The Blood-Vessels of the Developing Spinal Cord of *Xenopus laevis*

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WITH ONE PLATE

INTRODUCTION

STERZI (1904) studied the blood-vessels of the developing spinal cord in representatives of various vertebrate groups. He correlated the early development of the vascular plexus on the lateral aspect of the neural tube with the mitotic activity within its lateral walls. He also correlated the greater vascularity of the grey matter, compared with that of the white matter, with the greater functional activity of the former. From the observation that there are 15 separate vessels that are constant in position and time of appearance during the development of the spinal cord of the chick, Feeney & Watterson (1946) reached the tentative conclusion that the pattern of the blood-vessels is determined by localized structural or physiological changes, or both. Observations on mammals by Craigie (1925), Petren (1938), and Gyllensten (1959) indicated a marked increase in vascularity of the cerebral cortex while differentiation was proceeding. Quantitative observations on the blood-vessels of the spinal cord during development are lacking.

Craigie (1945) and Feeney & Watterson (1946) indicated the need for an experimental approach to the development of blood-vessels in the central nervous system. The present investigation was undertaken to establish the descriptive and quantitative features of the development of the blood-vessels of the spinal cord in an animal readily available in the laboratory as a foundation on which future experiments might be based.

MATERIAL AND METHODS

Tadpoles of *Xenopus laevis* were obtained, reared, and staged by the methods recommended by Nieuwkoop & Faber (1956). Specimens were taken at each stage and anaesthetized with M.S. 222. The vascular system was then injected with Monastral fast blue (B.N.V.S., Imperial Chemical Industries Ltd.) paste diluted with distilled water to a 1 in 5 solution. The injection was made with a fine glass micropipette into the third aortic arch of one side in stages 45–59, and into the

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atrium in stages 60–66. The aortic arches of the later stages were too thick to be pierced by a glass micropipette. In order to ensure complete injection the dye was introduced slowly into the blood-stream and allowed to mix thoroughly with the circulating blood while the heart was still pumping. As soon as the heart ceased to function the injection was stopped. It was not found possible to perform reliable complete injections at stages earlier than stage 45.

After injection the whole tadpole was fixed for 24 hours in an aqueous solution of 5 per cent. formalin and 0·5 per cent. glacial acetic acid. The spinal cord, together with the structures immediately surrounding it, was dissected out and dehydrated in ascending grades of alcohol, cleared in benzene, and embedded in paraffin wax. Some were bulk-stained with neutral red and others with alcoholic eosin. Serial sections were cut either transversely at a thickness of 100 μ, or sagitally and coronally at a thickness of 50 μ. For stages 45–53 a series in each of the three planes was cut for each stage and additional series were cut as follows: two transversely at stage 46; two transversely, one coronally, and one sagittally at stage 47; and five transversely at stage 49. For stages 54–66 only one transverse series at each stage was cut. The sections were dewaxed in xylol and mounted in balsam.

Sections prepared in this way allowed the vascular pattern to be examined in three dimensions with a stereoscopic dissection microscope. It was found that the particles of Monastral fast blue started to form long needle-shaped crystals about 3 months after mounting.

Monastral fast blue was injected into the atrium of one tadpole at stage 54 until the heart ceased pumping, and then a 1 in 5 solution of Monastral fast green (G.V.S., Imperial Chemical Industries Ltd.) paste in distilled water was injected into the 3rd aortic arch until it filled the systemic arteries. The animal was fixed and the central nervous system dissected out as before and bulk-stained with alcoholic borax carmine. After embedding in paraffin wax serial transverse sections were cut at a thickness of 25 μ. In this preparation arteries were filled with a green mass, veins with a blue mass, and the cells were stained red.

The direction of blood-flow in large vessels was clearly visible with the aid of a dissection microscope in the anaesthetized tadpoles at the earlier stages studied, and observations made in this way are included in the description of the findings.

QUANTITATIVE METHODS

For the purpose of this work the shape of a transverse section of the spinal cord was regarded as an ellipse and the central canal was ignored. The volume of cord segment in which the counts were made was calculated using the formula vol. $= \pi \frac{v}{2} \frac{t}{2} l$, where v = vertical diameter, t = transverse diameter, and l = length of cord.

Examination of the sections indicated that the vascular network increased in complexity by the addition of new capillary segments to those already present

by dichotomous divisions and junctions. If a segment is defined as the unbranched length of vessel between two points of division or junction, the number of segments is an index of the stage of development of the network. It must be emphasized that it is not an estimate of vascularity, i.e. of the length of vessel per unit volume. Attempts to count the number of vascular segments showed that it was easier to count the number of divisions and junctions and then to calculate the number of vascular segments using the formula $n = E+2A+B$, where n is the number of vascular segments, A the number of divisions, B the number of junctions, and E the number of entering arterial vessels.

