelin in nerves after section, contains numerous small granules and droplets of fatty material. Thus according to Mott, Halliburton and Edmonds ('04), "they (i.e., the nuclei of the neurilemma sheath) multiply, and later appear to share with phagocytes in the removal of the broken up myelin droplets." And Schröder ('08), basing his views upon the microscopic observations of Stroebe and Büngner and Schütte, has the following to say relative to the removal of the myelin clumps:

> In this purely degenerative process (i.e., the early clumping of the myelin) early progressive occurrences interpose themselves. The nuclei of the sheath of Schwann begin to proliferate already on the second day, according to Stroebe they attain their maximum increase in number through mitosis at about the eighth day; coincident with the proliferation of the nuclei protoplasm develops, it shoves itself into the breaches and spaces between the myelin clumps, flows around the clumps, then gathers itself together into a single round or oval, demarcated, single-, or many-celled structure, within whose interior the clumps of myelin rapidly diminish themselves to fine granules (according to Büngner and Stroebe). In this way arise frequently granular cells with round cell body and a fine latticed protoplasm in whose meshes the granules lie enclosed. Such elements, from about the fourth week on, are to be found in the lymph spaces around the neighboring vessels. Stroebe and Schütte mention, that the genesis of thse granular cells has been often incorrectly conceived; with predilection, one has declared them leucocytes or so-called wandering cells and has assumed that they arise from the vessels opened at the point of the primary injury to the nerve, move forward along the nerve sheath and then take up at all places the disintegrated myelin.

REGENERATION

Marked multiplication of the nuclei of the sheath of Schwann at an early stage of degeneration and the resulting embryonic nerve fiber have been constant findings with all those observers who since Waller, have studied degeneration and regeneration of medullated nerves after section. Upon this point there is complete agreement. The interpretation, however, of the significance of the increased number of nuclei and more particularly of the embryonic nerve fiber has given rise, as is well known, to the most heated controversies, often involving personalties. As

a result the most varied experiments have been conducted and a vast amount of evidence on all possible phases of the subject has been presented. Unfortunately, however, the question remains unsettled. Unprejudiced authors of text-books still include both theories.

Two distinct and opposite views relative to regeneration after the embryonic nerve fiber stage are at present current. According to one, most vigorously and ably advocated by Bethe, the embryonic nerve fiber is capable of producing per se a new medullary sheath and new axis cylinder, which, later making connection with the central stump, results in a regenerated and functioning medullated nerve fiber; in young animals at least, the regenerated fiber is capable of conducting impulses regardless of whether or not connection with the central stump is established. The supporters of the contrary theory claim that a new axis cylinder for the peripheral stump is attained only by a down growth of axis cylinders from the central stump. While there is some difference of opinion as to minor points, this is the main contention of those who advocate the 'outgrowth' theory. The sequence of events as interpreted by the adherents of this theory is briefly set forth by Halliburton ('07) as follows:

> From the microscopic study of the distal portions of divided nerve trunks, we arrived at the conclusion that the activity of the neurilemmal cells has some relation to the development of new nerve fibers. At an early stage in degeneration their nuclei multiply; later they participate with phagocytes in the removal of the broken up myelin droplets; subsequently they elongate and, becoming connected end to end, lead to the formation of what some have termed "embryonic" nerve fibers. . . . . We arrived finally at the conclusion similar to that which Howell and Huber reached fifteen years ago, that, although the peripheral structures are active in preparing the scaffolding, the axis cylinder which is the essential portion of the nerve fiber has an exclusively central origin.

Stroebe ('93), Huber ('95), Cajal ('05), Marienesco ('06), Ranson ('12) and others have described and illustrated microscopic preparations of medullated nerves in regeneration after section which seem to show beyond a doubt that outgrowth of the axis cylinder from the central stump does take place. On the other hand Bethe ('02), who bases his opinion mostly upon physio-

logical grounds, has met the objections of his opponents in a most creditable manner by repeated experiments and new evidence which appear irrefutable. Langley and Anderson ('04), Lugaro ('05) and Mott, Halliburton and Edmonds ('04) have repeated many of Bethe's experiments but oppose rather than support him in his contention for auto-regeneration in the peripheral stump. Langley and Anderson ('04) admit that the peripheral stump, after a sufficient length of time has elapsed, may be found capable of conducting impulses even when union with the central stump is successfully prevented. They explain this apparent auto-regeneration by an ingrowth of nerve fibers from the surrounding tissues. In every case where the peripheral stump became capable of conducting impulses, strong evidence was obtained to show that an ingrowth of fibers into it from other neighboring nerves had taken place. Mott, Halliburton and Edmonds ('04) have confirmed this finding and have found that in a piece of medullated nerve transplanted in the same animal in such a manner that an ingrowth of fibers from other nerves is prevented, regeneration fails to take place. They have further shown that in a regenerating nerve the medullary sheath "appears earliest at situations near the point where the ends of a nerve have been joined together, and reaches the distal portions later." Bethe ('07) has again repeated these experiments but can find no reason to abandon his former strong conviction that auto-regeneration takes place in the peripheral portion of a divided medullary nerve. The experimental work of Ballance and Purves Stewart ('01) lead them to declare for auto-regeneration, and Van Gehuchten ('04) has confirmed Bethe's results. Wilson ('09) after repeating some of Bethe's work, draws no specific conclusion regarding auto-regeneration of divided medullary nerves.

In short the main contention of the advocates of auto-regeneration hangs on whether there may be no ingrowth of foreign fibers from neighboring nerves into the peripheral portion of a divided nerve. This at bottom is the point in dispute between a class of able workers who hold to auto-regeneration and a group of equally acute observers who advocate the outgrowth of the axis cylinder from the central stump. So long as so cap-

able investigators are unable to obtain the same result in a given experiment, just so long will our theories on regeneration of divided medullated nerves remain at variance. One cannot help but suspect, however, that on account of this very difference in results and the very heated controversy on the subject, regeneration of medullated nerves after section has received more attention and investigation that the comparative importance of the subject would warrant.

It was with no desire to engage in such a discussion that the present work was begun, and the new points brought out by it in regard to degeneration will greatly outweigh the observations on regeneration. On the other hand, however, I considered the controversy of others no excuse for avoiding the subject when a promising experiment was thrown at my door.

As stated above, in fowls fed for a long time on polished rice, there results an acute degeneration in the medullated nerve fibers resulting in a breaking up of the myelin into large globules and droplets and a segmentation of the axis cylinder. This change takes place in from 12 to 18 days and bears the closest resemblance to degeneration in medullated nerve fibers after section.

Clearly then, if it could be shown that the process of degeneration in the nerve fibers of the rice-fed fowls was identical with degeneration after section and if regeneration takes place in the former, then regeneration of medullated fibers was accomplished without the possibility of an ingrowth of fibers from other nerves and the main ground for a difference of opinion on auto-regeneration was obviated. If, on the other hand, degeneration in the two is not identical nor comparable, it would still be of interest to know if regeneration in the rice-fed fowls is or is not concerned with the so-called 'embryonic nerve fiber,' '*Bandfaser*' or 'protoplasmic band;' if auto-regeneration obtains; or if the new axis cylinder results from an outgrowth of the central connection. Whatever the result arrived at, the facts collected from this new type of experiment would add evidence to one side or the other and argue for or against auto-regeneration.

While there could be found no other microscopic differences in degeneration in medullated fibers after section and in medul-

lated fibers of the rice-fed fowls at the time of paralysis, multiplication of the nuclei of the neurilemma sheath and the embryonic nerve fibers were conspicuous by their absence from the nerves of the latter fowls. Could regeneration be accomplished in such nerves, without a multiplication of the neurilemma nuclei, the significance of the embryonic nerve fiber would be minimized.

With the above questions in mind, regeneration was studied in the sciatic nerve of fowls which came down in 20 to 30 days with marked leg paralysis and which were, from time to time, placed on a regeneration diet. To recapitulate briefly certain observations noted above; in fowls of this class, those medullated fibers, presenting at the time of paralysis the most marked degeneration showed, at most, only a doubtful increase in the number of nuclei of the neurilemma sheath and no embryonic nerve fibers. Nerves of fowls killed after feeding from 4 days to 2 months on the regeneration diet never showed the marked multiplication of the nuclei of the neurilemma sheath or the embryonic nerve fiber, as has been constantly described for mammalian nerves after section, and as was found also in the nerves of fowls in which I had transsected the sciatic. Segments of the sciatic of one side cut out and compared with the sciatic of the other side at a later date, showed that in none of these cases had the looked for change in this respect taken place. In other nerves after 108, 125, 171 and 275 days in regeneration, the nuclei of the sheath of Schwann could not be said to be more numerous than in preparations taken at the time paralysis developed, and the embryonic nerve fiber could not be found. The close resemblance of the Marchi preparation from fowls 108 days and 171 days on a regeneration diet to those from fowls at the time of paralysis has also been pointed out and is clearly seen by comparing figures 2, 3, 4 and 5 with figures 13 and 14. Numerous large globules and small droplets of degenerated myelin are to be seen in each.

In view of this condition of the myelin, the uniform absence of embryonic nerve fibers, and the fact that a great majority of fibers of the sciatic do not show a breaking up of the axis cylinder at the time of paralysis, it was at first suspected that regen-

eration failed to take place in those medullated fibers showing the most marked degeneration; and that in its recovery the fowl gradually learned to do without these fibers. Further study convinced me, however, that this was not the case, and that those fibers which had shown such marked degeneration finally attained a new axis cylinder. This conclusion became evident after a close study of the axis cylinder in those fowls which had recovered from paralysis.

