

The Structure of Nerve-fibres.

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(Communicated by Professor Sherrington, F.R.S. Received July 31,—
Read November 22, 1906.)

Frog's nerve-fibres present very different appearances in different portions of their length, when teased and examined in Ringer's solution. The main distinctions affect the contents of the myelin sheath. To avoid misunderstanding I propose to speak of this portion of the nerve-fibre as the "intramyelin material," since the term more generally used ("axis-cylinder") has now become so completely identified with new appearances produced in this material by the action of reagents.

Briefly summarised the main differences observed are as follows :—

(a) In places the intramyelin material exhibits the perfect transparency and homogeneity of a clear solution.

(b) In places this limpidity is clouded by a fine punctate granulation.

(c) At others the granular appearance is much more marked. The individual granules are of different sizes, and vary from obvious spherules to just perceptible points.

(d) In other places again large hemispherical vacuoles make their appearance, their bases upon the myelin sheath and their convex surfaces pointing inwards towards the central axis of the fibre. These marginally placed vacuoles are situated at irregular distances from one another, and are of irregular size. They lie now on this side, now on the other side of the fibre. The remainder of the intramyelin material, the more central portion, is here less translucent and more granular.

(e) The vacuoles here have extended, and by their more numerous formation and communication with one another have joined to form an irregularly spiral channel. The central and less translucent portion of the intramyelin material also presents the appearance of a spiral, as it bends now to this point, now to that point upon the myelin sheath at places where a separation from the myelin sheath has not yet occurred. The curves of this irregular spiral are now short, now long. The material of which it is formed also varies in diameter, more swollen here, more condensed there. The central mass is much longer than the straight tubular myelin sheath within which it lies.

(f) In other places this central coagulum is of more uniform diameter and forms a straighter line. It has undergone a diminution in length and in thickness.

The evidence of this change of length is sometimes very complete. Imagine a stretched elastic string placed in the long axis of a tube with flexible walls. Release the string and so let its elasticity come into play. As the string contracts, the flexible tube will become arranged as a spiral curled around the axial string. Such a figure would be the reverse of the appearance described above in which a straight tube enclosed a spiral string. I do not say that I have seen evidence of tension so complete as this, but I have frequently seen something similar, if something less.

Thus when the process of teasing has left nerve-fibres arranged upon the slide in undulating curves, the clot which forms within the intramyelin material sometimes shortens to a length less than that of the fibre. In this case the clot does not follow the curvature of the fibre, but passes straight across from one point of maximal curvature to another.

Sometimes this central coagulum may be seen to show the usual signs of longitudinal strain in a viscous cord. It is faintly marked by fine lines in the main direction of the strain, and has a finely fibrillated appearance which has led to a belief that this central mass is formed by the agglutination of a number of originally separate "neuro-fibrils." There is, however, nothing in the appearances seen in teased nerve-fibres to warrant such a view. The most severe test to which this view can be put is to seek, in terms of it, for an explanation of the alterations in length which this central mass undergoes. Such an explanation would require not only an imagination of separate fibrils, but also their new endowment with the property of elasticity.

In nerve-fibres teased in Ringer's solution these appearances are usually distributed in a definite order. The immediate vicinity of the cut end is granular, this granulation is succeeded by a tract of homogeneous intramyelin material, and then all the successive appearances due to a gradually increasing degree of coagulation occur in the order given above. The order in which the appearances are arranged is sometimes complicated by less extensive and subsidiary sets of appearances of the same kind arranged around interpolated nodes of Ranvier.

This balancing of appearances around injured points and nodes of Ranvier introduces a complexity into the description of the normal character of teased nerve-fibres. If there is any hurry to identify some one of these appearances as the "normal" one, it must be remembered that teased nerve-fibres are traversed by the "current of injury" and must be expected to exhibit evidence of consequent polarisation.

Nerve-fibres Teased in "Normal Saline" Tinged with Toluidine Blue.