The centrifugal nature of the blood-flow in the spinal cord of Anura allowed the counts to be made in the direction of flow from entering arterial vessels towards the periphery. A division was taken as a point where one vessel becomes two, and a junction as a point where two vessels converge to form one. For vessels running parallel to the long axis of the cord, those passing cranially from a transverse vessel were regarded as leaving a division and those passing caudally from a transverse vessel were regarded as leaving a junction. The formula was derived by arguing that the number of vascular segments in a network is equal to the number of entering arterial vessels, plus twice the number of divisions (each of which gives rise to two vascular segments), plus the number of junctions (each of which forms one vascular segment).

A count of these values was made at every stage from 49 to 66, inclusive, in three consecutive sections between the origins of the 2nd and 3rd segmental nerves, which supply the forelimbs, and at stages 56, 61, and 66 in three consecutive sections between the origins of the 5th and 6th, and 8th and 9th segmental nerves, which supply the trunk and hind limbs respectively. At the same time the mean of the vertical diameters and the mean of the transverse diameters of the spinal cord, measured with a 1-mm.^{2} graticule in the focal plane of the eye-piece, were found. The number of vascular segments in a volume of 1 mm.^{3} of spinal cord was then calculated for each stage of development.

Drawings of the same sections were made at a magnification of $\times 200$ with the aid of an Edinger projection apparatus at every stage from 52 to 66. After checking the accuracy of the drawings the combined lengths of all the vessels and parts of vessels were measured on the drawings with a map-measuring instrument. The length of vessel in mm. in a volume of 1 mm.^{3} of spinal cord was then calculated for each stage of development, and thus an estimate of vascularity was obtained.

OBSERVATIONS

Superficial vessels

At stage 45 branches of dorsal intersegmental arteries pass to the groove formed on each side between notochord and neural tube. On reaching the

groove they divide into cranial and caudal branches which join with the relevant adjacent vessel to form a longitudinal anastomotic vessel. At intervals branches from the longitudinal vessel pass dorsally over the lateral surface of the neural tube until they reach its dorso-lateral region, where in turn they divide into cranial and caudal branches, which join the relevant neighbouring vessel to form a series of arcades. From these arcades vessels pass to the veins of the body-wall. The dorsal direction of the blood-flow in these vessels was seen in the anaesthetized tadpole at this stage.

By the next stage a single median vessel has appeared on the dorsal surface of the cord, into which the vessels of the lateral surface drain. This vessel will be referred to as the 'dorsal spinal vein'. In the anaesthetized tadpole the blood in it was seen to flow cranialwards into the choroid plexus of the 4th ventricle. No direct arterial supply to this plexus was found in this material.

Between stages 46 and 47 a single median vessel is established on the ventral surface of the cord, and this will be referred to as the 'ventral spinal artery' (Plate, figs. A, B). No transitional stages were found to indicate its method of formation. Large vessels from the dorsal intersegmental arteries to the ventral spinal artery accompany the first segmental nerves of both sides and the 4th segmental nerve of one side; the vessels accompanying the other spinal nerves are small. The dorsal spinal vein at stage 47 has established connexions, which accompany the 5th and 10th spinal nerves, to the body-wall veins. During this stage a vessel, which joins the plexus on the ectomeninx covering the saccus endolymphaticus, develops closely applied to the meningeal surface of the ectomeninx over the first four segments of the spinal cord. In the anaesthetized tadpole the blood in this vessel was seen to flow cranially into the plexus over the saccus. This vessel will be referred to as the 'dorsal dural vein'.

By stage 49 the large vessels on the surface of the spinal cord have acquired the pattern found in the adult. The ventral spinal artery is situated in a shallow ventral median fissure and receives large arteries which accompany the first spinal nerve on both sides, and yet another large artery which accompanies the 5th or 6th spinal nerve on one side.

From the ventral spinal artery the blood is conducted over the lateral surface of the cord through a wide-meshed plexus to the dorsal spinal vein.

The dorsal dural vein (Plate, fig. F) extends over the entire trunk region of the cord and receives blood from the dorsal spinal vein, and from the plexus on the surface of the cord by vessels arising from the region in which the rootlets of the dorsal roots enter the cord. At this stage the saccus endolymphaticus is not situated over the spinal cord.