Mallory's phosphomolybdic acid hematoxylin, carmine, Cajal's new silver impregnation method for axis cylinders, and Ranson's modification of the last were used for staining the axis cylinder. The preliminary treatment (i.e., hardening in absolute alcohol) called for in the silver methods produced such shrinkage of the fibers that it was often impossible to obtain satisfactory teased preparations. The degenerated myelin was also dissolved out to such an extent, that together with the shrinkage, relations were so distorted that it was usually difficult to distinguish an old from a new axis cylinder and to tell the relation of the latter to the globules of myelin. With the first two methods it was possible to stain a preparation in bulk, clear and tease out without passing through the higher alcohols or xylol. The globules of degenerated myelin were thus preserved. With the phosphomolybdic acid hematoxylin, which gave beautiful results after proper fixation with Müller's fluid, the procedure was as follows: Fix in Müller's fluid (small pieces as fresh as possible), wash 1 to 2 hours in running water, partially tease out a segment of the nerve not more than 1 mm. long to permit rapid infiltration of the stain, place in a ripened solution of Mallory's phosphomolybdic acid hematoxylin 20 minutes to over night, blot off excess of stain and differentiate in 50 per cent alcohol made slightly alkaline with ammonia, pass through two or three changes of 91 per cent alcohol, clear in origanum oil, tease, blot, and mount in xylol balsam. Carmine preparations were prepared in a similar manner but differentiated in weak alcohol without the ammonia. For longitudinal and cross sections, pieces of nerve after washing were rapidly dehydrated and cleared and mounted in paraffin and stained as indicated. Tissues were also fixed in

alcohol and in Bensley's chrom-sublimate solution for carmine staining.

It had been previously shown by me ('12), that, in degeneration in the rice-fed fowls at time of paralysis, there is just as large a percentage of fibers showing advanced degeneration in the sciatic as in its peripheral rami. In regeneration, then, there should be, in the earlier stages, a greater percentage of fibers containing no axis cylinder in the peripheral branches than in the sciatic itself; provided of course, the new axis cylinder is the result of an outgrowth. To determine this, segments of the sciatic and its peripheral rami were cut out in the above series of regenerating fowls for comparison with each other and with the sciatic of the other side at a later date.

The first indication of a regeneration of the axis cylinder was obtained from fowl No. 54 which developed marked leg paralysis on March 4, 1912. The animal was placed on the regeneration diet on March 7. By May 3, all signs of neuritis had disappeared and complete use of the legs had been regained. On this day, on the left side, segments of the nerve were cut out from the upper part of the thigh, and from near the foot. An examination of transverse sections of these two pieces revealed a greater proportion of medullated fibers devoid of axis cylinder in the peripheral segment than in the segment from the thigh. Out of 742 fibers in the peripheral portion, the axis cylinder was wanting in 11; in 1365 fibers (counted at random) in the proximal portion, the axis cylinder was wanting in 9. The fowl was killed 114 days later, on August 25. Transverse sections of the sciatic of the opposite side on this date, revealed an axis cylinder in practically every fiber. In 5788 fibers the axis cylinder was wanting in 8. Similar data were obtained from the nerves of other rice-fed fowls, No. 52, No. 57 and No. 64. However, as degenerated fibers may not be evenly distributed throughout the sciatic, one peripheral nerve may contain a larger percentage of degenerated fibers than its neighbor and relatively more than the sciatic of which it is a branch. As misleading results might thus be obtained, this method of comparison was not prosecuted further.

These findings having indicated that, under favorable conditions, regeneration of the axis cylinder may take place in the degenerated nerves of the rice-fed fowls, more definite evidence of regeneration was sought. This was a difficult and tedious task because it was necessary to show beyond doubt that an axis cylinder in a particular nerve was a new and not an old axis cylinder. Therefore a most careful search was made of sectioned and teased preparations of nerves taken at varying lengths of time after recovery from paralysis.

The sciatic of fowl No. 38—108 days after regeneration diet was begun and 49 days after paralysis had disappeared—was particularly studied because, in addition to being a typical case of peripheral neuritis, the axis cylinders stained exceptionally well and many globules and droplets of myelin were shown by the Marchi stain (fig. 13). In a large fiber containing several large globules of degenerated myelin along its course, a well staining axis cylinder was seen running a tortuous course to one side of the large globules which often occupied almost the entire diameter of the fiber. Two such vesicular globules of degenerated myelin in close proximity to the axis cylinder were seen, which contained in their center segments of a structure which was identified as the old axis cylinder. In figure 17, *m* shows one of the globules in question with its axis cylinder contents. In this figure, *m* clearly represents a single globule of degenerated myelin which has been cut on the tangent by the microtome knife. Part of the old axis cylinder was also probably taken away. The different portions of *a* and *a'*, the new and the old axis cylinders, were not in focus at the same time and the drawing has been constructed, with the aid of a camera lucida outline, to show as nearly as possible in one plane, the relations of these structures. In this figure, *a'* is the exact counterpart of *a* in figure 6 which is readily recognized as broken up axis cylinders within large globules of degenerated myelin. That *a*, figure 17, represents an axis cylinder there can be no doubt.

This observation has been confirmed in other fibers of this same nerve, as well as in the fibers of the sciatic of fowl No. 51, No. 54, and No. 61. In each of these there could be no

doubt about the identity of the structures. When it is remembered that the axis cylinder in advanced degeneration is often quite difficult to stain, it is not surprising that such clear pictures as the above (fig. 17) were not frequently found. The tortuous course of the new axis cylinder around the globules, as well as the different focal levels of fragments of the old, makes it extremely difficult to get a photomicrographic representation which will show both structures in one picture. Figure 18 shows a fragment of the old axis cylinder, a', inclosed within a large globule of degenerated myelin, m, in close proximity to the new axis cylinder a. In figures 19 and 20 the same condition is shown: a', the fragment of the old axis cylinder; m, a globule of degenerated myelin and a, the new axis cylinder. It is more frequently the case that the remains of the old axis cylinder are represented only by a mass of granules or fragments enclosed within the globule.

Further confirmation of these observations was readily obtained by a study of cross-sections of the sciatic of these same fowls. In such sections the new axis cylinder could be seen at one side of the myelin globules while segments or fragments of the old were to be seen within the globule. In some fibers the new axis cylinder was a very small structure, 0.5μ or less in diameter, and located quite at the periphery of the sheath. In other fibers it was larger and with its surrounding concentric lamellae occupied an equal proportion of the sheath with the globules of degenerated myelin. Figure 21, fowl No. 54, shows a new axis cylinder, a, the old axis cylinder, a', and a globule of degenerated myelin, m, in the same cross-section. Figure 22—fowl No. 38—shows a large new axis cylinder, a, with its concentric lamellae, s, by the side of the old axial tube, a; a' is also surrounded by concentric lamellae and no large globules of myelin are seen here. It is probable that this is a section of a nerve devoid of myelin globules at this place and in which the axis cylinder has degenerated as a result of its interruption by myelin globules at another level. Figures 23 and 24 are photomicrographs respectively of the same preparations as figures 21 and 22.

All these observations speak strongly for a new axis cylinder in recovery; the greater percentage of fibers with axis cylinders in the sciatic than in its peripheral rami argues also for an outgrowth of the axis cylinder. Further evidence of outgrowth of the axis cylinder was soon obtained. Before I was able to confirm the first observation that a new axis cylinder and segments of the old were to be found in the same fiber at the same time, another fiber was observed in the same preparation, in which growth activity was apparent. This fiber is shown in figures 27 and figures 28 and 29 (photomicrographs) all of the same fiber. It will readily be seen that *b* is an outgrowing branch of the axis cylinder *a*. That *a* is a new axis cylinder is proven by the presence of a fragment of the old axis cylinder, *a'*, (fig. 27 and fig. 29) between the new, *a*, and its branch, *b*. Both axis cylinder and branch stained equally well and much better than the remnants of the old. An end bulb is seen on the tip of the branch. It might be pointed out here that Cajal and Marienesco have observed a similar branching of the outgrowing axis cylinder in medullated nerves after section. These branches, of which there may be one or more to each fiber, often take an abortive course and have been observed to grow in a recurrent direction up the central stump. Whether, in the present case, *b* is an outgrowth from *a* or whether at an earlier stage both were outgrowing buds of approximately equal size is purely a matter of speculation. It should be added that in cross-sections old sheaths were observed which contained two axis cylinders of approximately equal size, each surrounded by a secondary sheath of its own. But more frequently one is much larger and occupies a more central position than the smaller which may be located quite near the periphery of the sheath. Figures 25 and 26 from the sciatic of fowl No. 54, show in transverse section two axis cylinders in the same nerve sheath, and apparently in the same portion of the fiber that was formerly occupied by the old axis cylinder. Whether the zone around each represents a newly acquired myelin sheath I have not determined. Nuclei, however, have not been observed in this zone.

Other than the rami as just noted (fig. 27), an outgrowing axis cylinder with its end bulb, as described by Cajal ('07), has been observed only once by me. Figure 30—from a teased preparation of the sciatic nerve of No. 38—shows in *a*, a structure with an end bulb which stained intensely with phosphomolybdic acid hematoxylin and which is lodged in a band of poorly staining tissue rich in nuclei. Whether the band of tissue is a group of embryonic nerve fibers or non-medullated fibers in regeneration, I cannot say.

In the light of this group of evidence which bespeaks an outgrowth of the axis cylinder into the old nerve fiber sheath, certain observations of Cajal ('07), Marienesco ('06), Ranson ('12) and others gain an additional interest. These investigators found that, almost immediately after section, the axis cylinders of the central stump showed evidence of growth activity. "Marienesco, in one of his recently published papers has demonstrated that the lengthening of the regenerating fibers (i.e., axis cylinders) is demonstrable twenty-four hours after a nerve has been cut" (Halliburton). Ranson ('12) says:

> On the first day after the lesion some of the axons grow out into the exudate and break up into many branches (fig. 16). Others on the first day, give off fine branches from their surface within the sheath in the immediate neighborhood of the lesion (fig. 17), some of which find their way into the exudate. Thus from the end of the first day on, fine nerve fibers, which are demonstrably branches of the medullated axons of the proximal stump, are present in the developing scar, and . . . . running for the most part within the sheath of the old axon from which they arose, they arrange themselves into fascicles, etc.

Cajal's beautiful figures illustrate most clearly the axis cylinder growing down into the old sheath of that portion of the central stump which showed degeneration after section. Howell and Huber ('92) also observed a similar growth of the axis cylinder in the central stump. Branches of these axis cylinders are also to be seen growing up the medullated fiber in a central direction; others burst through the sheath into the interfibrillar tissue, and still others after invading the blood clot and inflammatory tissue between the sutured central and peripheral stumps

are seen to grow down into the old fiber sheaths of the peripheral stump. All this may take place before there is any marked increase in the number of nuclei of the neurilemma sheath and long before embryonic nerve fibers develop. It is further a common observation (as pointed out by Langley and Anderson ('04)) that, unless very special precautions are taken to prevent it, the peripheral stump is invaded by foreign fibers from neighboring nerves; and Forsmanns has shown that even macerated brain tissue exerts a chemotactic influence on the outgrowing axis cylinders.

