THE NERVE SUPPLY OF BONE

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BONE has many qualities which suggest that it has a nerve supply. It is one of the most actively living tissues of the body even in the adult. Excepting white fibrous tissue it is the only one which is repaired with its own material. The repair is a highly intelligent and co-ordinated one, a reconstruction. Trabeculae originally laid down to take the stresses and strains to the best mechanical advantage with the minimum of material are taken away and new ones put up in a better place, suggesting that the laying down of bone is a response to stimuli arising through bending or stretching. In old age, with its lessened bodily activity, the bones rarefy. They do so even in youth if the subject is confined to bed, but here they recover again when they begin to be used actively. Too rigid fixation of fracture fragments almost inhibits union. Constant stimuli arising through the use of the bones therefore seem necessary to their maintenance.

The sensitivity of bone is mostly stated to be low, yet fractures and inflammation of bone are very painful. Clinical experience on this subject is summarized by Leriche (1930), who points out that by Böhler's (1929) technique of a single large injection of novocaine between the ends of a fractured bone anaesthesia and relaxation of muscle spasm is quickly produced, even while everything from periosteum to skin is still sensitive, showing that the pain is arising deep in the bone. He also says that osteotomy under local anaesthesia requires the introduction of more novocaine for each few millimetres of cutting, and that the marrow spaces and periosteum are well known to be very sensitive.

The question of the existence of specifically trophic nerves is raised by the occurrence of atrophic processes in bone in such diseases as tabes dorsalis and syringomyelia. The clinicians incline to believe that such nerves do exist. Most of the literature on this aspect of the subject may be found reviewed by de Castro (1925, 1930), Kuré (1936) and Müller (1931), but it may be briefly stated here that the confusion arises from so little having been done to trace the nerves of the "passive tissues" to their origins and terminations. At present no one can say whether an atrophy is due to loss of specifically trophic fibres, or is merely secondary to vasomotor upset, or due to loss of ordinary sensation and the protection which pain affords, or is an atrophy from disuse.

PREVIOUS WORK ON THE NERVES OF BONE

The chief worker on this subject was de Castro, who evolved a technique for silver impregnation of nerves in tissue which had been through decalcifying acids, and described nerves supplying growing bone. He found nerve fibres entering with the irrupting vessels and traced them along to the final capillary loops. As the vessels burrowed deeper he found that they left nerve twigs connected to the osteoblasts by a loop form of ending in contact with the cell protoplasm. All the osteoblasts were not found to have a nerve supply, and this he explains by saying that these cells are probably not discrete units, but are interconnected. He states, however, that "There are no nerves in fully formed bone, because, as the bone consolidates and the osteoblasts become included in it so as to become osteocytes, they lose the nerve supply which they had by a retrograde degeneration of the fibres." By silvering whole rat and mouse heads he traced the nerves of the developing basi-occipital and petrous temporal bones to the superior cervical sympathetic ganglion and the vagus and glossopharyngeal nerves. In his view the nerves of developing bone are from the autonomic nervous system only.

Other workers have confirmed de Castro's findings in growing bone although they were unable to find the special form of ending he described. Schartau (1936) used sections of 40–50 μ which showed the general arrangement but no detail. Miskolczy (1926), working mainly on the periosteum, saw nerve fibres entering the mouths of the Haversian canals as well as the bone surface at other points, but he could follow them no farther.

The general textbooks of histology state that there are nerves in the Haversian canals and that they are probably mostly vasomotor, but that some go on to the endosteum and the marrow. Stöhr (1928) makes a similar statement and quotes Miskolczy. He also mentions Lushka's finding that the nerves to the vertebral bodies come from the cranial and sympathetic nerves.

TECHNIQUE AND MATERIAL

The working out of a method of staining nerves in bone was the first problem to be solved. No claim is made that this has been fully done, but that the way has been cleared for further work.

Only block impregnation with silver nitrate and serial sectioning in paraffin were tried extensively, since it was realized that only serial sections could be the basis of nerve demonstration in bone. The Bielschowsky-Gros and Cajal methods for frozen sections were tried after fixation-decalcification by de Castro's method, but without success.

