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BY

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For some years past I have been engaged, in conjunction with Major Lamb, I.M.S., on an investigation into the action of venoms of different species of poisonous snakes on the nervous system, and our results have appeared from time to time in the *Lancet*,[1] in a series of six papers in all. This work has furnished me with a large number of microscopic sections illustrative of acute degenerative changes in ganglion cells as a result of toxic poisoning, and I have ventured to think some of these of sufficient interest to permit of me bringing them before the notice of this Section. In doing so I wish to express the debt I owe to Major Lamb for so freely placing at my disposal the tissues from the animals killed in the course of this inquiry; for their histological examination I myself am entirely responsible.

Monkeys were the animals used in these experiments. They were injected with varying doses of one or other of the venoms; the toxic symptoms were observed and any corresponding changes in the nervous system subsequently investigated. Perchloride of mercury was the fixing agent almost invariably adopted, and the sections were stained with thionin according to Nissl's method. Thirty-nine monkeys were in this way examined, and by way of controls similar sections were prepared from four normal monkeys.

The following were the venoms employed: (1) Cobra venom, (2) venom of *Enhydrina valakadien* (common sea-snake), (3) venom of

[1] *Lancet*, 1904, i, p. 20, ii, p. 518, 1146; 1905, ii, p. 883; 1906, i, p. 1231; 1907, ii, p. 1017.

Bungarus cæruleus (common krait), (4) venom of *Bungarus fasciatus* (banded krait), and (5) venom of *Daboia russellii* (chain viper). The first four of these venoms had without doubt a direct action on the central nervous system, and the symptoms produced by any one of them had much the same characters as those produced by any one of the other three. Large doses of any of the four would cause generalized convulsions, with death supervening in three to four minutes. But with smaller doses death would be delayed several hours, and there would be no stage of preliminary excitement; indeed, the first symptom was now one of drowsiness. Paralysis of the extremities would next appear, more marked in the hind than in the fore limbs. Then the paralysis would become so marked that the animal could not move. Respiration is next involved, being first slow with inspiratory effort, and later it ceases altogether. Whilst, therefore, there is a widespread paralysis with all these venoms, the cause of death is paralysis of respiration.

The similarity in action of the first three venoms in particular was specially marked, for it was almost impossible by observing the symptoms to say with which of the three the animal had been injected. It has been determined, however, that there are some minor differences. For example, the venom of the common krait has a distinct action on the vasomotor centre, causing paralysis of this centre and marked fall of blood pressure, whilst cobra and sea-snake venoms have no such effect. Again, cobra venom has a considerable action on the vagal cardio-inhibitory centre, whilst the common-krait venom has only a slight action and sea-snake venom none at all. The longest time that any animal lived after receiving a minimal lethal dose of any of these three venoms was: with cobra venom, six and half hours; with sea-snake venom, six and three-quarter hours; and with common-krait venom, sixteen and a half hours.

With the fourth venom, that of the banded krait, the symptoms differed in some respects from the other three. With a large dose there were convulsions and death in a few minutes, due in this case to intravascular thrombosis; with a smaller dose the symptoms were those of cobra-venom poisoning—that is, paralysis of the limbs and later of respiration, only with the krait venom death was much longer delayed, one of our animals not dying till forty-four hours after injection. But with a still smaller dose death might be delayed for twelve days. When this was so there would be an interval of two to six days when there were no symptoms except that the animal seemed depressed and off its food. Then it would begin to emaciate till the emaciation assumed

the characters of muscle atrophy with corresponding muscle weakness; and before death, in some of the animals, there seemed to be more or less complete paralysis. The symptoms suggested, therefore, an acute and progressive muscular atrophy.

The fifth venom investigated—that of *Daboia russellii*—showed little action on the nervous system. In large doses it produced an extensive intravascular thrombosis, the animal dying in a few minutes. With smaller doses the animal might live for several days, and then there is no such thrombosis, and no paralysis of the limbs or of respiration. Death, indeed, seemed to be due to cardiac syncope: Rogers holds that it depends on a vasomotor paralysis of central origin.