Clearly then the importance per se of the embryonic nerve fiber in the regeneration of medullated nerves has been greatly overestimated.

To summarize briefly, there have been observed in the nerves of this series of fowls which have recovered from a pronounced paralysis of the legs brought on by a prolonged diet of white rice, the following: sections of nerves, taken from the sciatic and its peripheral branches at various times during recovery of the fowl, showed a greater percentage of fibers possessing axis cylinders in the sciatic than in its peripheral branches. In regeneration, a new axis cylinder was acquired by those nerve fibers in which a long series of observations prove that the axis cylinder and myelin sheath had undergone marked degeneration. The large globules and small droplets of degenerated myelin persisted several months after complete recovery of the fowl. Multiplication of the nuclei of the neurilemma sheath and the resulting embryonic nerve fiber were lacking or were of the greatest infrequency in regenerating as well as degenerating medullated fibers. A new axis cylinder and segments or fragments of the old and globules of degenerated myelin were found together in the same nerve fiber, whose neurilemma sheath showed no increase in its nuclei. A new axis cylinder, a branch of the same ending in a bulb, fragments of the old axis cylinder and globules of degenerated myelin (and with no multiplication of the nuclei of the neurilemma sheath) were all seen in the same portion of a regenerating fiber. Two axis cylinders in the same fiber and indications of an outgrowing axis cylinder were observed.

From these facts it is clear that neither the nuclei of the sheath of Schwann nor the embryonic nerve fiber could have taken any part in the formation of the new axis cylinder. Consequently auto-regeneration in so far as it signifies the formation of a new axis cylinder by the embryonic nerve fiber does not obtain with fowls in regeneration after paralysis from polished rice.

The same observations which show that the new axis cylinder, in these experiments, is not acquired through auto-regeneration, also demonstrate that it is attained by outgrowth. The presence of a new axis cylinder and segments of the old in the same *old* medullary sheath; the presence of two new axis cylinders in an *old* sheath, and the occurrence of a new axis cylinder with an outgrowing branch, and of an outgrowing axis cylinder with an end bulb, can only mean that, in the absence of auto-regeneration, the new axis cylinder has grown out from its central stump.[6]

Regeneration in the cord

Before considering the possibility of regeneration in the fibers of the cord, it is necessary to refer to the degenerative changes in the medullated fibers and nerve cells of the cord described by me in a recent study of "Polyneuritis Gallinarum" ('12). Here it was found that a very small per cent of the fibers of all columns of the cord presented as advanced myelin degeneration, as the fibers of the peripheral nerves. A still smaller per cent of the fibers also showed a disintegration or breaking up of the axis cylinder. The nerve cells of the ventral horn and the basal

[6] The question naturally arises, at what point does this outgrowth of the axis cylinder begin? I have not been able to answer this satisfactorily. One would suppose that outgrowth would begin at the peripheral end of that segment of the axis cylinder (still connected with the nerve cell) which did not undergo degeneration; if such a segment exists. As stated below, I have not been able to determine if that portion of the axis cylinder running between the anterior horn cells and the periphery of the cord ever shows segmentation, such segmentation not having been observed. Both ventral and dorsal nerve roots, on the other hand, have been frequently observed in which segmentation and disintegration of the axis cylinder and clumping of the myelin were clearly visible.

portion of the dorsal horn experienced marked changes in their chromophile, Nissl, or tigroid substance, the globules or flocculi had given way to a uniform, finely granular mass collected at one side of the nerve cell, usually at the base of one of the processes of the cell. I was not able to determine whether that portion of the axone between the motor nerve cell and the periphery of the cord ever underwent segmentation.

A close examination of sections of the cords of Nos. 38, 57, 61 and 64 has revealed a persistence of globules of degenerated myelin in the medullated fibers of all columns of the cord for 59, 108, 275 and 379 days. These globules were frequently very large and occupied the entire diameter of the fiber. Furthermore, a careful search has failed to reveal any evidence of regeneration in the fibers of the cord. No such proof of regeneration as was found in the fibers of the sciatic and illustrated in figures 17 to 30 was found in the cord. Nothing suggestive of a new axis cylinder, an outgrowth or branching of the same was seen and there were not observed two axis cylinders in the same fiber.

On the other hand, fibers in degeneration in all columns of the cord were found in which no new axis cylinder nor fragments of the old were observed. Figure 31 is a cross-section of a degenerated fiber 49 days after complete recovery of fowl No. 38 (108 days in regeneration) was attained. A large globule of degenerated myelin completely fills up and distends the sheath and no indication of the axis cylinder is to be seen.

On comparing cross-sections of the lumbo-sacral cord of fowls taken at the time paralysis developed with sections from the same region of other fowls several months after complete recovery, the data included in table 1 were obtained.

From this table it will be seen that there are as many degenerated fibers with no axis cylinder in the cords of those fowls killed several months after recovery, as in the cords of fowls killed at the time paralysis developed.

In the absence of any positive evidence of regeneration, the persistence of degenerated fibers with no axis cylinder, in as great numbers as at the height of degeneration, strongly suggests the

conclusion that regeneration in the medullated fibers of the cord of the rice-fed fowls fails to take place.

Regeneration in the cord was included in this study because, after regeneration had been shown to take place in the peripheral nerves by an outgrowth of the axis cylinder into the old medullary sheath and in the absence of the embryonic nerve fiber, no reason was now apparent why the same thing could not occur in the fibers of the cord as well. According to the majority of investigators, regeneration in the fibers of the cord is, to say the most, doubtful. Schäfer ('08) declares "regeneration does not occur within the central nervous system, or at most in a very incomplete manner. This fact may be associated with the circumstance that the fibers within the spinal cord and brain have no nucleated sheath of Schwann, and the conducting path which the cells of this sheath form in the peripheral nerves for the outgrowing axis cylinders is therefore absent." According to Halliburton ('07), as noted above, the neurilemma is 'non-existent' in the medullated fibers of the central nervous system and "as is well known, regeneration does not occur" in these fibers. But

TABLE 1

Showing degeneration in the lumbo-sacral cord at time of paralysis and after recovery

FOWL NO.	NUMBER OF DEGENERATED FIBERS WITH NO AXIS CYLINDER IN THE CORD OF THE FOWL KILLED		REMARKS
	At time of paralysis	After complete recovery	
14	77		
1	65		
13	28		
38		59 67	108 days in regeneration 49 days after recovery
57		62	379 days in regeneration 300 days after recovery
64		57	275 days in regeneration 256 days after recovery
61		117[1]	59 days in regeneration; no recovery

[1] The cord of this fowl also contained a great many fibers which appeared considerably swollen and which are not included in this count.

as noted, if regeneration in the peripheral nerves was accomplished without the embryonic nerve fibers, then clearly in these fowls the absence from the cord of the neurilemma sheath and its derivative, the embryonic nerve fiber, would afford no explanation of a failure or regeneration in the fibers of the cord, and an outgrowth of the axis cylinder in the fibers of the cord might well be expected. Such, however, as the evidence shows, is probably never the case.

Although at the time of paralysis the marked changes described above were seen in the nerve cells of the lumbo-sacral cord, it is doubtful that this should be termed degeneration. The mitochondria seem to have undergone no alteration whatsoever. They were just as numerous as in the nerve cells of the cord of the normal fowl. In the cells of the cord of fowl 79, killed as soon as all signs of paralysis had disappeared (after 30 days on a special regenerative diet), the tigroid bodies again presented an appearance similar to the normal. This was also true for fowl No. 57 (10 months after complete recovery). No 'shadows' or other evidences of degenerated nerve cells were found in either case.

GENERAL SUMMARY

In the experiments described above degeneration of medullated nerve fibers was brought about in fowls by a prolonged feeding of polished rice, and regeneration was accomplished by a return to an adequate nutritive diet.

In such fowls the fibers are intact during degeneration and all traumatic and inflammatory effect produced by cutting the tissues and the nerve or of tying the latter are obviated; the process of degeneration can be stopped at almost any stage or greatly prolonged, and several stages of degeneration are to be observed in different fibers of the same nerve. In regeneration the possibility of an ingrowth of fibers from other nerves into the regenerating nerve under observation is eliminated and repair of the medullated nerves can be induced after any stage of degeneration.

Ten to 20 per cent of the medullated fibers of the nervus ischiadicus showed a complete fatty change of their medullary

sheaths into globules of degenerated myelin and a segmentation or granulation of their axis cylinders. No multiplication of the nuclei of the neurilemma sheath could be observed and consequently no embryonic nerve fibers or Band-fasern.

During recovery these degenerated fibers attained new axis cylinders and the medullary sheaths returned to normal. In other words, regeneration has been observed to follow degeneration in medullated nerve fibers without passing through the embryonic nerve fiber or Band-faser stage.

By prolonging the degenerative process there resulted a multiplication of the nuclei of the neurilemma sheath. This and other experiments described tend to show that the embryonic nerve fiber may be coincident with a late stage of degeneration in medullated nerve fibers. It may not represent an early stage of regeneration and its presence does not signify an attempt at regeneration on the part of the medullated nerve fiber.

In the absence of the embryonic nerve fiber, the degenerated myelin was absorbed with extreme slowness, persisting as droplets after 1 year and 14 days. On the other hand, where the embryonic nerve fiber was formed the degenerated myelin quickly disappeared from the fiber. The conclusion is reached that the proliferating nuclei of the neurilemma sheath participate in the resorption of the degenerated myelin.

In regeneration a new axis cylinder was attained by outgrowth and in the absence of the embryonic nerve fiber. The new axis cylinder grew down the old medullary sheath which latter still contained large globules of degenerated myelin and fragments of the old axis cylinder. The outgrowing axis cylinder was seen to branch, and in cross-sections of the nerves two new axis cylinders were observed within the same old medullary sheath. The embryonic nerve fiber could, of course, play no part in the formation of the new axis cylinder either by auto-regeneration or by outgrowth.