I have, in a previous note, described the manner in which these nerve-fibres are affected by the addition of neutral red to the "normal saline" in which they were teased. This dye characteristically stains all injured points and some of the nodes of Ranvier. As time elapses it also stains granules which form at gradually increasing distances from these points. The most notable fact about neutral red staining is the slow invasion of the nerve-fibre by this process of granule formation. When I first replaced this basic dye by another one of an apparently not very different character—toluidine blue—I was surprised by the complete difference in result obtained. It is true that here and there some stained plugs of material were visible at injured points, but I failed to find any trace of the progressive granule formation so evident in the other case. The characteristic effects of toluidine blue staining were obtained in quite another region of the teased nerve-fibres, and had a very different character. This dye stains all those regions of the nerve-fibre in which the coagulative changes occur, whereas neutral red yields a picture of a slowly progressing change gradually affecting that first portion of the nerve-fibre in which the intramyelin material is at first seen homogeneous and transparent.

In these regions of coagulative change toluidine blue gives rise to appearances which vary *pari passu* with the extension, and with the intensity, of the coagulation present. Regions such as in unstained fibres would be definitely granular, are loaded with blue granules distributed with much precision across and along the intramyelin material. Such granules leap suddenly into sight over long stretches of the fibre, and are not originated therefore by any process which can be described as an invasion. The tract of developed granules is bounded on the side nearer to the injury by a clear, unstained and homogeneous region, the line of demarcation is sometimes remarkably sharp. On the other side the granular tract passes into a region in which coagulative changes have led to the development of a central rope now stained an intense blue colour.

This curiously complementary behaviour of these two basic dyes is therefore of obvious interest in mapping out two different tracts of the teased nerve-fibre. It certainly does not diminish this interest that in case of either dye an appropriate adjustment in the saline solution in which the dye is introduced to the nerve-fibre may be made to overcome this dissimilarity. If Ringer's solution is substituted for "normal saline," or, better still, if a small quantity of sodium carbonate, 0.02 per cent., is added to the Ringer, or even when the sodium carbonate is simply added to "normal saline," or even when

the basic dye is itself added in increased quantity, then toluidine blue may be made to reveal the complete set of appearances. In these solutions the invasion of the homogeneous region may be watched in a nerve-fibre in which the sudden appearance of granule formation in more distal regions and the intensely stained coagulum is also marked. If, on the contrary, Ringer's solution is substituted for "normal saline" in the case of neutral red, nothing whatever, or at the most only faint traces of granule formation are obtained even in the region near to the cut end. The neutral red stain is enabled to penetrate the whole nerve-fibre best when present in small quantity, 0·01 per cent., and when presented in unboiled "normal saline" rendered faintly acid by the presence of carbonic acid. Toluidine blue solution must be rendered faintly alkaline, or, at least, the calcic phosphate of the Ringer's solution must be present to prevent a development of acidity. Neutral red is, on the other hand, ineffectual in the presence of a reagent, preventing the development of acidity, and can be made to stain the distal coagulated stretch of the fibre only when presented in association with a slight trace of acid. There is an obvious conclusion to be drawn from this series of facts.

There is one region of the nerve which neutral red stains when unaided by any addition of acid. This is the point of actual injury, and a limited region in its neighbourhood which gradually extends with lapse of time. The inference is clear, that this is a tract of the nerve in which a certain amount of acidity develops, or, at least, that it is the least alkaline portion of the nerve. The behaviour of toluidine blue in the same region is also seen to support this inference. Here it refuses to stain until alkalinity is obtained. This might lead to the additional inference, that the region of coagulation which toluidine blue stains unaided is itself more alkaline than any other tract of the fibre.

It would seem therefore that at present we had done no more than map out the teased nerve-fibre into regions of homogeneity and of coagulation, into regions of less and greater alkalinity, and also that we have associated these two separate attributes. There is a homogeneous region close to each injured point which is gradually invaded and rendered granular by an acid tide sweeping in from the point of injury. There is a more alkaline region, in which different grades of coagulation occur.

The Distribution of Potassium Salts in Teased Nerve-fibres.