Between stages 54 and 58 the saccus on each side extends caudally as far as the 4th spinal nerve. The dorsal dural vein is not separated from the dura as the sacci extend alongside it (Plate, fig. G). The vessels from the spinal cord to the dorsal dural vein pass ventrally to the sacci. The specimen at stage 54 injected with two different colours showed that at this stage the plexus on the lateral

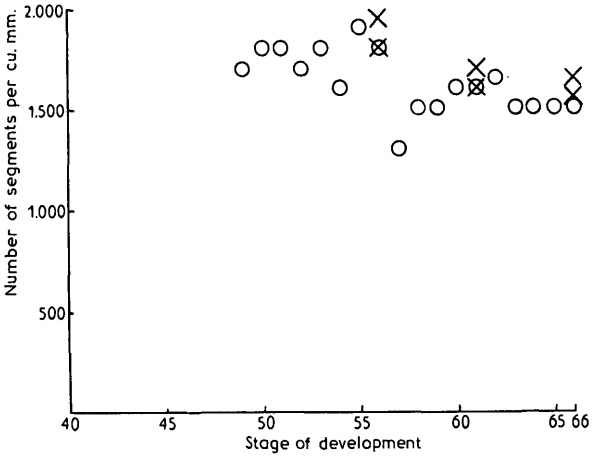
surface receives blood only from vessels within the cord and none from the ventral spinal artery, there being no other arterial supply to the spinal cord.

Vessels inside the spinal cord

The first vessels injected in the substance of the spinal cord were found in one of five specimens at stage 46, in five of seven specimens at stage 47, and in all specimens at later stages. They arise from the ventral spinal artery and enter the cord just lateral to the midline (Plate, fig. E). Then they pass dorsally to the plane between the ependymal and mantle layers at the level of the ventral limit of the spinal canal. In this situation they run cranially or caudally for a short distance before passing round the canal in the same plane to leave the dorsal surface in the midline. The first vessels are not paired, and the vascular pattern within the cord is asymmetrical.

At stage 48 a series of such vessels is present in the trunk region of the cord, and parts of a longitudinal anastomotic vessel have developed at the level of the ventral extremity of the spinal canal (Plate, fig. D). The longitudinal anastomotic vessel extends along the whole trunk region of the cord at stage 49 (Plate, fig. C). The vessels approaching the longitudinal vessel from the ventral surface are at different levels from those leaving it and running to the dorsal surface. The precise vascular pattern within the cord remains asymmetrical and inconstant from segment to segment and from specimen to specimen.

In the following stages new vessels appear which either run from existing vessels to those on the surface of the cord or run from one existing vessel to another. It is not possible to discern any individual vessels that are constant in position and time of appearance. Their only constant features are that branching and joining are dichotomous, and the vascular tree thus formed has

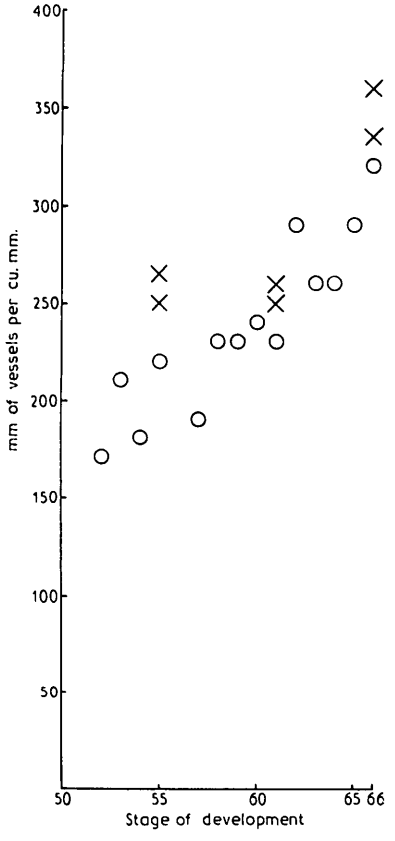


TEXT-FIG. 1. Graph showing the number of vascular segments per mm.^{3} in the developing spinal cord of *X. laevis* at each stage of development. ○, level of 2nd–3rd segmental nerves; ×, levels of 5th–6th and 8th–9th segmental nerves.

its trunk at the ventral median fissure, while its terminal branches leave the cord surface on all aspects to join the superficial plexus.