No indications of regeneration were observed in the fibers of the spinal cord.

Anatomical Laboratory, The University of Chicago,
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BIBLIOGRAPHY[7]

Ballance, C. A., and Stewart, P. 1901 The healing of nerves; London (cited after Mott, Halliburton and Edmonds).

Bethe, Albrecht 1903 Neurol. Centralbl. Bd. 22, January, p. 60.

1907 Neue Versuche über die Regeneration der Nervenfasern. Archiv f. d. ges. Physiol., Bd. 116, p. 385.

Cajal, Ramón y 1906 Mécanisme de la régéneration du nerf. Compt. rend. Soc. de Biol., vol. 59, p. 420.

1905 and 1907 Mecanismo de la regeneración de los nerviós. Trabajos del laboratio de investigaciones biologicas de la Universidad de Madrid. (Illustrations shown by Schäfer in Quain's anatomy).

Eijkman, C. 1897 Eine Beri Beri-ähnliche Krankheit der Hühner. Virchow's Arch., Bd. 148, p. 523.

Frazer, Henry, and Stanton, A. T. 1911 The etiology of beri-beri. Studies from the Institute for Medical Research, Federated Malay States, no. 12.

Halliburton, W. D. 1907 Nervous degeneration and regeneration. Brit. Med. Jour., vol. 1, pp. 1111 and 1460.

Howell, W. H., and Huber, G. C. 1892 A physiological, histological and clinical study of the degeneration and regeneration in peripheral nerve fibers after severance of their connections with the nerve centers. Jour. Physiol., vol. 13, p. 335.

Huber, G. C. 1895 A study of the operative treatment for loss of nerve substance in peripheral nerves. Jour. Morph., vol. 11, p. 629 (cited by Ranson).

Langley, J. N., and Anderson, H. K. 1904 On autogenetic regeneration in the nerves of the limbs. Jour. Physiol., vol. 31, p. 418.

Lugaro, E. 1906 Neurol. Centralbl., Bd. 25, p. 786 (cited after Ranson).

Marienesco, G. 1906 Jour. f. Psychol. u. Neurol., Bd. 7, p. 141 (cited after Halliburton).

Mott, F. W., Halliburton, W. D., and Edmonds, Arthur 1904 Regeneration of nerves. Jour. Physiol., vol. 31, p. vii.

1906 Regeneration of nerves. Proc. Roy. Soc. B., vol. 78, p. 259.

Nageotte, J. 1911 Betrachtungen über der tatsächlichen Bau und die künstlich hervorgerufenen Deformationen der markhatigen Nervenfaser. Archiv f. mik. Anat., Bd. 77, Abt. 1, p. 245.

[7] This list of references is meant to include only such of the available literature as has a direct bearing on the subjects discussed.

NAGEOTTE, J. 1911 Rôle des corps granuleux dans la phagocytose du neurite, au cours de la dégénération Wallérienne. Comp. rendu. Soc. de Biol., vol. 71, p. 251.

RANSON, S. WALTER 1912 Degeneration and regeneration of nerve fibers. Jour. Comp. Neur., vol. 22, p. 487.

SCHÄFER, E. A. Quain's elements of anatomy, eleventh edition, p. 41. New York and London.

SCHRÖDER, PAUL 1908 Einführung in die Histologie und Histopathologie des Nervensystems. Jena.

STROEBE, H. 1893 Experimentelle Untersuchungen über Degeneration and Regeneration peripherer Nerven nach Verletzungen. Beitr. z. path. Anat. u. z. allegem. Pathol., Bd. 13, p. 160.

VAN GEHUCHTEN, A. 1904 Bull. de l'Acad, Roy. de Med. de Belgique, p. 50 (cited after Langley and Anderson).

VEDDER, EDWARD B., AND CLARK, ELBERT 1912 A study of polyneuritis gallinarum. Phil. Jour. Science, vol. 7, sec. B, p. 423.

WILSON, J. GORDON 1909 The present position of the theory of auto-regeneration of nerves. Anat. Rec., vol. 3, p. 27.

PLATE 1[8]

EXPLANATION OF FIGURES

1 Photomicrograph of a teased preparation of the nervus ischiadicus of a normal fowl. Marchi method. Zeiss 4 × 16 mm.

2 Photomicrograph of a teased preparation of the nervus ischiadicus of fowl No. 2, after 24 days on an exclusive diet of polished rice. Every fiber shows degeneration. Advanced degeneration is seen in 4 fibers. Marchi method. Ziess 4 × 16 mm.

3 Fiber from the nervus ischiadicus of fowl No. 6—3 days in paralysis—showing marked degenerative changes, a swelling of the fiber at *a*, *b*, etc., and no nuclei of the neurilemma sheath. *m* is a hollow globule of degenerated myelin containing a segment of the axis cylinder. Marchi method. × 200.

4 Fiber from the nervus ischiadicus of fowl No. 1—showing complete alteration of the medullary sheath into globules of degenerated myelin. The laminated appearance of the larger globules is seen at *m*. *n* and *n'* are nuclei of the neurilemma sheath. Marchi method. × 312.

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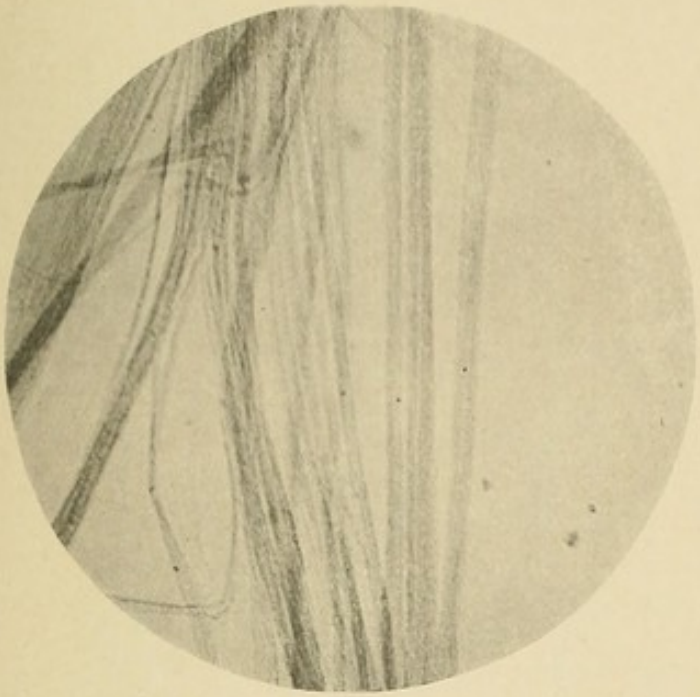
[8] Camera lucida outlines were used in the preparation of all the drawings. The photomicrographs are by Martin and Cortes.



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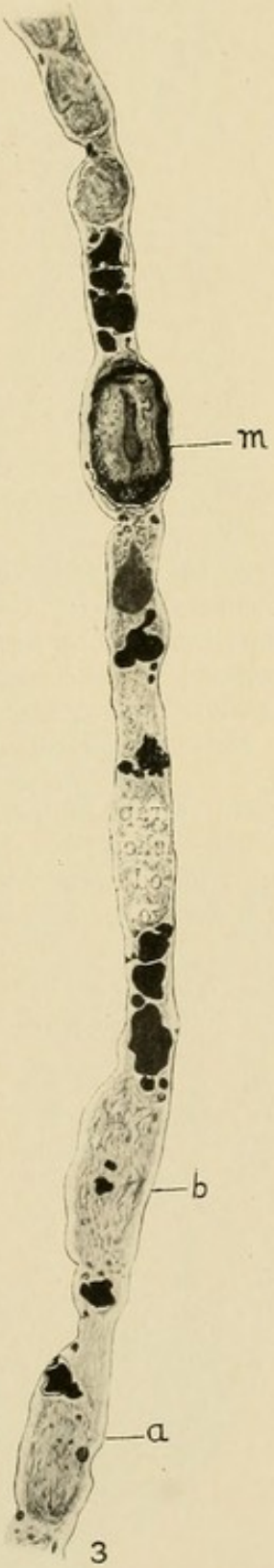

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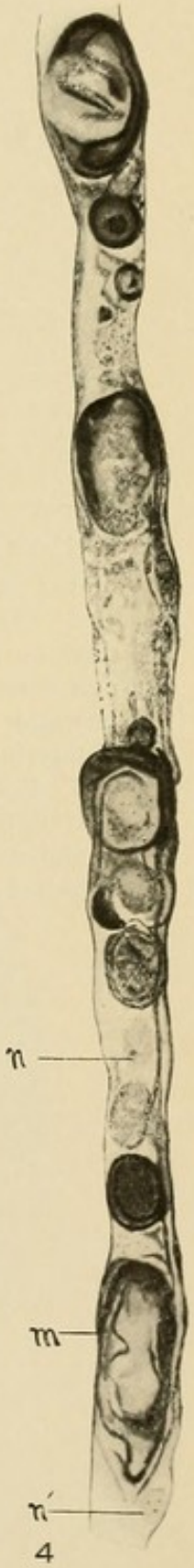

