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MICROSCOPIC INNERVATION OF FIXED VASCULAR GRAFTS

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A series of experiments was performed by us in the course of the last few years to examine whether fixed vascular grafts are suitable for bridging a defect in the aorta and, if so, how the new vessel wall is being formed and what part the graft was playing in its formation. Dogs differing in breed, age, and sex were used. The experiments furnished convincing proof that vessel segments kept for longer or shorter periods in different preservative media act for some time merely as connecting tubes in the wall of the recipient vessel, whereafter they gradually become transformed, taking on a histologic architecture ensuring a perfectly undisturbed blood circulation. Generally, the impression was gained that, in case of successful operations, the transplant turned into a vessel wall fully efficient for undisturbed blood flow. This transformation having been histologically confirmed in accordance with foreign publications the question arose whether under the given histological conditions the nervous system was growing into the newly developed vessel wall and, if so, how the nerve fibers were adopting themselves to meet the demands of the new environment, in what manner they were attaching themselves to the tissue layers and, thereby, to the adjacent portions of the recipient vessel.

To study the problem fixed segments of dog aorta were transplanted into the abdominal aorta of dogs. These were allowed to survive for varying periods before being sacrificed. At autopsy, the grafts with the adjacent portions of the recipient aorta were excised and the nerve connections examined. The results obtained on a series of 5 dogs were as follows :

Dog No. 1. A portion of 2 cm length was excised from the abdominal aorta and was substituted by an aortic graft, which had been kept for 2 months in a 0,1 per cent mercury oxycyanide solution. At autopsy, 1 month and 6 days after the transplantation, an aneurysm-like dilatation was found around the transplant. On gross inspection it seemed that the graft was destroyed and blood circulation was maintained by a temporary segment formed by the surrounding tissues. A piece was then cut out of the aneurysmal area so as to include the two adjacent portions of the recipient artery. The specimen was fixed, according to *Lavrentiev*, first in a mixture of alcohol, formalin and arsenic acid, and subsequently in 20 per cent neutral formalin. Thereupon, tangential sections 20 to 30 micra thick were prepared and impregnated according to *Bielschowsky-Schultze-Gross*. It was revealed that the nerve fibres grew into the area of the aneurysm ; moreover, in some of the sections a vast sympathetic-type ganglion was found in the temporary vascular wall together with the afferent sympathetic trunk. In addition, it could be seen in the preparations that regeneration had started over the whole aneurysmal area, and that large masses of nerve fibres had grown into the transplant which was degenerating, yet at the same time undergoing transformation (Fig. 1*). From this it was inferred that had the animal been kept alive for some additional time, the dilated arterial wall would

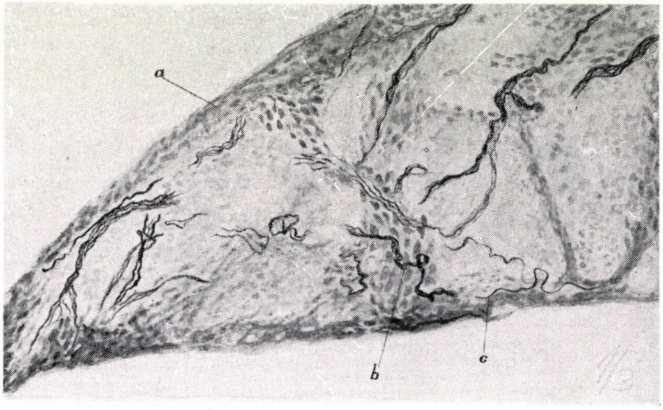

Fig. 1. Dog. Innervation of fixed aortic graft. *a)* connective tissue, *b)* nerve, *c)* nerve fibre. Bielschowsky's method. ×200. Photographically reduced 3 to 1

have been strengthened by the trophic influence of the nervous system to an extent that it could have secured a permanent, unhampered path for blood circulation. In this case, the graft acted as a temporary connecting tube which gave rise by its effect in the surrounding tissues to the development of a boundary layer capable of substituting the excised vessel wall as a whole, in spite of its incapacity of pulsatile motion, due to the lack of the non-regenerating layer of smooth muscle.