I have already pointed out that Macallum's reagent may be used to prove the existence of potassium in every portion of the length of a nerve-fibre

provided only that the portion tested be first injured previous to the application of the test. The circumstance of injury reveals the presence of a highly concentrated solution of potassium salt within the intramyelin material, which makes an immediate appearance at every injured point. The bizarre appearances produced by this method of mapping out injured points had been observed by Macallum, but was left completely unexplained by him. Their relation to injury is, however, unmistakable when sought for. Since these points of injury can be multiplied indefinitely so as to include the whole length of the nerve-fibre, it follows that potassium really exists everywhere within the intramyelin material in some manner hidden from the action of the reagent, and that the processes attendant upon injury are capable of presenting it in a new condition ready to take part in the reaction. I have discussed the probable meaning of this fact in a previous communication, and the important conclusions which can be drawn from it as to the nature of nerve-function.

A slight modification in the method of use of this reagent brings out further points of considerable interest. For if nerve-fibres are teased in "normal saline" and allowed to lie in this solution for some time before being tested with Macallum's solution, the potassium solution is found much more widely distributed. It is now found in all those regions of the fibre, in which toluidine blue can be used to reveal its own characteristic effects. Under these conditions potassium is discoverable at each site of injury, and in a neighbouring tract to which it has diffused from this site. Potassium is also now detected in all those regions in which I have described the occurrence of coagulative change. We are, therefore, introduced to a new and very important conclusion. Potassium salts are also discoverable in regions of the fibre distant from points of injury, provided that time is given for their appearance.

Although not situated at injured points this more distal region of the fibre has a secondary relation to injury of a most interesting kind. This relation is at once made evident on reference to Bethe's observations upon the staining capacity of polarised nerve-fibres.

Bethe has made use of an ingenious experimental method, whereby the "fixation" of a nerve is secured during the traverse of a polarising current. The nerve is then prepared for examination under the microscope, and is stained in toluidine blue or in some other similar basic dye. Prepared in this way the fibres are found to exhibit a remarkable distribution of staining capacity. The anodal region is pallid, the indifference point is studded with granules, and the kathodal region is intensely stained. It will be seen that this set of appearances is exactly similar to that which I have observed in

teased nerve-fibres stained with the same dye. Now I have already produced evidence in favour of the view that teased nerve-fibres are traversed by an electrical current—their own injury current. Bethe's observations enable me therefore to identify this distribution of staining capacity in teased nerve-fibres as due to polarisation caused by this current. The pallid stretch of homogeneous intramyelin material is the anodal region of the self-polarised nerve. The deeply-stained region, in which coagulative changes are observable, is the kathodal region where the injury current leaves the nerve-fibre to traverse the external circuit of the salt solution. We can now return to the evidence obtained as to the distribution of potassium.

Injury suddenly elicits the appearance of strong solution of potassium at the injured point. Given time, this solution diffuses into the neighbouring stretch of the nerve-fibre. This phenomenon is accompanied by an electrical phenomenon, the injury current. The traverse of the injury current polarises the fibre and gives rise to the appearance of a new mass of potassium solution, accompanied by appearances characteristic of coagulation at the kathodal region.

Macallum has more recently published an account of the distribution of the inorganic chlorides present in nerve-fibres. With the assistance of Miss Menten he has penetrated the false character, which the vagaries of the slowly-diffusing silver nitrate confer upon their apparent distribution, and has discovered the fact that the inorganic chlorides are everywhere present in great quantity in the intramyelin material. The drawings accompanying this paper also reveal a very remarkable addition to this statement of chloride distribution. In most of these illustrations it is easy to observe indications of effects produced by the nature of the reagents made use of. The proportion existing between the diameters of the myelin sheath, the intramyelin material, and the whole fibre, are alone sufficient basis upon which to make this statement. The intramyelin material does not occupy one-half of its usual relative cross-section. Where this is not the case, and the right proportions are maintained, the authors describe the fibres as swollen. Wherever, in the illustrations, this swollen, but normal, appearance of the fibre is present, the distribution of the chloride coincides exactly with the appearance of teased nerve-fibres stained with basic dyes, and also with appearances obtained by the use of Macallum's reagent for potassium. I have no hesitation whatever in urging, upon this basis of evidence, the necessity of conjugating these facts. The salt present is potassium chloride. It is present in extraordinary quantity. It is present in some masked position in the structure of the intramyelin material. From this position there is direct evidence that it is enticed by injury, and by the kathodal state.

Staining Due to Salt Concentration.