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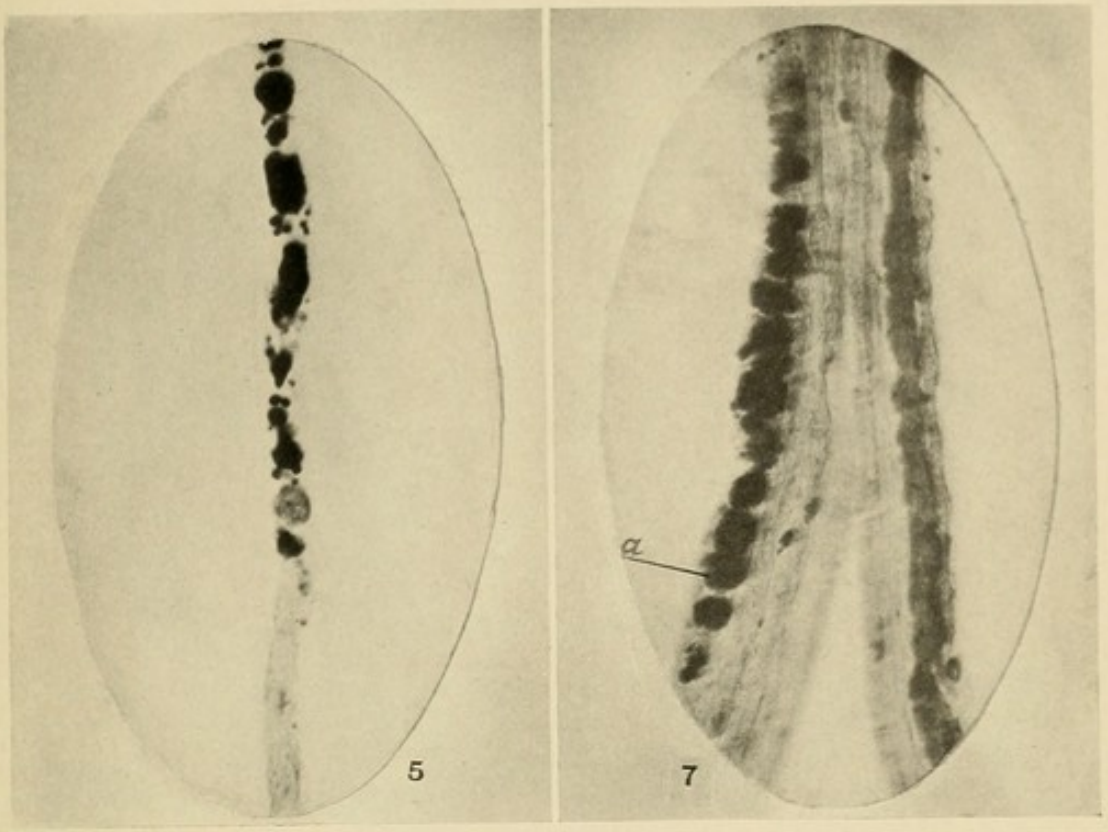
PLATE 2

EXPLANATION OF FIGURES

5 Fiber of the nervus ischiadicus of fowl No. 6. No multiplication of nuclei of the neurilemma sheath is seen. Marchi method. Zeiss 4 × 4 mm.

6 Photomicrograph of section of nervus ischiadicus of fowl No. 9, *G*—52 days on polished rice and calcium lactate. *a*, *a'* segments of discontinuous axis cylinders enclosed within enlarged portions (globules of degenerated myelin) of the fiber. Mallory's phosphomolybdic acid hematoxylin. Zeiss 4 × 2 mm. oil immersion.

7 Photomicrograph of teased preparation of nervus ischiadicus of fowl No. 6 —24 days on polished rice. Several stages of degeneration are shown. Fiber, *a*, showing advanced degeneration lies side by side with one showing only slight degenerative change. Marchi method. Zeiss 4 × 4 mm.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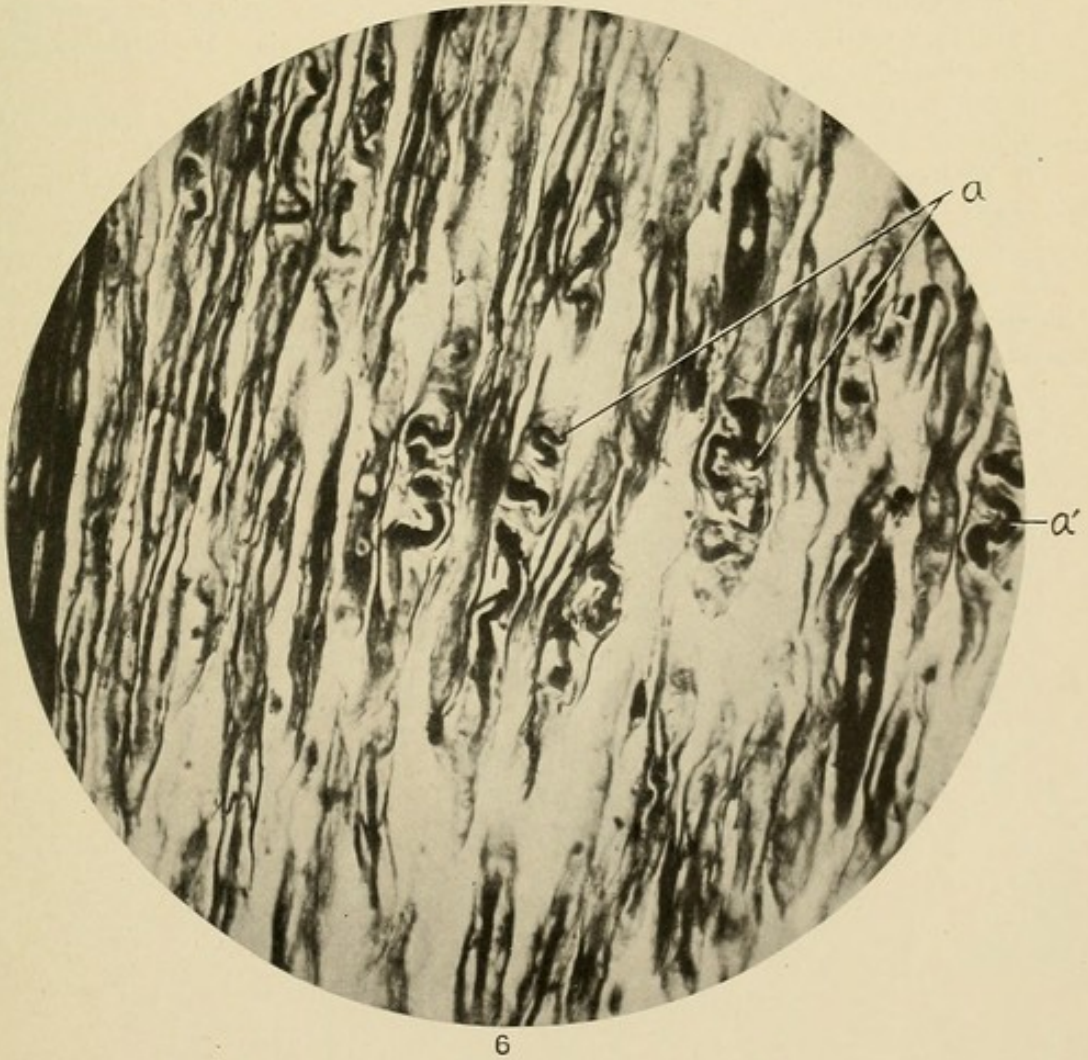
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PLATE 3

EXPLANATION OF FIGURES

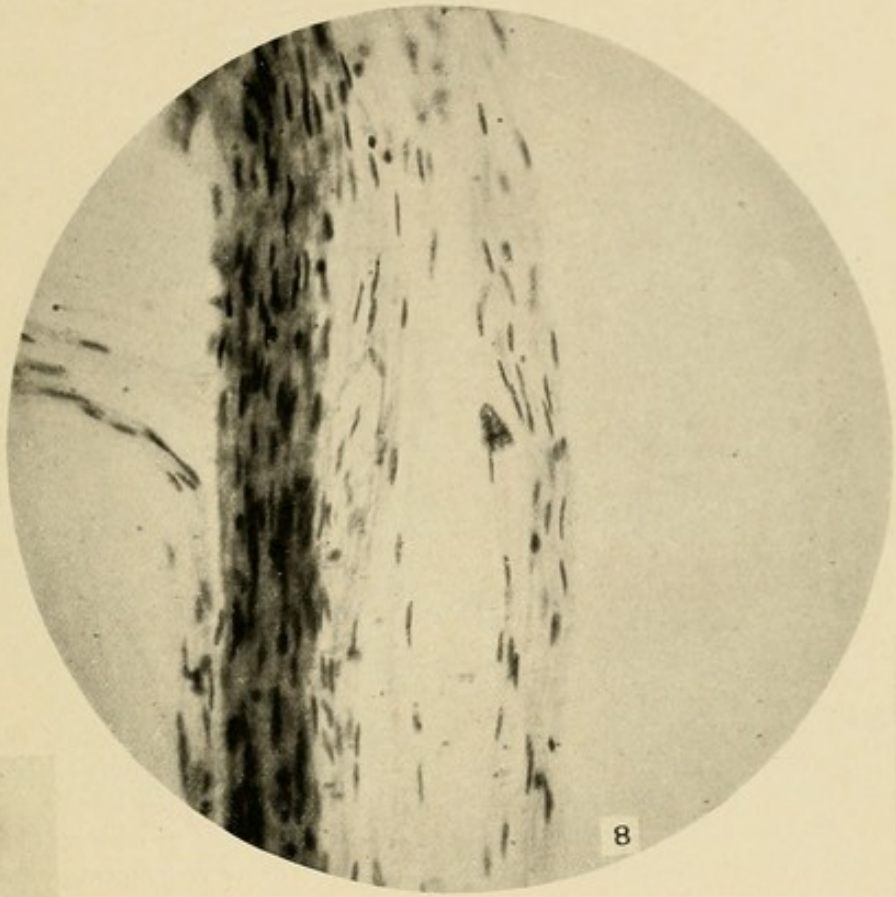
8 Photomicrograph of teased preparation of nervus ischiadicus of fowl No. 78—dry gangrene in left leg which had been bandaged for 24 hours 28 days before death. Slender embryonic nerve fibers with numerous spindle-shaped nuclei have entirely replaced the medullated fibers. Delafield's hematoxylin, Zeiss 4 × 2 mm. oil immersion.

9 Photomicrograph of embryonic nerve fibers in the nervus ischiadicus of fowl No. 17, *G*—60 days on polished rice and calcium lactate. Delafield's hematoxylin. Zeiss 4 × 2 mm. oil immersion.

10 Embryonic nerve fiber from the same nerve as figure 9. Droplets of degenerated myelin are seen at *m*. Delafield's hematoxylin. × 255.

11 Embryonic nerve fiber from a teased preparation of the nervus ischiadicus of fowl No. 9, *G*—52 days on polished rice and calcium lactate; droplets of degenerated myelin are seen at *f*; *n*, nucleus. Delafield's hematoxylin. × 250.