On examining the specimens the question arose where the sympathetic trunk may have come from, seeing that in the aortic wall proper there occurs no parietal ganglion, at least not in the section involved. It cannot of course be assumed that the ganglion is the outcome of simple regeneration. The only reasonable explanation would therefore be that the correspondingly situated ganglion of the sympathetic trunk grew into the aneurysm, or vice versa, and that their fusion was also the cause of the regeneration that started from the

* All figures were drawn by assistant Miss Z. Moller, to whom thanks are due for her careful work.

trunk towards the aneurysmal area, and of the proliferation of fine nerve fibres initiated by the new environment.

Dog No. 2. An aortic segment kept for 102 days in 4 per cent neutral formalin was transplanted in the aorta, again in the lumbar region. Autopsy showed the transplantation to have been a complete success, without any signs

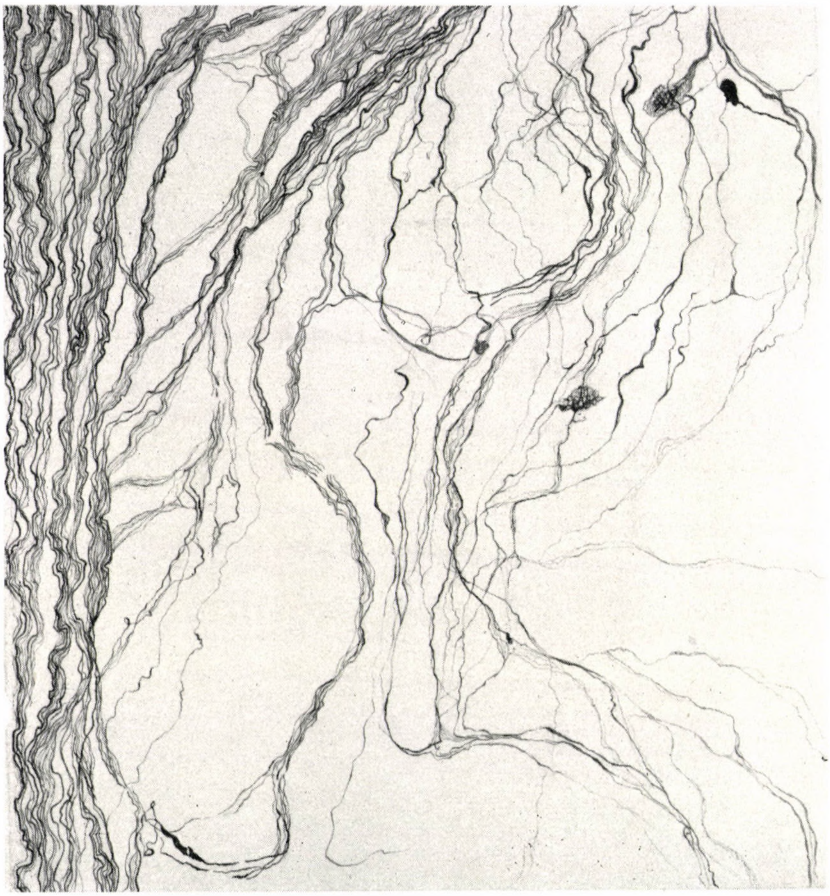

Fig. 2. Dog. Innervation of fixed aortic graft. Bielschowsky's method. × 400. Photographically reduced 2 to 1

of aneurysm or obliteration. The dog survived the operation by 3 months and 6 days. In this case, too, at necropsy the graft was excised together with a portion of the recipient vessel at each end. The material was fixed and impregnated in the manner described above. The impregnation, also, was a success. The abundance of nerves in the preparation exceeded many times the usual nerve supply to the area. In addition, quite conspicuous were the embryonic characteristics, such as the vagueness, disorientation, exquisite delicacy and undulation of the fibres. These marks differed considerably from the characteristics peculiar to definite vegetative fibres (Fig. 2).

Particularly towards the centre of the transplant there appeared in large masses neurofibrillar end formations, loops, clubs, terminal ramifications