That, then, is a brief résumé of the symptoms produced by the several venoms, and now we come to consider the histological changes met with as a result of their action on the nervous system. And first in regard to Daboia venom. Briefly, it had no recognizable action on the motor ganglion cells of the cortex, pons, medulla or cord, even in an animal that lived for sixty hours after injection of the venom. The suggestion that death was due to vasomotor paralysis made us pay very special attention to the "vasomotor area" in the medulla. And so we examined this area by means of serial sections in four of the monkeys. In two the sections were cut transversely, and in two in longitudinal direction. For comparison serial sections were cut through the corresponding areas of four normal monkeys. But careful comparison of the sections from the normal monkeys with those from the animals killed with the venom failed to show in the latter any certain signs of even an early degeneration in ganglion cells. Histological examination, therefore, was negative as regards giving evidence of Daboia venom having a selective action on the ganglion cells in that part of the bulb within which the vasomotor centre is supposed to be situated. The sections, then, from the monkeys killed with Daboia venom fail to supply us with specimens of degenerative lesions in ganglion cells; yet they are not without their value, for they serve as controls with which to compare the sections from the animals killed with the other venoms.

Now as to the other four venoms, we have just seen that they each produce symptoms of a widespread action on the central nervous system; and histological examination confirms this by showing a correspondingly extensive degenerative change in the motor ganglion cells of cortex, pons, medulla and cord. The type of cell degeneration varies little with these several venoms, only they do not all produce their

histological effect with equal rapidity. Thus, in an animal dying one and a third hours after injection of sea-snake venom, the ganglion cells showed undoubted chromatolytic changes; whereas in the animals dying two hours after cobra venom, or four and a half hours after the venom of the common krait, their cells presented little abnormality. Speaking generally, however, the longer the animal lived after injection, the more marked were the histological changes, and hence within certain limits these changes were in inverse proportion to the dose of the venom. In other words, the histological changes in the cells seemed to depend less on the quantity of venom than on the length of time it had to act. The action of certain venoms, too, seems much more enduring than that of others. For example, after injection of a small dose of cobra venom, if the animal does not die within two to three days at the longest, recovery invariably takes place, and recovery, too, from any paralysis that may have developed is very rapid. With the venom of *Bungarus fasciatus*, on the other hand, symptoms need not appear till six days after injection, and death may be delayed till six days later still. If, then, the changes in the ganglion cells are in proportion to the length of time the animal lives, it was thought that by studying the cells in animals dying at varying intervals, one might be able to trace the stages of this degenerative process, for we have sections showing changes in the cells from animals dying one and one-third hours, four hours, six hours, six and three-quarter hours, ten hours, twelve hours, fourteen and a-half hours, forty-four hours, three and a-half days, five days, six days, eight days, and ten days after injection. It is, however, only generally true that the changes in the cells are in proportion to the time the animal lived, for the degenerative process in the animals that lived for days was rather less acute than in those that died in a few hours; and so one finds examples of more extensive disintegration of the ganglion cell in those animals that died in six to seven hours than in the monkey that lived for ten days.

The process of degeneration, as far as can be made out, is something as follows (*see* Plate, figs. 5 to 26): In the first place the cell stains with basic dyes more intensely than normal, and one was forced to recognize that the sections from the paralysed monkeys took longer to decolorize than those from the control animals. This staining, while deeper, is possibly rather more diffuse than normal, and it gives the appearance of deeply-stained tigroid bodies in a less deeply-stained protoplasm. The tigroid bodies next appear as if they were being dissolved in the cell protoplasm, and they suggest the idea of pieces of