12 Fiber of the nervus ischiadicus of fowl No. 14, showing infiltration by numerous wandering cells. *S* is a nucleus of the neurilemma sheath. Delafield's hematoxylin. × 125.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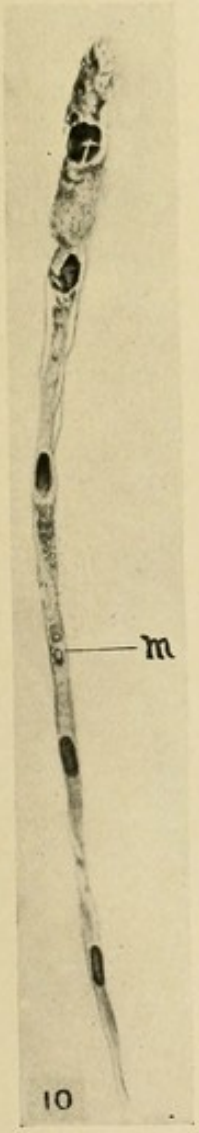
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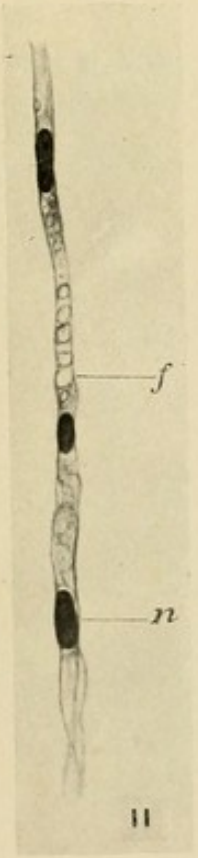
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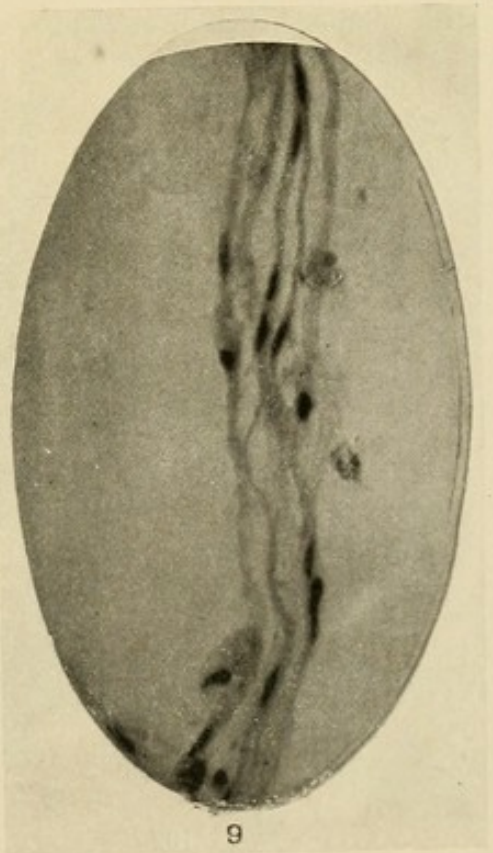
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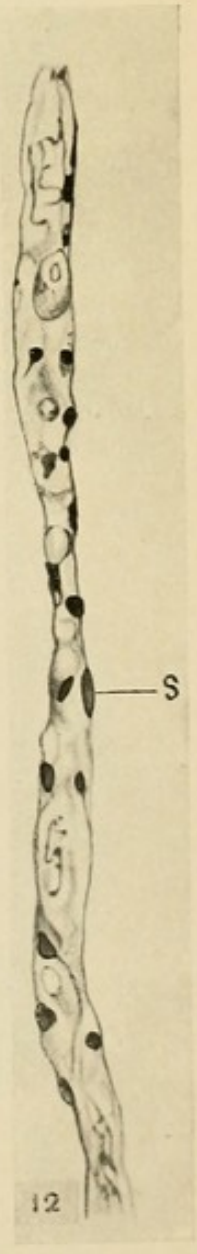
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PLATE 4

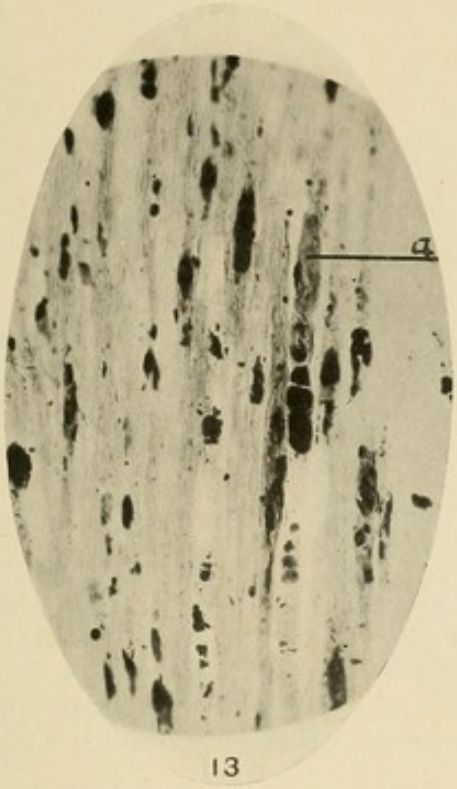
EXPLANATION OF FIGURES

13 Photomicrograph of a section of the nervus ischiadicus of fowl No. 38 —108 days in regeneration and 49 days after recovery was apparently complete—droplets or globules of degenerated myelin are to be seen in every fiber. *a* resembles a fiber in advanced degeneration. To be studied in connection with figures 14, 15 and 16, showing the slowness of absorption of degenerated myelin. Marchi method. Zeiss 2 × 16 mm.

14 Teased preparation of nervus ischiadicus of fowl No. 54—171 days in regeneration. Marchi method. Zeiss 2 × 16 mm.

15 Teased preparation of a motor root of the nervus ischiadicus of fowl No 57—1 year and 14 days in regeneration—compare with figure 16. Marchi method. Zeiss 2 × 16 mm.

16 Teased preparation of sensory root of same nerve as figure 15. Marchi method. Zeiss 2 × 16 mm.



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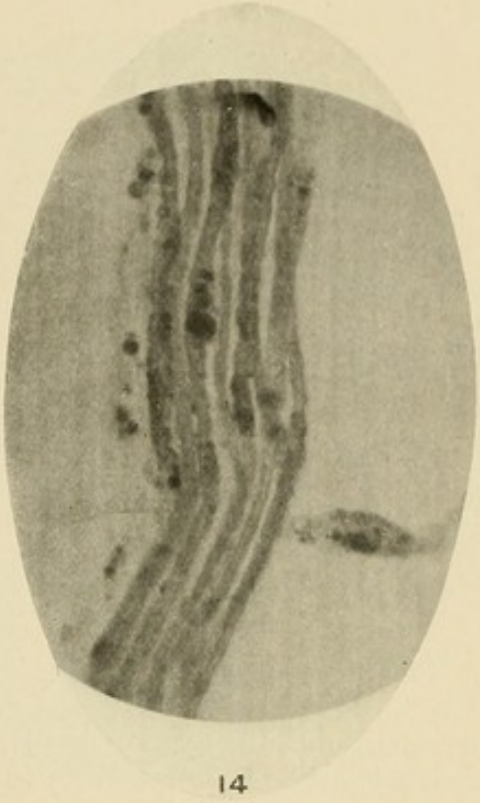

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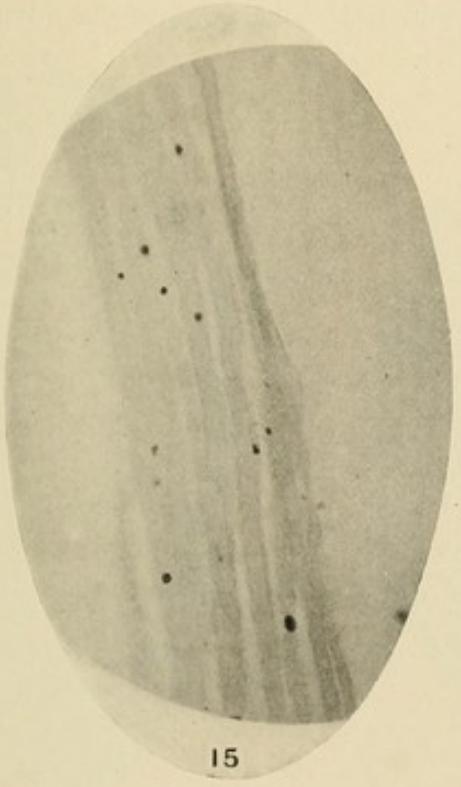

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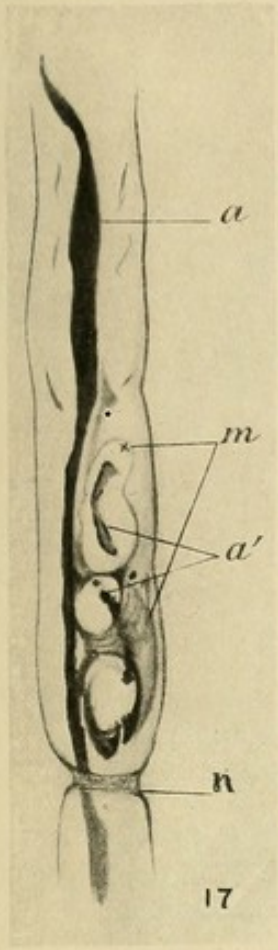
PLATE 5

EXPLANATION OF FIGURES

17 Fiber from the nervus ischiadicus of fowl No. 38—108 days in regeneration—*a*, new axis cylinder; *a'*, segment of old axis cylinder enclosed within a large globule of degenerated myelin, *m;* *n*, node of Ranvier. Mallory's phosphomolybdic acid hematoxylin. × 500.

18, 19 and 20 Photomicrographs of sections of the nervus ischiadicus showing new axis cylinders, *a*, and fragments of the old, *a'*, in globules of degenerated myelin, *m*. Figures 18 and 19 from fowl No. 38. Figure 20 from fowl No. 54, 171 days in regeneration. Phosphomolybdic acid hematoxylin. Zeiss 4 × 2 mm. oil immersion.