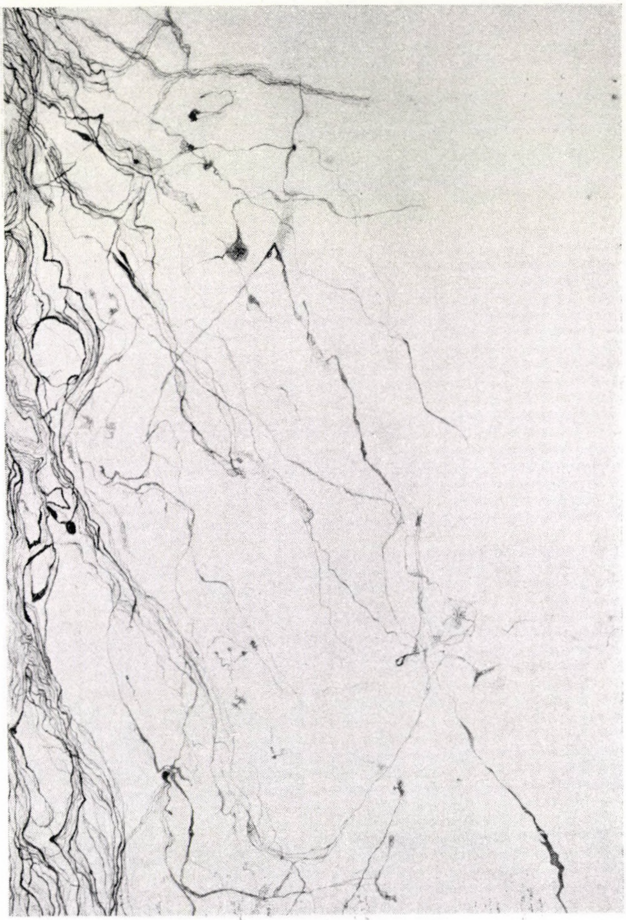

Fig. 3. Dog. Innervation of fixed aortic graft. Bielschowsky's method. × 400. Photographically reduced 2 to 1

neurofibrillar plates, and other similar structures of varying size which in their entirety appeared to impart a quite particular sensory character to the terminal branches of the fibrous system. The groping for a path to follow, on the one hand, and the course-determining and formative influence of the regenerate, on the other, reflected themselves conspicuously in the structural character and the assumed configuration of the entire nerve-fibre system (Fig. 3). Even more interesting and characteristic were perhaps those structures which presented themselves quite near to the termination of the regenerating fibre system. Special terminal clubs, neurofibrillar plates inserted into the fibres, peculiar loops, and loose whirl-like formations were visible in this area. In general, the

Fig. 4. Dog. Innervation of fixed aortic graft. Bielschowsky's method. × 400. Photographically reduced 2 to 1

entire nervous network was distinguished by extraordinary fineness, abundance in many places by a marked clearness of the neurofibrils, and in some by a peculiar and unusual texture. All this is very accurately and distinctly visible in Fig. 4 which is the direct continuation of Fig. 3.

Among the various interesting and peculiar end-structures, which might be characteristic of nerve regeneration in other areas as well, there occurred not infrequently some formations in the shape of a corkscrew. Their structure, indeed, reflected the character, shape, course, and trend of regeneration. They should be visualized as exceedingly fine fibres, almost of the tenuity of neurofibrils, which at the end of a longish stretch form into ascending spirals giving

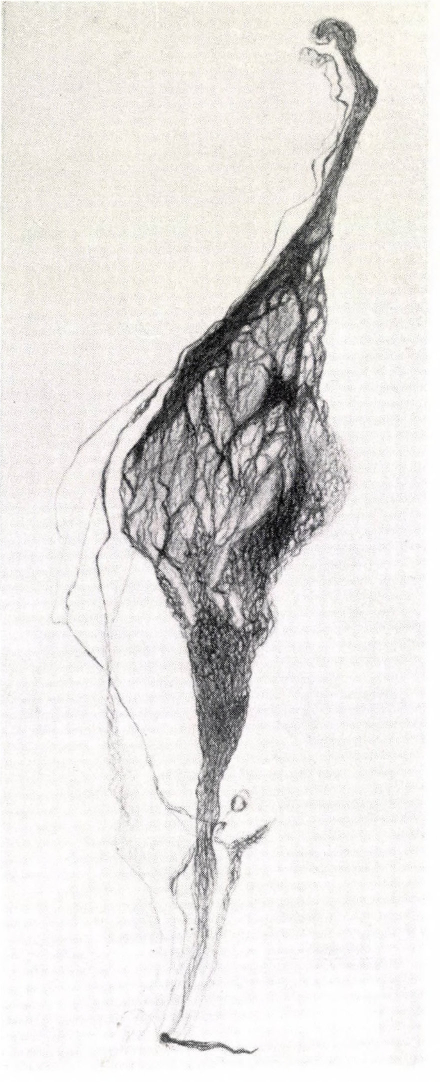

Fig. 5. Dog. Innervation of fixed aortic graft. Nerve regenerate. Bielschowsky's method. × 900. Photographically reduced 3 to 2