The ordinary methods for silver impregnation very seldom show any nerve fibres even in muscle, etc., after the block has been through decalcifying acid. Stewart (1936) reported one such success using Cajal's ammoniated alcohol technique on decalcified dentine. Cajal (1933) says that nitric acid in formol, used for 1 day on embryos and small foetuses, seems actually to better the neurofibrillar action of silver. Huber & Guild (1913) thought the failure of silver methods on bone was due to imperfect fixation in the interior of solid bone. They used a slightly modified Ranson technique and injected the fixative into the arteries of anaesthetized animals. They were able to stain nerves passing through bones but have described none distributed to bone or its contents. Their method is unfavourably criticized by de Castro and was not tried by the writer.

In the writer's hands the only methods which can be relied upon to show nerve fibres anywhere with constancy after decalcification were de Castro's or those using his addition of hypnotic drugs, chloral hydrate, somnifaine, etc., to the fixing-decalcifying bath. But de Castro's method, even when it gave a strong impregnation of the nerves of the soft parts of a block, never showed nerve fibres in bone matrix. As implied above, he himself never succeeded in impregnating such nerves.

It became clear by degrees that there were probably two causes at work preventing the staining of nerve fibres after acids, the first being some effect overcome by de Castro's addition of the hypnotics to the acid bath, allowing the nerves of the soft parts to be shown, the second being simple failure of the silver nitrate and other solutions to penetrate the piece of bone after its fibrous matrix had been swollen during decalcification, washing, etc. The use of alum to prevent the swelling here suggested itself.

It also seemed that, since de Castro's method often failed to show up very fine fibres and nerve endings, more strongly acting methods such as those in use for embryonic material should be tried after fixing and decalcifying by de Castro's method. The combination thus arrived at is given in the table below, and a summary of materials and other technique tried is given later. The method given is de Castro's fixation-decalcification, using a high alcohol because de Castro's 60 per cent alcohol allows maceration if used for more than a few days, followed by Cajal's ammoniated silver technique with a stage of 5 per cent potash alum. This method gave one strong impregnation of nerves in bone matrix, but all the soft parts of the block are choked with a heavy precipitate of silver. Attempts to repeat the impregnation have all failed, and there is always much precipitation of the silver, so that it is certain that, though one success has shown that the reason for using alum to prevent swelling of the bone matrix by the acid is probably correct, alum is not a good agent for the purpose because it interferes with the working of the silver afterwards.

TECHNIQUE USED

1.	Fix in 100 c.c. 96 per cent alcohol + 4 c.c. somnifaine + 6 c.c. nitric acid, until decalcified	
2.	100 c.c. 96 per cent alcohol + 2 drops liquor ammoniae fort.	24 hours
3.	50 per cent formol	24 hours
4.	100 c.c. 96 per cent alcohol + 2 drops liquor ammoniae fort.	24 hours
5.	5 per cent potash alum	24 hours
6.	Pyridin	3 days

7.	Distilled water, many changes	2 days
8.	3 per cent. silver nitrate at 37° C. in darkness	7 days
9.	Ammoniated silver nitrate, changed daily (10 c.c. of 10 per cent silver nitrate, 20 drops of 40 per cent NaOH; wash precipitate in three changes of distilled water, 400 c.c. distilled water, ammonia till precipitate is almost all dissolved.)	2 days
10.	Wash in distilled water + 0·2 per cent glacial acetic acid	12–24 hours
11.	20 per cent formol Wash and embed in paraffin.	3 days

SUMMARY OF MATERIALS AND TECHNIQUES EXPERIMENTED WITH, AND THEIR RESULTS

At first the humerus and femur of new-born kittens were used on account of their small size and ease of decalcification, and about twenty blocks put through most of the established techniques, Cajal, de Castro, Ranson, Blair & Davies (1935) modification, Bielschowsky, etc., trying the effects of different acids, longer or shorter times in pyridin, silver nitrate, etc.