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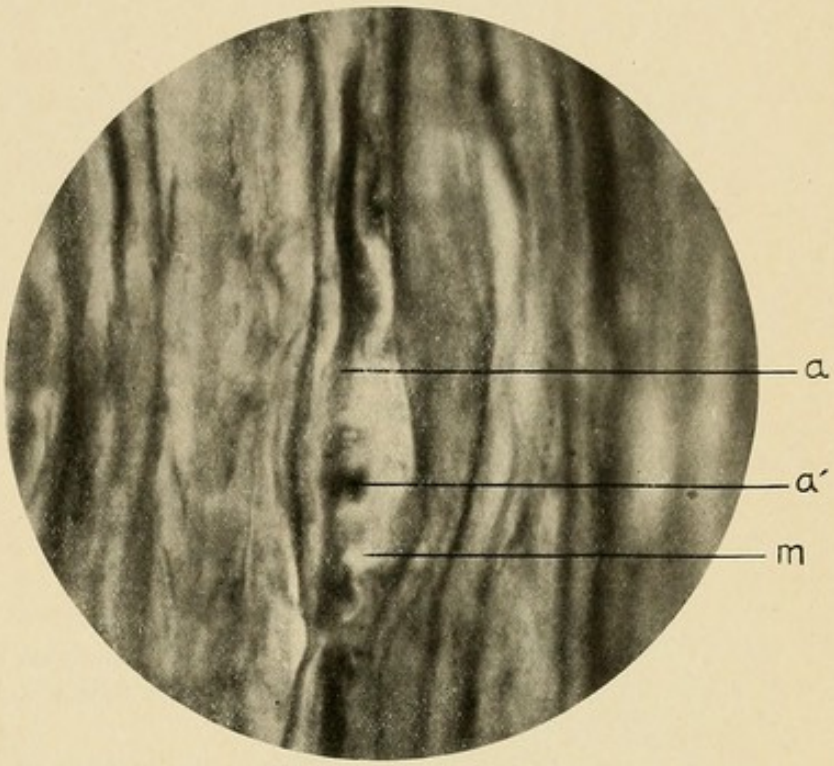

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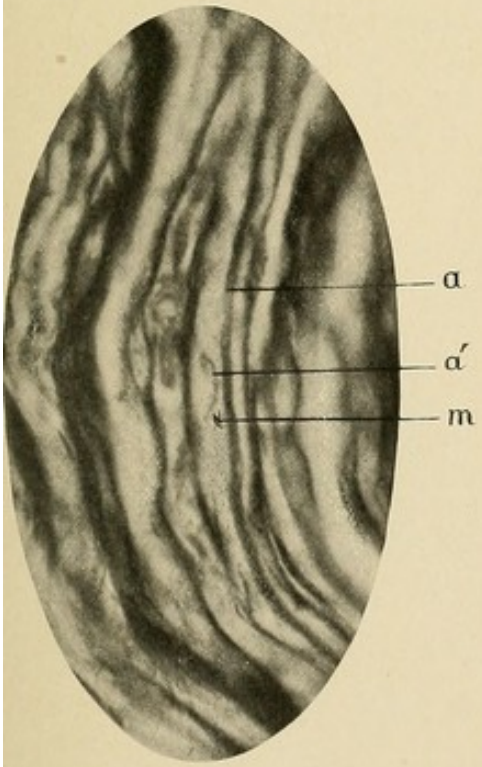

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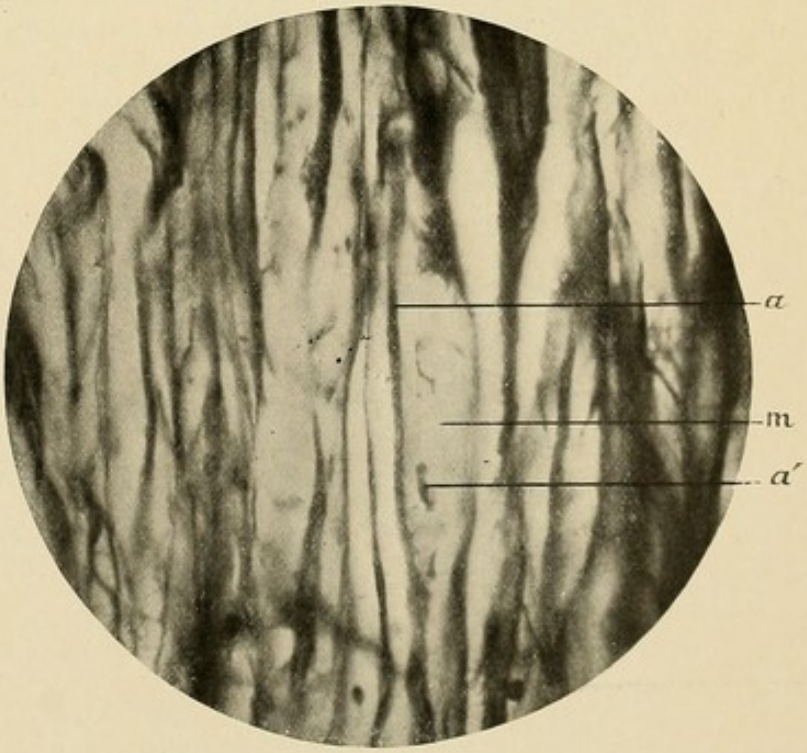

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PLATE 6

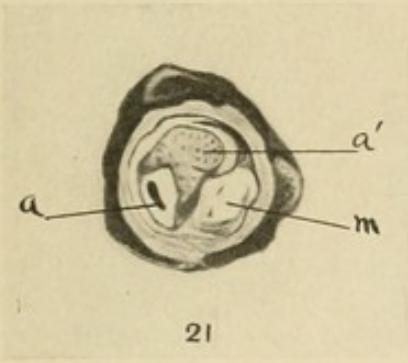
EXPLANATION OF FIGURES

21 Cross-section of fiber of nervus ischiadicus of fowl No. 54. A new axis cylinder, *a*, lies adjacent to the old, *a'*, which still contains neurofibrillae. *m*, a globule of degenerated myelin. Phosphomolybdic acid hematoxylin. × 555. (See also fig. 23.)

22 Cross-section of fiber of nervus ischiadicus of fowl No. 38. *a*, new axis cylinder with newly formed medullary sheath, *s*; *a'*, remnants of old axis cylinder; *n*, neurilemma sheath; *c*, connective tissue fibrils. Phosphomolybdic acid hematoxylin. × 1000. (See also fig. 24.)

23, 24 and 26 Photomicrographs respectively of the same fibers shown in figures 21, 22 and 25. Zeiss 4 × 2 mm. oil immersion.

25 Cross-section of fiber of nervus ischiadicus of fowl No. 54. Two new axis cylinders, *a*, are seen within the old myelin sheath, *m*, and in the position previously occupied by the old axis cylinder. Each has acquired a secondary myelin sheath. Phosphomolybdic acid hematoxylin. × 1000. (See also fig. 26.)



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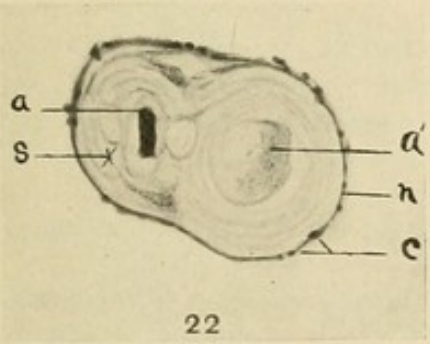

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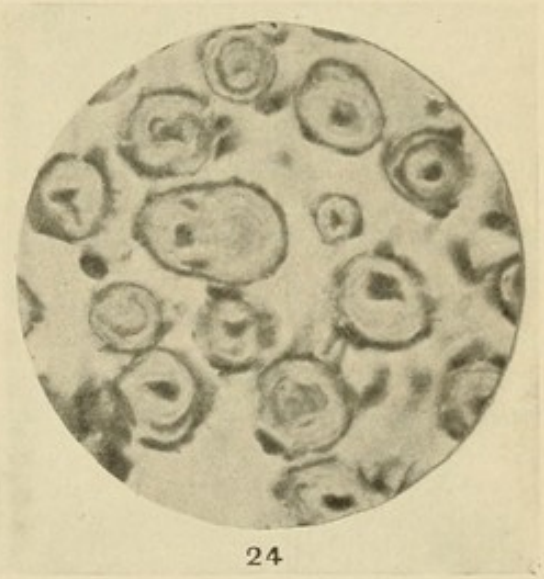

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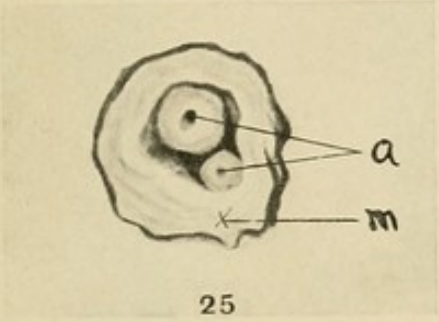

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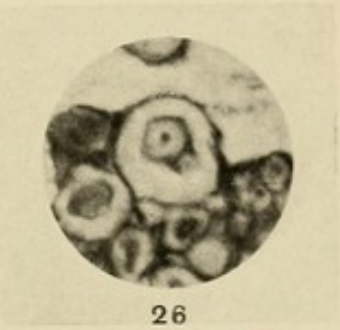

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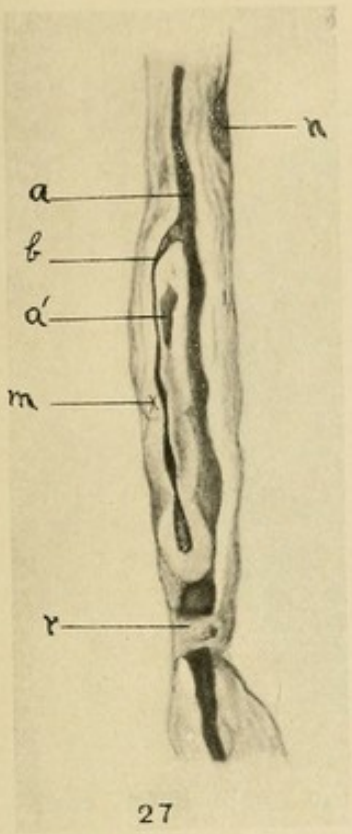
PLATE 7

EXPLANATION OF FIGURES

27 Fiber of the nervus ischiadicus of fowl No. 38. *a*, new axis cylinder with a branch, *b*, growing down into a long globule of degenerated myelin, *m*; *a'*, remnant of old axis cylinder; *n*, nucleus of neurilemma sheath; *r*, node of Ranvier. Phosphomolybdic acid hematoxylin. × 500. (See also figs. 28 and 29.)