exactly the impression of closely winding coils around a rather long, solid, cylindric shaft. Along with them there are large, flat, fibrillate varices, special branches, end-plates and clubs, interstitial lamellae, loose, whirl-like terminal ramifications, and similar structures occurring in great numbers and variety at the end-system of the advancing fibres. Among these diverse neurofibrillary formations, which in this case really deserve to be called pioneers, or trail blazers, there is one which is quite peculiar, spindle-shaped, of conspicuous size, blunt at the end, differing from all other terminal structures. This was seen in but a single preparation, and because of its uniqueness it was studied by stronger magnification. It was found to be really the end plate of a single fibre the neurofibrils of which were multiplying, opening out and uniting again. Further magnified, it was seen to consist of a close plexus of delicate neurofibrils, which in some places, arranging themselves in bundles, formed long-shaped bunches, while in others they intersected each other forming windowlike openings. Another characteristic of this peculiar regenerate in question is that the terminal body proper is surrounded by a system of neurofibrillar plates which, blunted at their ends, conclude with a glomus (Fig. 5).

Dog No. 3. Aortic segments that had been kept in Jores solution in a refrigerator for 58 days were transplanted in the same region of the aorta as above. The animal was allowed to survive the operation by 5 months and 18 days. The transplantation once more succeeded very well. No deficiencies, no anomalies were observed. Impregnation was also unobjectionable. The nerve fibres were well discernible in the graft. Regeneration occurred, and although no aneurysm had developed, there was again a powerful sympathetic ganglion present in the tissue undergoing transformation. Naturally, interpretation was this time less easy than in the case of dog No. 1, for in the absence of an aneurysm it was difficult to imagine that with one of its ganglions the sympathetic trunk had grown onto the new vessel wall, or vice versa. One should rather suppose that the sympathetic trunk and, with it, the ganglion, found their way to this site from the periadventitial plexus accompanying the aorta. To be decided, the problem awaits further experimental studies and careful anatomical observations, which will then also determine at what stage regeneration of the nerves really begins in the graft, and how much time is required for the innervation to take its final form in these vascular segments. At the same time, these studies will have to throw light upon the questions what the definitive nervous network is like, and whether in view of the new environment it differs from the normal conditions of innervation.

Dog No. 4. Aortic portions preserved in 4 per cent neutral formalin for 19 days were transplanted into the aorta of the animal. The dog was sacrificed 7 months and 10 days after the operation. Autopsy showed the recipient vessel to have become obliterated along a length of 2 cm. both cranially and caudally. In spite of this, the graft underwent transformation in the usual way. Growing in of the nerve fibres and regeneration, respectively, were clearly visible in the impregnated sections in this case as well.

Dog No. 5. An aortic segment which had been kept in 4 per cent neutral formalin for 60 days was transplanted in a dog's aorta. 15 months and 14 days later the animal was examined. The recipient vessel had become obliterated. The nerve fibres were found to have grown into the graft, like in the preceding case.

From what has been said above and from the figures it can safely be concluded that whichever the medium used for preserving the graft, nerve fibres will grow into the transformed aortic wall, they will supply it richly everywhere, and will establish close contact with the layers. These phenomena raise the question what the real role of the nervous system may be in view of the fact, confirmed by histological examinations both in this country and abroad that the smooth muscle elements of the media disappear in the transplant and even later do not become replaced, so that proper contraction, dilatation, and changes in luminal diameter cannot really take place in this vascular segment. This question is justified, and there is an answer to it. While the most important function of the nervous system in the vessel is undoubtedly concerned with the movement of the wall, there are other circumstances as well which justify and make it by all means necessary that the nervous system should grow into the new aortic wall. These are as follows.