As it became clear that there was no great advantage in using young bone and that adult bone might differ from young bone, use was next made of pieces of the chest wall of adult guinea-pigs, rats, mice and cats. Small blocks, each a part of two ribs and the space between, were put down in batches of one to two dozen in the techniques and variations in the last paragraph, as well as in others from the large numbers now published, such as Agduhr's modification of Bielschowsky's technique, Cajal's ammoniated silver technique, de Castro's with 1–2 days pyridin after the ammoniated alcohol (a useful introduction in the much better differentiation it gives between nerve and connective tissue), and de Castro's with a stage of 50 per cent glycerin in distilled water for 7 days after the ammoniated alcohol, which gives an even better differentiation than pyridin, leaving bone matrix a clear yellow. None of these gave good impregnations of nerves anywhere except de Castro's. Even it falls short on fine twigs and terminations and fails to show nerves in bone matrix.

Because these small ribs show almost no Haversian bone adult cat femur was next tried. This bone has about twice as much ground lamellae as human, but the Haversian systems are well developed. It was at this time that the use of alum suggested itself, so six pieces were used in modifications of Cajal's ammoniated silver method, with and without alum. Block 1 was fixed in ammoniated alcohol, decalcified in 6 per cent nitric acid in the formol, and then carried on as in the table above. Block 2 was fixed in alcohol with 2 c.c. per cent of somnifaine, decalcified in the formol, and the rest as in the table. Block 3 was treated as in the table. Blocks 4, 5 and 6 were given the same treatment as 1, 2 and 3 but without alum. Block 3, a piece of the femur of a fully grown cat, age unknown, but without signs of growth in the bone, is the one specimen obtained showing nerve fibres in the bone matrix.

The method is long, since decalcification in high-percentage alcohol is slow. It has been repeated six times unmodified and each time with 5–6 blocks in modifications such as putting the alum bath between different stages, and using smaller bones such as rat and guinea-pig, but without further success. The successful specimen was obtained from a cat. The tissues of this animal were anoxaemic and contained chloralose, death having been brought about by this anaesthetic. The technique has been tried again on similar pieces of bone and on bone from a rat killed with chloralose, but without success.

It seems very probable, however, from this one result and from experience with de Castro's technique which stains nerves so constantly in the soft tissues of a block, that the problem remaining to be solved is a purely physical one, to prevent swelling of the fibres of the bone matrix and at the same time to avoid shrinking it as a whole.

FINDINGS

It was felt very undesirable to base much on one specimen. Publication was, therefore, delayed for more than a year while efforts were being made to produce more results. The block was cut longitudinally and serially at 12μ, and the sections were thoroughly examined and shown to others. There is no room for doubt that the structures described are nerves. The nerve impregnation and the differentiation from connective tissue are good even where Sharpey's fibres are shown. These are brush-like and more or less at right angles to the periosteum, while the nerves run in or parallel to the Haversian canals, finally diverging from them into the matrix. One can follow the nerve fibres from the periosteum along the canals to their endings in the matrix and close to the bone cells. The nerves which enter with the nutrient vessels by the main nutrient foramen seem all destined for the marrow or its vessels. The fibres destined for the bone matrix accompany the vessels which enter the Haversian canals here and there over the surface. At all these points one or two fibres enter closely applied to the vessels while others run into the bone parallel to the canals, but often as much as the width of the canal away from it, suggesting that the canal had once been wider and that the nerve fibres had become included in the bone (Pl. II, figs. 2, 3, 4). In the canals in the depths of the bone the nerves retain this relationship, some lying inside and some just outside the lumen (Pl. II, fig. 6, and Pl. I, fig. 4). The actual boundary of a canal is hard to define, and the presence of lacunae has been taken to mark definite bone from canal or from periosteum. There is usually also an indication of surface in a darker deposit of silver.

The nerve fibres are then distributed to either side, covering well over half the distance to the next adjacent canal (Pl. II, fig. 1, and Pl. I, figs. 3, 6). There is even an indication in places of communication between the nerves of such a pair of canals. The branching is free and irregular. In many places there are curious coiled hanks of fibres not far from the canals, with thick fibres giving off thin terminal twigs (Pl. I, fig. 5). The fibres lying in the bone matrix show most of the histological characters of unmyelinated nerves, varicosities, alternate thickening and thinning, beading due to neurolemmar nuclei, etc. (Pl. I, figs. 1, 2, and Pl. II, fig. 1). They are of medium thickness. The thickness of nerve fibres in the same region and same species of animal seems, however, to vary greatly with the technique used. They appear thicker after nitric than after formic acid.