28 and 29 Photomicrographs at different focal levels of the same preparation shown in figure 27.

30 New axis cylinder, *a*, with end bulb, *b*, among a group of nucleated bands (embryonic nerve fibers or non-medullated fibers?). *n*, nuclei. Phosphomolybdic acid hematoxylin. × 555.



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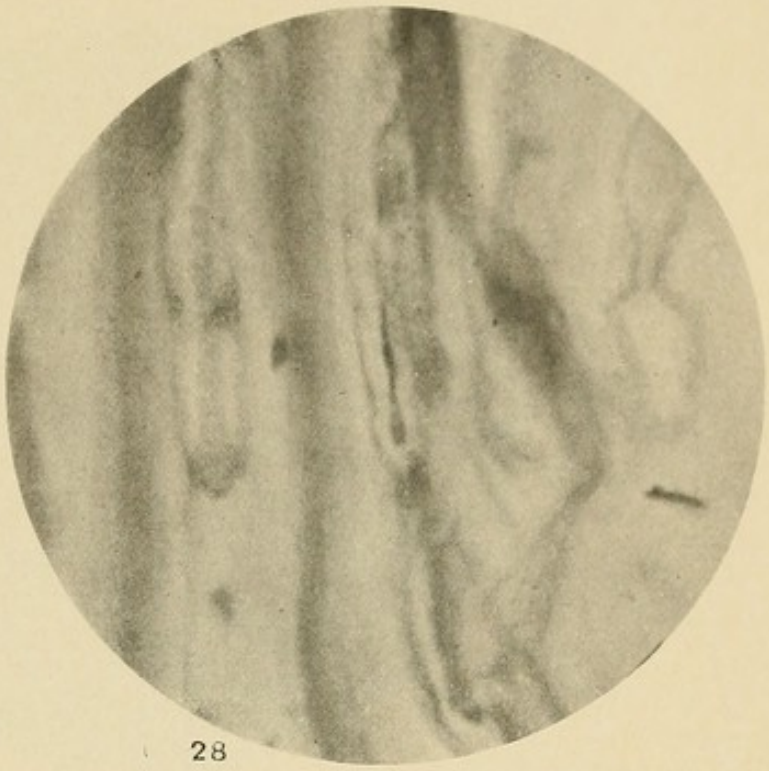

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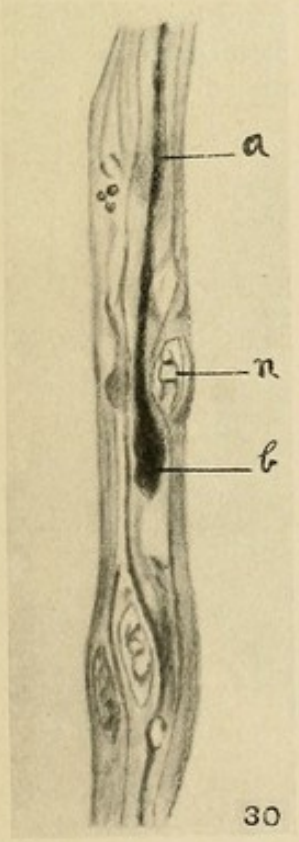

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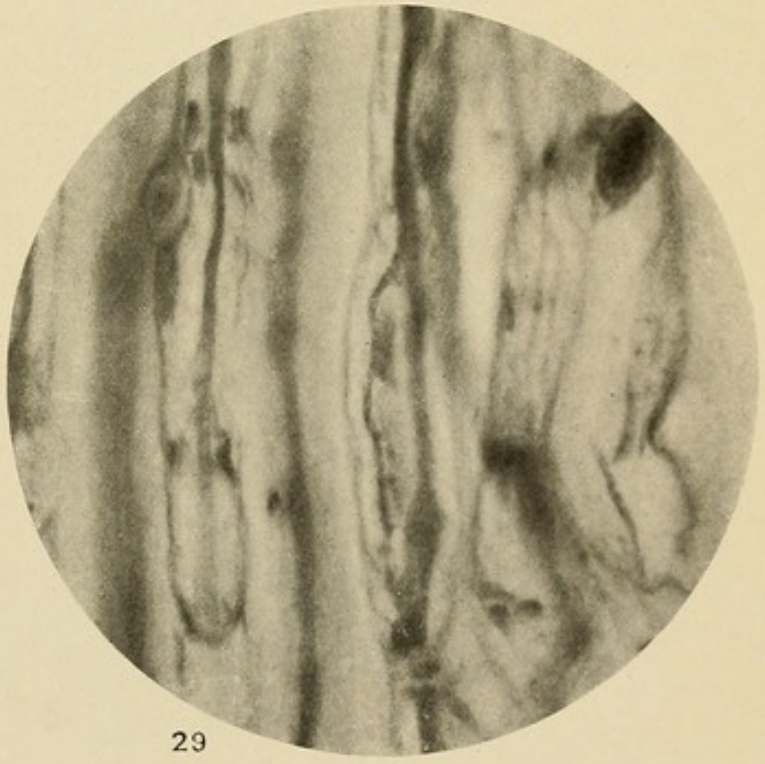

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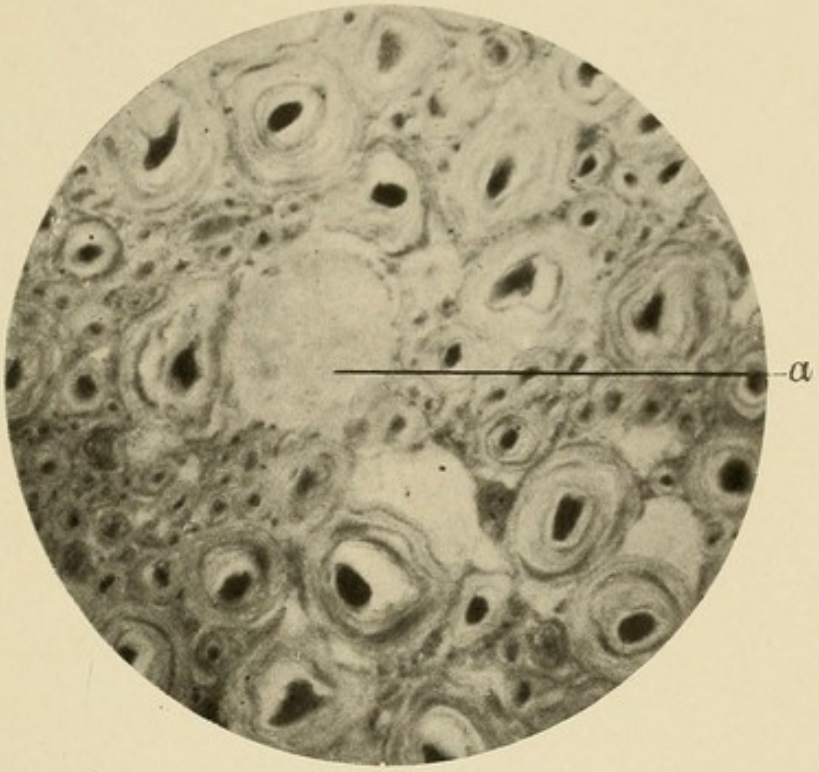
PLATE 8

EXPLANATION OF FIGURES

31 Photomicrograph of a cross-section of fibers of the lumbo-sacral cord of fowl No. 38—108 days in regeneration—*a*, is a fiber greatly distended by a large globule of degenerated myelin. No indication of an axis cylinder is visible. Phosphomolybdic acid hematoxylin. Zeiss 4 × 2 mm. oil immersion.

32 Photomicrograph of teased preparation of nervus ischiadicus of fowl No. 24—7 days on polished rice. Two droplets of degenerating myelin are seen in fiber *a*. Marchi method. Zeiss 4 × 2 mm. oil immersion.



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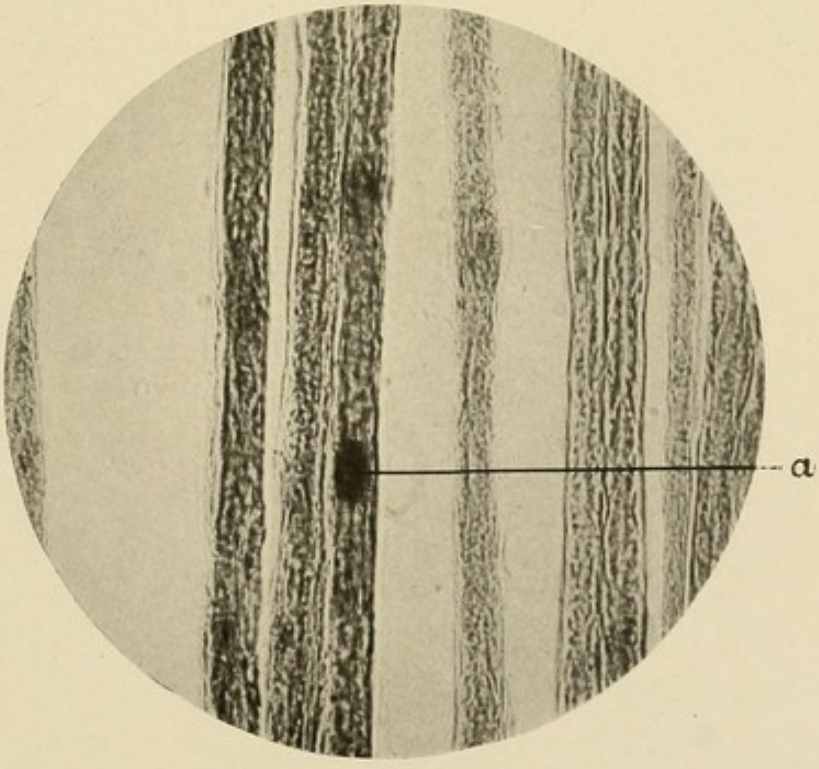

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