metal being acted on by an acid medium. The chromatic granules are now smaller than normal, but there is never the dust-like chromatolysis that one sees in the more chronic degenerations; neither is there enlargement nor globular deformity of the cell itself. The granules and diffuse staining next disappear, and leave a skeleton cell with its margin, reticulum, and nucleus well differentiated, though rather deeply stained. The cell reticulum has sometimes a granular appearance, but in any case it stains well with the basic dye. Later on the cell stains much more faintly, and is then the typical "ghost" cell. During almost any of these stages vacuoles may appear in the body of the cell, and its margins frequently become indented, as if little pieces had been bitten out of them. Finally, the cell begins to disintegrate, and portions entirely disappear, leaving behind little more than the nucleus, with perhaps some adherent stroma attached to it. Vacuolation, as well as disintegration of the cell, is seen mostly in the pale (ghost cell) stage, but in the most acute degenerations it is frequently met with while the cell is still deeply stained. During the whole of this degenerative process the nucleus, at least in a large proportion of the cells, seems to be little affected otherwise than is shown by a varying intensity and diffuseness of its staining. It remains central in the vast majority of the cells, and shows little change in size or shape. The nucleolus, too, shows little change. In the more rapid degenerations, for instance, with cobra or sea-snake venom, the Nissl granules seem to be more or less uniformly affected throughout the whole cell, and hence it could not be said that the chromatolysis was either "peripheral" or "perinuclear." With the more slowly-acting venoms, on the other hand—e.g., *Bungarus cæruleus* venom—a proportion of the cells suggested that the chromatolysis was, to begin with, perinuclear, and that it extended from thence outwards, for in these cells the perinuclear area was devoid of granules, whilst granules were still present at the periphery of the cell.

There was nothing specially distinctive in the histological appearances in the monkeys killed with the venom of banded krait, and which lived from five to ten days after injection. Such a length of time should permit of extreme changes developing in the ganglion cells; also it might almost give time for trophic symptoms to appear in the muscles and other tissues. General emaciation was very noticeable in these animals, some of them losing from 20 to 25 per cent. of their weight in a few days; but it is very doubtful if this emaciation can in any way be related to the changes in the anterior horn cells. We do not know on what day the degeneration in these cells first appeared, but in

Monkey No. 19 signs of paresis were not noted till the fifth day, and paralysis was not complete till two days later. Neither were we able to show that the venom was having a selective action, affecting certain ganglion cells before others. In all the animals killed with this venom practically all the cells in the anterior horns of the cord were entirely free from granules. Some cells were faintly stained, and some deeper stained, skeletons, and many showed considerable disintegration of their protoplasm. It could not, however, be determined that there was any actual loss in the number of cells in the anterior horns, and there was little difference in the appearances of the cells in the monkey that died in ten days from those of the animal that died in three and a half days after injection. It is, of course, difficult to be certain as to what is the change in the cell that constitutes loss of function. In some of the monkeys killed with cobra venom—e.g., Monkey No. 10—there was definite paralysis, but no recognizable structural change in the ganglion cells of the motor cortex or cord; and, on the other hand, it is recognized that there may be almost complete loss of Nissl granules without paralysis or other loss of function.

We attempted, but not with much success, to get some observations on this subject by examining the ganglion cells of monkeys that had recovered after being paralysed. The first of these monkeys was injected with cobra venom and it showed signs of paralysis in two hours ten minutes. Antivenomous serum was then given intravenously, and paralysis was still present fifty minutes later. The dose of antivenom was repeated and one and a half hours later all signs of paralysis had gone. The animal was at once killed. On examining the ganglion cells of the motor cortex and anterior horns of the cord it was found that quite 50 per cent. of the cells showed some deficiency of their Nissl bodies, and many of them had these bodies fragmented, giving a much finer granulation to the cell protoplasm than was normal. In some cells the granulation was quite dust like, but all gradations were found between these and the cells with Nissl bodies of normal size. Generally, the cells had the appearance of a much more chronic chromatolysis than seemed to be present in other cases of cobra poisoning; also there was little diffuse staining of the cell, and the staining was not so intense as in the cases not treated with antivenom. Two other monkeys were similarly injected, first with venom, and when paralysis was complete with antivenom one animal was killed twenty hours and the other forty-four hours after signs of paralysis had disappeared. In both of these the ganglion cells were practically normal. The Nissl bodies in a

proportion of the cells were possibly slightly deficient in number and not quite so large as in a normal animal, but, on the other hand, some of the adjacent granules seemed to have become fused together, forming masses of chromatic material considerably larger than normal. In none of the cells was there any appearance of fragmentation of granules, as in the first animal.