The endings of the fibres in the matrix show the same characters as those always found by the writer in periosteum using de Castro's method. Most of the fibres grow thinner and thinner and end in scattered specks of silver which can be traced no farther with an oil immersion lens and 20 × eyepieces in a binocular microscope. Other fibres run into very close relationship with the bone cells (Pl. I, figs. 1, 2, and Pl. II, fig. 1), but with the same power nothing could ever be seen resembling the ring terminals described by de Castro on the osteoblasts of growing bone.

An idea of the richness of the supply may be gained from the fact that in the best part of the block no part of the field of a 1/6 in. lens is without nerve fibres. Here also the nerves extend to about two-thirds of the thickness of the wall of the shaft, but nothing should be based on this, for the impregnation is obviously incomplete in other places.

With only one impregnation any attempt at interpreting what function these fibres can have must only be very tentative. De Castro's work has established that nerve fibres are at least closely connected with the osteoblasts of growing bone. He suggested that these nerves are probably concerned with the modelling of the bone and may even be the site of action of the endocrine secretions affecting bone. Without being too speculative one may say that the endings in adult bone described in this paper suggest, in conjunction with the properties of bone mentioned in the introduction, that they may be the sensory and effector endings required for a reflex control of bone growth and maintenance. The free nerve endings are a type of termination associated with protopathic sensation (the only type of sensation elicited in bone clinically), and those in relation with the osteocytes are evidently the same as those seen by de Castro related to the osteoblasts of young bone, but supposed by him to degenerate when these cells became enclosed in the bone as osteocytes.

The nerve supply of bone marrow has been partly worked out by de Castro and others. The writer can confirm de Castro's findings that most of its nerves go to the blood vessels, but that there are others which end among the marrow cells, their manner of ending being unknown. Further work will be carried out on them at the same time as that obviously still much needed on the nerves of bone matrix.

SUMMARY

1. Nerve fibres were traced into and along the Haversian canals of adult bone and into the bone matrix. Their distribution, characters and endings in the bone matrix are described. Some fibres end blindly in the bone matrix, some in close relation with the bone cells.

2. A short account of the materials and techniques experimented with is given, leading to those eventually successful in one case, a piece of adult cat femur and a combination of de Castro's method with Cajal's ammoniated silver method, with a stage of 5 per cent potash alum.

3. De Castro's addition of hypnotics to the decalcifying bath solves the difficulty of producing silver impregnation of nerves after decalcification. The remaining difficulty is the finding of the most suitable agent which will prevent the swelling of the white fibrous matrix of the bone in the acid, so that it may be permeable to the reagents used later. Alum is the type of substance needed for the purpose but it interferes with the working of the silver afterwards.

4. A suggestion is put forward tentatively that the nerve fibres found may be the two ends of a reflex arc governing bone growth and maintenance.

My grateful thanks are due to Profs. Blair and Davies at whose suggestion the work was commenced, as well as to Prof. Nicol and Mr Stibbe for their advice and criticism.

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EXPLANATION OF PLATES I AND II

All sections are longitudinal

Plate I

Fig. 1. Terminal piece of a nerve in bone matrix, showing the histological characters. On the left of the field a fibre is seen to pass practically in contact with a bone cell (a very common feature in these sections) and then to pass on to another bone cell. × 1095.

Fig. 2. End of a nerve fibre in the bone matrix. Shows the histological characters, varicosity and beading. A little below the middle of the left side of the field is the outline of a bone lacuna to which the nerve fibre ends are going. × 1500.

Fig. 3. Nerve fibre leaving a canal and running into the matrix. × 840.

Fig. 4. Section tangential to a Haversian canal. Shows nerves accompanying the canal but lying in the bone outside it. × 840.

Fig. 5. Coiled hank of nerve with fine bare endings. The section is just tangential to a canal (dark upper part of background). × 840.

Fig. 6. Shows the extent of the distribution of a nerve fibre in the matrix between the two Haversian canals marked by arrows. × 588.