We may now turn to another striking fact which has made its appearance in the course of these investigations. Basic dyes, toluidine blue, and neutral red have been made use of to map out the distribution of a material, which, upon further examination, turns out to be potassium chloride. Set forth in these direct terms, there is nothing surprising in this statement, since both dyes can be "salted out" from their solutions by the addition of potassium chloride. Neutral red is a most insoluble body when tested in this manner, and it was for this reason that I selected its staining effects as a guide to the distribution of the potassium chloride solutions in nerve-fibres. Toluidine blue is more soluble, nevertheless it is easily capable of being "salted out" by solutions of the concentration that my electrical experiments have led me to anticipate. I have also since found that it is more easily "salted out" by potassium chloride in the presence of small traces of alkali, 0.03 sodium carbonate. This latter point has an obvious bearing upon the salting out of toluidine blue in alkaline cell-structures, and more especially at kathodal points.

This definite coincidence between toluidine blue staining and results obtained by the use of reagents precipitating potassium and "chloride" renders it possible at once to advance a general proposition of some interest: *All the appearances made evident in the nerve-fibre by the use of toluidine blue are due to the manner in which this dye is salted out from its solutions by potassium chloride.*

There is also a further point. The appearances which Bethe developed in nerves fixed in alcohol, or in alcohol and ether, I have obtained in nerves freshly teased in "normal saline." Bethe's results coincide with mine, and must be equally accepted as providing a picture of solutions of different concentrations of potassium chloride. Nor is this to be wondered at. Potassium chloride is only slightly soluble in alcohol, although extremely soluble in water. The addition of alcohol, the rapid dehydration which ensues, and the replacement of the water by alcohol, must be definitely expected to leave the salts present as precipitates. Wherever it exists the potassium chloride is precipitated practically in the same quantity as it was originally present in solution. Alcohol fixation, therefore, is precisely calculated to leave *in situ* precipitates of salt for subsequent micro-chemical investigation with toluidine blue and similar dyes.

It is impossible to leave this point without remarking upon the general bearing of this conclusion. The nerve-fibre cannot be the only case in which considerations of this kind are worthy of examination. Granules in the

cell-bodies of secretory cells, or in the bodies of nerve-cells, are no doubt elicitable by similar means, which also have the same significance. It is possible that Nissl granules are of this order of importance. Failing definite evidence upon which to make any assertion, it must at least be said that the well-known appearances accompanying "chromatolysis" are extremely suggestive in character. They would seem to be the consequences of such changes in osmotic pressure as would result from the sudden liberation of salts into solution.

Neurofibrils.

It is now possible to deal with the phenomena observed in that tract of the teased nerve-fibres which corresponds with Bethe's indifference point and its granules. In teased nerve-fibres this region is naturally much more extended, since the polarising "injury current" has a comparatively small value and the changes in the intrapolar region are more gradual. Throughout the whole of this region dense granule formations occur, and these are of such a striking appearance when examined with high powers of the microscope as to absorb a major share of attention. In my own case their fascination left me at first with scant respect for the polar changes described above. To a certain extent this interest is justified when it is considered as the region least involved in polar changes, and altered, therefore, only from the "normal" resting condition in so far as it is involved in the transmission of an electrical current. For the present, however, it is sufficient to deal with the light which these granule formations throw upon the artificial structures known as neurofibrils.

In a previous communication I reported the fact that in nerve-fibres teased in saline solution I had never seen structures bearing any very close resemblance to the neurofibrils described as making their appearance in nerve-fibres "fixed" and stained in special reagents. Since making this statement I have, however, seen structures which cannot be described as granules, since their length is considerable and their thickness much too minute, and which yet cannot be called "neurofibrils," since they are too short, too irregularly arranged, and since they anastomose. The resemblance which they bear to the "neurofibrils" described as found in fixed nerve-fibres is very much that borne by the anastomosing networks of fibrils described as structural components of some nerve-cells. Fibrils such as this I have only seen in some few out of the many nerves that I have teased. Much more frequent, but still only of comparatively occasional occurrence, are structures conveniently classed as bacillar granules, shorter but more

characteristically much thicker than the fibrils. The latter are always in much smaller number in each unit length of the fibre. Other forms seen far more frequently are short ovoid and spherical granules and flakes of the precipitated dye. When all these forms are present they occur in a definite order. The fibrils are found in the first portion of this stained region abutting upon the unstained anodal tract, then follow bacillar granules, then ovoid granules always associated with tracts of the fibre in which definite kathodal staining and coagulative changes are found. In this latter region spherical granules may also occur, but they are placed not in the central coagulum but in the clearer fluid surrounding it. In one and the same portion of the kathodal region ovoid granules may be seen enclosed in the central mass, flakes may be seen upon its surface, and spherical granules may be seen in the clear fluid surrounding it.