In these three monkeys I take it that there had been at least slight chromatic changes in the ganglion cells before the antivenom had been injected, for other animals dying paralysed in two hours after injection with the same venom showed such slight changes. The second animal would seem to show that the cells return to their normal within twenty hours after the paralysis disappears. But it is doubtful what interpretation one should give to the appearances in the first of the three. It may be that they represent a regeneration of the chromatic material. This, however, is not probable, for though changes had almost certainly commenced in the cells before the antivenom had been given, these changes must have been slight and not nearly so marked as those seen in the animal after recovery from its paralysis. It is more likely that the antivenom inhibited the venom and so modified the degenerative reaction in the cell, for the appearances were such as one associates with a much more chronic chromatolysis than that produced by ordinary cobra venom.

The *connective-tissue elements* (glia cells) of the grey matter seem to play an entirely secondary part in the degenerative changes produced by snake venoms. These glia cells may be slightly increased in number round the ganglion cell in its earliest stages of chromatolysis, but this increase is not in the least considerable, and it is not till vacuolation comes on and the cell begins to disintegrate that they are seen to cluster definitely round the disappearing ganglion cell. During this later stage, and sometimes also at an earlier stage, the glia cells are found indenting the margins and sometimes they are right inside the body of the cell. It is doubtful if there is any definite increase in the number of the glia cells throughout the rest of the grey matter, and they did not seem more numerous in animals living eight to ten days than in those dying in a few hours. The individual cells surrounding the effete ganglion cells are distinctly increased in size as compared with ordinary glia cells, and there seems little doubt that they are derived from pre-existing glia cells and that they are neuroclastic in function.

Nerve fibres from the peripheral as well as from the central nervous system were stained in various ways so as to demonstrate any change

in their myeline or axis cylinders. The peripheral nerves examined were the vagus in the cervical region, the median near the elbow, and the posterior tibial from the lower leg. In all the animals the sections were stained (1) with osmic acid, according to the method of Marchi, or that of Busch; (2) with thionin or Congo red, according to Nissl's method; and (3) with hæmatoxylin and eosin, or van Gieson's method. In none of the animals was there a genuine degenerative reaction with the Marchi or Busch method. In some sections there was slight "blackening" of some of the fibres, but I could not satisfy myself that this was not an artefact, and in any case the axis cylinders in these fibres were shown to remain intact. There was not even a definite "Marchi reaction" in the monkeys that lived for eight and ten days after injection of the *Bungarus fasciatus* venom. It is, of course, difficult to know what effect chromatolysis in a ganglion cell has on its nerve fibre, but in any case the absence of a secondary degeneration in the nerve fibres in these animals can be explained on the supposition that the degenerating ganglion cells had not lost their trophic functions till, say, forty-eight hours or so before the death of the animal. With the other stains there seemed to be, in a number of the monkeys, a slight increase of the internodal nuclei of the peripheral nerves. This was perhaps most definite in the animals that lived longest—for example, in the monkey that lived for ten days. With the view of showing the earliest recognizable degeneration in myeline and axis cylinders, nerve fibres from certain of the monkeys—those killed with sea-snake and with common-krait venom—were also stained by Donaggio's method. With this stain (*see* Plate, figs. 27 to 31) it seemed definitely shown that a considerable proportion of fibres, of the central as well as the peripheral nervous system, gave the appearances of an early degeneration. These fibres stained much more intensely and resisted much longer the decolorizing agents than do the nerve fibres of a normal animal. In the brain and spinal cord the deep staining seemed to affect chiefly the axis cylinders. These structures, in addition to their deep staining, were in places wavy in outline, and occasionally showed slight swellings so as to form at intervals spindle-like thickenings. But the myeline also retained the stain more tenaciously than normal, though less uniformly than the axis cylinders. This patchy staining of the myeline seems to give the fibre an uneven outline, constrictions alternating with apparent swellings. In some of the fibres the myeline and axis cylinders stain the same colour and with the same intensity, so that one could not be distinguished from the other, the two seeming to

have merged into one. In the peripheral nerves and nerve-roots something of the same appearances were to be seen ; but as a rule the deep staining of the axis cylinders was less in evidence, and what stained most intensely seemed to be a fragmented myeline, as represented by deep-stained granules, most often arranged at the margin of the fibre, but sometimes scattered through its whole diameter. This granular appearance was most marked in the monkeys that lived for the longer periods, whilst in Monkey No. 39, which lived for one and a third hours, there was less granulation visible and more of the deep-stained axis cylinder. I take it, then, that the first change in the nerve fibres in snake-venom poisoning shows itself by a deeper staining of the axis cylinders, and that later this quality passes to the myeline, which later still may show some signs of fragmentation. There is therefore an early degeneration in these nerve fibres, but it seems to be chiefly chemical in nature, for there was little structural alteration to be demonstrated. The axis cylinders are in places somewhat swollen, and the myeline uneven in outline, with rarely some granular disintegration. But there was no definite Marchi reaction and certainly no evidence of Wallerian degeneration, even in the animals living for eight and ten days after injection.