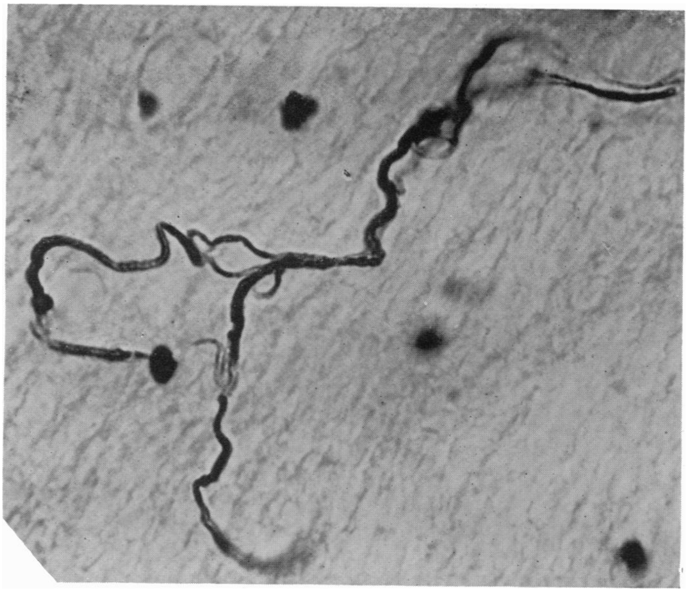

Fig. 1

Fig. 2

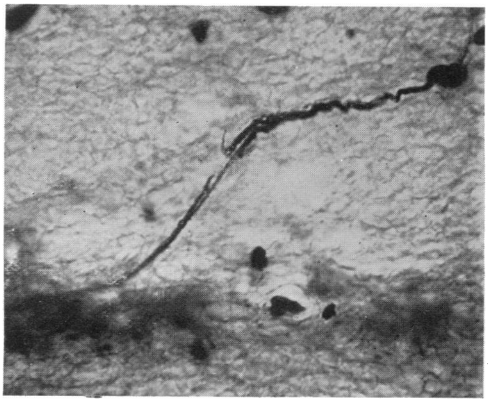

Fig. 3

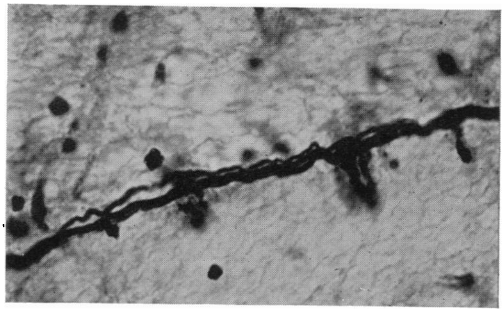

Fig. 4

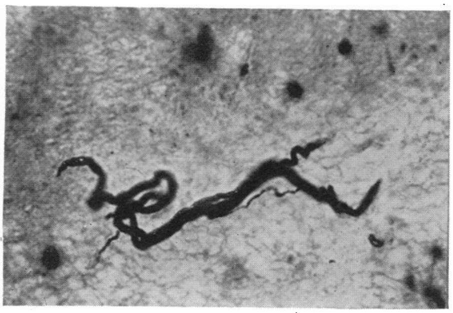

Fig. 5

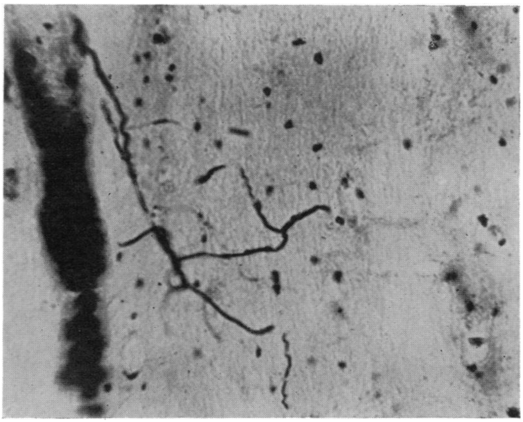

Fig. 6

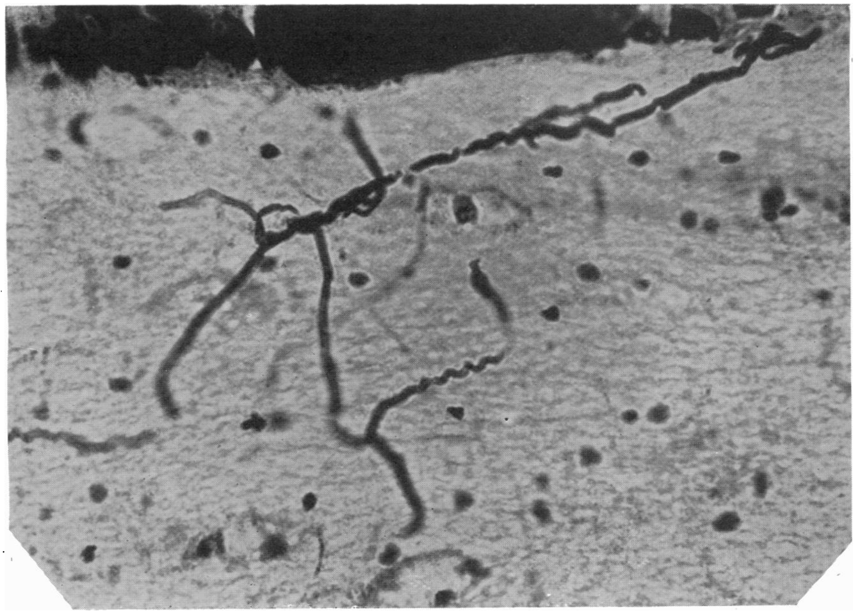

Fig. 1

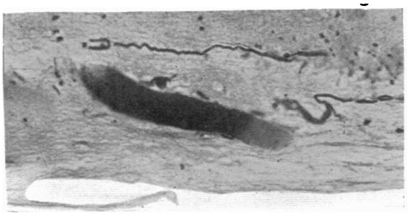

Fig. 2

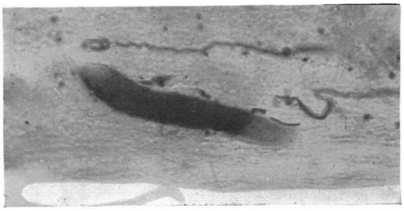

Fig. 3

Fig. 4

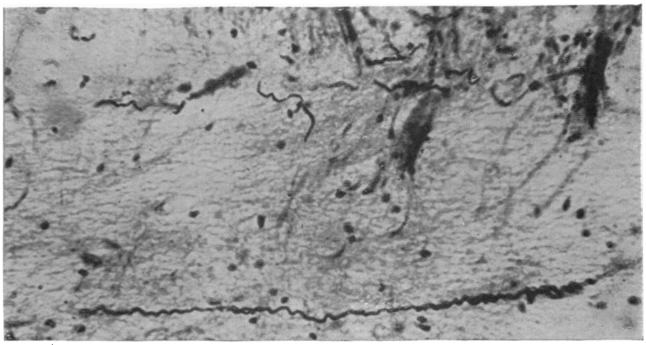

Fig. 5

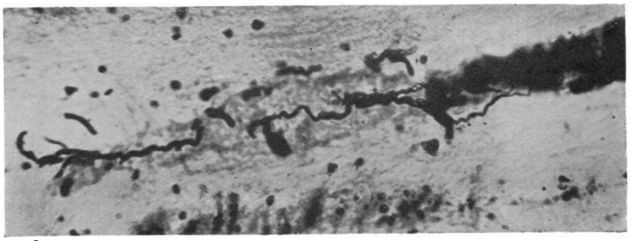

Fig. 6

Plate II

Fig. 1. Shows the histological characters of the nerve fibres. In the middle of the lower edge of the field will be seen the outline of a lacuna with the cell body and nucleus shrunken to the upper left side. The fibre coming down can be traced as far as the cell body. × 1190.

Figs. 2 and 3. Two views of the same field showing the entry of a Haversian canal and nerves from the periosteum. Fig. 2 is focused on a nerve entering the bone parallel to the canal, Fig. 3 on twigs applied to the vessels in it. × 306.

Fig. 4. Shows a small piece of nerve fibre applied to a blood vessel in the commencement of a Haversian canal. A larger nerve fibre is also seen entering the bone matrix well outside but parallel to the canal. × 588.

Fig. 5. This section is from the heavily impregnated side of the block. Shows Sharpey's fibres together with nerve fibres in the matrix. × 468.

Fig. 6. Shows nerve fibres lying in an obliquely cut Haversian canal. × 542.