To understand the meaning of these granule formations and this difference in their character, it is essential to remember the manner in which they are thus associated with different degrees of coagulation. At the distal extremity of the homogeneous anodal tract is placed the region in which the first approach to visible coagulation takes place. In this place there is no separation between a core and a surrounding fluid. Each unit of volume still contains almost its normal amount of uncoagulated material. The threads of coagulum are still surrounded by an almost normal intramyelin solution. It is in this region, where "solid phase" is still surrounded by a great mass of "fluid phase," that the fibrils are sometimes seen. Before I had puzzled out the meaning of the adjacent unstained anodal tract, in which no granule formation takes place except as the result of diffusion from the source of the injury current, I was greatly exercised by this constant position of such fibrils as I had found. The bacillar granules probably represent a further stage of degradation, in which, however, the main mass of material is almost normally fluid in character. In both these cases the dye is precipitated upon the outer surface of threads of coagulum.

As we proceed into districts nearer and nearer to the kathode an opposite set of conditions arises. The greater portion of the proteid material is precipitated or coagulated as a "solid phase." Within the interstices of this mass there are, however, entrapped collections of solution. It is probable that here the granules represent such collections within which the dye is precipitated.

At one end of the scale of changes solid threads are stained upon their surfaces. At the other there is a similar staining upon the surface of a coagulated mass, and within this again are intensely stained vacuoles.

In no place are neurofibrils of indefinite length existent, simply by reason

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of the gradually varied conditions present in the district where coagulation is taking place.

REFERENCES.

- A. B. Macallum, "On the Distribution of Potassium Salts in Animal and Vegetable Cells," 'Journal of Physiology,' vol. 32, pp. 96-128, 1905.
 A. B. Macallum and Miss Menten, "On the Distribution of Chlorides in Nerve Cells and Fibres," 'Roy. Soc. Proc.,' B, vol. 77, pp. 165-192, 1906.
 Albrecht Bethe, "Allgemeine Anatomie und Physiologie des Nervensystems," pp. 272-298, Leipzig, 1903.
 J. S. Macdonald, "The Structure and Function of Nerve Fibres," 'Roy. Soc. Proc.,' B, vol. 76, 1905.

The Influence of Increased Barometric Pressure on Man.—II.

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(Received October 6,—Read November 8, 1906.)

In a previous communication, we gave reasons for thinking that decompression symptoms may be avoided by maintaining a steady rate of 20 minutes per atmosphere during this process.

The actual time selected was purely empirical, being based on the statistics of Caisson Works, Diving Operations, and Laboratory Experiments. It is clear that a more scientific foundation would be obtained if we could determine (1) the rate at which the tissue fluids are saturated with nitrogen; (2) the rapidity with which dissolved gas escapes during decompression. The most direct method would be to analyse samples of arterial blood under various pressure conditions; but this is not, unfortunately, practicable in the case of man. Another way is the examination of venous samples under similar circumstances. This plan can be followed, and we hope to communicate some results in another paper, but the technic is difficult and still in need of improvement. A third line of research is the indirect determination of the tissue gases, and this will be discussed in the present communication.

If a condition of diuresis be produced by drinking considerable amounts of water, the profuse secretion of urine which results will afford some measure of the dissolved tissue fluid gases. We have proceeded in the following way:—The subject of the experiment drinks at least a quart of warm water, and, after an interval of 10 to 15 minutes, enters the pressure chamber, the pressure being then raised a definite amount. Directly the desired pressure has been attained, he empties his bladder. Ten minutes later the bladder is emptied again. Samples of the urine passed are then run