In the wall of every artery and vein, and in the connective tissue immediately surrounding it, there are four plexuses to be distinguished, viz. the periadventitial, the adventitial, the interlamellary, and the intramuscular plexuses. With respect to the vessel wall's function, the greatest significance attaches of course to the last one, the nerve plexus of the media. Accordingly, if there is a defect — and there actually is —, then this plexus will be the first the fibres of which will be groping in vain for masses of executive cells in the graft that underwent transformation. For, as revealed by histology, the smooth muscle cells disappear between the longest persisting elastic fibres and even later do not become replaced. On the other hand, the periadventitial plexus is a nerve-supplying network providing the vessel wall segmentally with the necessary fibres, and so represents the structure of which the fibres of the vascular wall will have to be replaced. The adventitial plexus, originating from the former, has partly the same role to fulfill but in addition it is the site for the sensory nerve endings in the vascular wall and, at some places, there are in it fibres of central origin as well as sensory end-apparatuses. The elements of this plexus divide into branches, and serve the purpose of innervating the collagen fibres and their intermediate substance. Although they are no pressorreceptors, they feel the dilatation of the vessel wall and the intraluminal pressure exerted upon the corresponding layer, and thus secure the vascular reflexes. Moreover, amongst them are the end-organs of the wall mediating sensations of pain. Although there is nothing to support it morphologically, it might be possible that at least part of these fibres is derived from the sympathetic nervous system. The interlamellary plexus, too, serves the innervation of the adventitia and, like to some extent the former plexuses, supplies sensory and motor fibres to the walls of the vasa vasorum.

On the ground of what has been said above, the regenerated vessel is indeed justified to require nervous elements. This is particularly so in respect of the vast number of ingrowing capillaries vascularizing the graft, loading the foreign tissue of the sutures with lymphocytes and, just like in perochondrial osteogenesis, practically dissolving the preserved transplant. These capillary vessels must be followed by the corresponding sympathetic fibres, for the capillaries too pulsate ; a phenomenon which cannot even be imagined without a nervous stimulus. And indeed this does reflect itself in the transplants, where the capillary vessels are followed by an immense number of nerve fibres proceeding singly or in trunks. However, there is another bearing, which we usually call the trophic aspect and which in its essence agrees with what has been expounded in the foregoing. According to this, a nervous stimulus is absolutely necessary to elicit tissue metabolism ; a point which we have never failed to stress, and lately would emphasize particularly. If the stimulus is lacking the tissue will slowly degenerate and necrotize or, with the lapse of time, cease functioning. Trophic action is in our opinion a vascular action, in the first line one of the capillary vessels. This will always show when the nerve to the tissue is cut or paralysed permanently, for thereby not only the cerebrospinal connection of the receptors and effectors of the tissue will be interrupted, but the receptors and effectors of the vascular system will be disconnected too. Although in the small vessels, and especially in the capillaries, we have no means of morphologically differentiating these multiform and multistructural end-structures by virtue of the uniform morphological and physiological outlook, we deem it justified and in the present case even necessary to assume their presence.

Apparently, these theoretical considerations have fully been confirmed by the experiments which revealed that nerves had been replaced abundantly, astonishingly rapidly and, under the given circumstances, completely. From this fact we are now entitled to conclude that preserved transplants turn gradually into viable, perfectly functioning vessel walls.

Summary

1. Aortic segments which for varying lengths of time had been kept in 0,1 per cent mercury oxycyanide, 4 per cent formalin, or a Jores solution, respectively, were transplanted in the abdominal aorta of dogs of different breed and sex.

2. In their majority the operations were successful. The animals survived them by several months. The grafts necrotized gradually, and were replaced by new vascular walls.

3. After a month nerve fibres were found to begin growing in and to supply abundantly the single layers.

4. Since from the point of view of blood circulation the transplants appear to turn into perfectly efficient vascular walls, there is reason to believe that after being examined on humans it will be possible to perform successfully the operation in case of injury sustained at accidents or war casualties.

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МИКРОСКОПИЧЕСКАЯ ИННЕРВАЦИЯ ФИКСИРОВАННЫХ ТРАНСПЛАНТАТАХ КРОВЕНОСНЫХ СОСУДОВ

А. АБРАХАМ и Л. ШИН

1. Авторы пересаживали кусочки аорты, содержанные более или менее продолжительное время в 1%-ом растворе оксицианистой ртути, в 4%-ом нейтральном растворе формалина или же в растворое Jores, в брюшную аорту собак различной породы и различного пола.

2. В большинстве случаев операция оказалась успешной, животные остались в живых в течение месяцев, пересаженные кусочки постепенно разрушились и были замещены новыми стенками сосудов.

3. По истечении одного месяца нервные волокна начинают врастать в трансплантат и обильно снабжают их отдельные слои.

4. Ввиду того, что с точки зрения кровообращения пересаженные кусочки аорты становятся полноценными стенками сосудов, то после соответствующих испытаний и соблюдая необходимую осторожность, такую операцию можно успешно провести и у человека при несчастных случаях или в случае боевых поранений.

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