Nerve terminals were examined in monkeys killed with Daboia venom, in those killed with sea-snake venom, as well as in those killed with common-krait venom. The nerve terminals were fixed and stained in osmic acid. With the first of these three venoms the terminals were practically normal. With the second a considerable proportion of the fibres were also normal, but others had their myeline rather wavy in outline and segmented at frequent intervals. In a few other fibres still the myeline seemed entirely fragmented and to be represented by small granules occupying the whole diameter of the fibre. With the venom of the common krait, especially in Monkey No. 31, which lived for sixteen and a half hours, the same sort of granular appearance was to be seen, but a larger proportion of fibres was affected. Some few fibres stained diffusely, showing no differentiation of myeline or axis cylinder, and they looked as if their contents were in solution. The nerve terminals would seem then to show in a proportion of their fibres the appearances of a commencing degeneration.

DESCRIPTION OF PLATE.

Figs. 1, 2, 3, and 4.—From monkeys killed with Daboia venom. These cells may be regarded as being normal.

Figs. 5 and 6.—From Monkey No. 35, which died in six and three-quarter hours after injection of sea-snake venom. Shows some fragmentation of the Nissl granules and the whole cell rather diffusely stained.

Fig. 7.—From Monkey No. 36, which died in six and three-quarter hours after injection of sea-snake venom. Cell paler with disappearance of the Nissl granules from a part of the cell.

Figs. 8, 9, and 10.—From same monkey as fig. 7. Show deep-staining reticulum, but almost complete loss of granules. In fig. 8 there is a neurophage at the margin of the cell.

Fig. 11.—From Monkey No. 37, which died six and three-quarter hours after injection of sea-snake venom. Shows loss of Nissl granules as well as vacuolation of nucleus.

Fig. 12.—From same animal as fig. 11. Shows pale cell with no appearance of nucleus of limiting membrane.

Fig. 13.—From Monkey No. 38, which died four hours after injection of sea-snake venom. Shows a skeleton cell in the process of disintegrating; there is vacuolation and a neurophage visible.

Fig. 14.—From same monkey as fig. 13. Shows later stage of disintegration.

Figs. 15 and 16.—From same monkey as figs. 13 and 14. Shows little more than nucleus surrounded by neurophe cells.

Figs. 17, 18, and 19.—From Monkey No. 39, which died in one and a third hours after injection of sea-snake venom. Shows Nissl bodies breaking up and disappearing; vacuolation in fig. 19.

Figs. 20 and 21.—From Monkey No. 33, which died in ten hours after injection of common-krait venom. Fig. 20 shows fragmented granules; in fig. 21 the granules have disappeared.

Fig. 22.—From Monkey No. 31, which died in sixteen and a half hours after injection of common-krait venom. Shows cell deeply stained, with sort of reticulum, but no granules.

Figs. 23 and 24.—From Monkey No. 18, which died in six days after injection of banded-krait venom. Fig. 23 shows a few granules; in fig. 24 they have almost disappeared, and there is a vacuole and a neurophe cell.

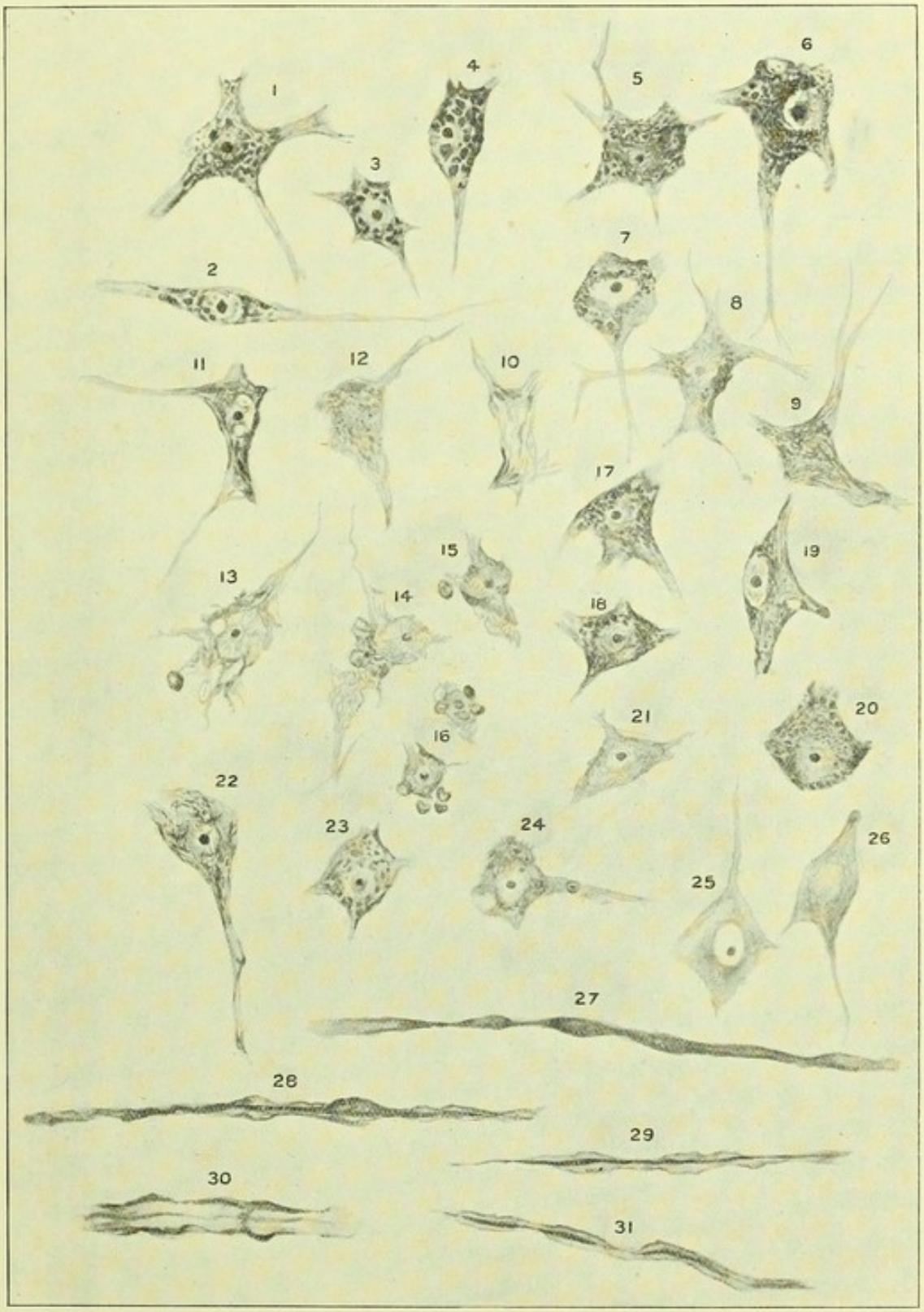
Figs. 25 and 26.—From Monkey No. 20, which died in ten days after injection of banded-krait venom. Show "ghost" cells without granules.

Fig. 27.—From Monkey No. 37, which died six and three-quarter hours after injection of sea-snake venom. Shows nerve fibre irregular in outline with axis cylinder and myeline not differentiated.

Fig. 28.—From Monkey No. 36, which died in six and three-quarter hours after injection of sea-snake venom. Shows axis cylinder deeply stained and differentiated from foam-like myeline.

Fig. 29.—From Monkey No. 39, which died one and a third hours after injection of sea-snake venom. Shows axis cylinder deeply stained, with myeline faintly indicated.

Figs. 30 and 31.—From Monkey No. 32, which died in twelve hours after injection of common-krait venom. Fig. 30 shows both axis cylinder and myeline; fig. 31 shows spindle-shaped swelling of axis cylinder.



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