Quantitative observations

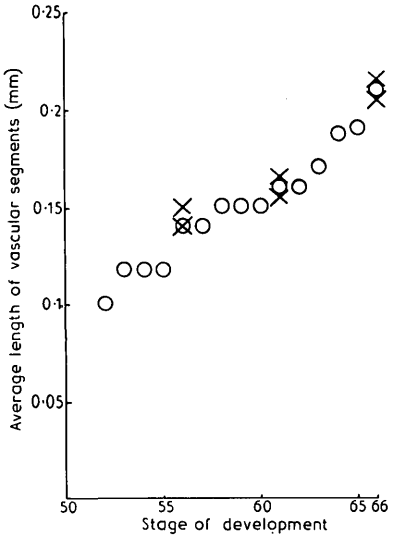
The number of vascular segments per mm.^{3} of spinal cord between the 2nd and 3rd segmental nerves at each stage of development is illustrated in the graph of Text-fig. 1. The points on the graph are scattered, but the number of vascular segments per mm.^{3} is slightly less for the later stages than for the earlier stages of development. The two additional estimates for each of stages 56, 61, and 66 are within 15 per cent. of the estimates for the level between the 2nd and 3rd segmental nerves.



TEXT-FIG. 2. Graph showing the combined length of vessels in mm. per mm.^{3} in the developing spinal cord of *X. laevis* at each stage of development. O, level of 2nd–3rd segmental nerves; ×, levels of 5th–6th and 8th–9th segmental nerves.

The length of vessel per mm.^{3} of spinal cord at the level between the 2nd and 3rd segmental nerves for each stage of development from stage 52 to 66 is shown on the graph of Text-fig. 2. The values for stages 49–51 were not measured, as the volume of spinal cord involved was so small. Again the points are scattered, but there is a definite increase in vascularity as development proceeds. The two additional measurements at each of stages 56, 61, and 66 are within 15 per cent. of the measurements for the level between the 2nd and 3rd segmental nerves.

Inspection of these two graphs suggested that the average length of the vascular segments increases during development. The average length of the vascular segments was calculated by dividing the length of vessel per mm.^{3} by the number of vascular segments per mm.^{3} The results obtained are presented in the graph of Text-fig. 3. There is a gradual increase in the average length of the vascular segments, as development proceeds, to a value at stage 66 which is double that at stage 52. The amount of scatter of the points in the graph is less than in the previous graphs. The average length at any given stage is greater than the average length at any earlier stage and smaller than at any later stage. The values for the additional levels at each of stages 56, 61, and 66 are within 10 per cent. of the values for the level between the 2nd and 3rd segmental nerves.



TEXT-FIG. 3. Graph showing the average lengths of the vascular segments in mm. in the developing spinal cord of *X. laevis* at each stage of development. O, level of 2nd–3rd segmental nerves; ×, levels of 5th–6th and 8th–9th segmental nerves.

DISCUSSION

The development in *Xenopus* of a pair of longitudinal vessels in the groove between the spinal cord and notochord, followed later by that of a single ventral spinal artery, resembles that described for *Rana esculenta* by Sterzi (1904). He found, however, that the dorsal spinal vein of *R. esculenta* became separated from the cord as the meninges develop, whereas in *Xenopus* the dorsal spinal vein is never separated from the cord and the dorsal dural vein appears as a separate entity growing caudally from the plexus over the saccus endolymphaticus.

The site at which the first vessel penetrates the cord of *Xenopus* and its course between ependymal and mantle layers resembles that for all other vertebrates, the Urodela excepted, as Sterzi (1904) emphasized. Its exit from the dorsal rather than the lateral surface may be associated with the relatively early development of a plexus over the dorsal surface of the cord.

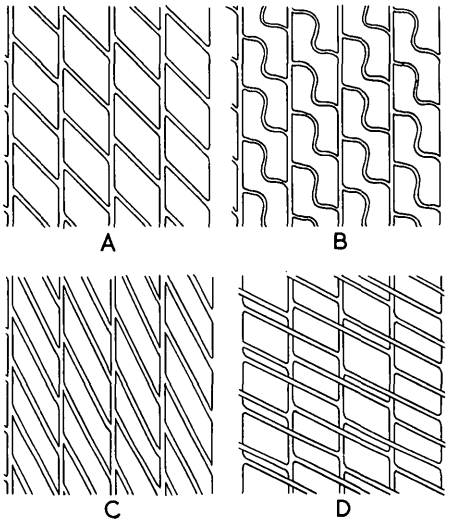
In *R. esculenta* Sterzi (1904) found two paired longitudinal anastomotic vessels, one at the level of the ventral limit and one at the level of the dorsal limit of the ependyma, while in *Xenopus* only one paired ventral longitudinal vessel was found. Feeney & Watterson (1946) found a similar anastomosis in the chick and suggested that there may be something peculiar about this part of the cord as it is so constantly associated with such a vessel. It is suggested

here that the plane between the ependymal and mantle layers is peculiar in being the region farthest away from the superficial vascular network.

The absence of individual vessels that are constant in position and time of appearance contrasts with the observations of Feeney & Watterson (1946) on the chick embryo. The differences in the external and internal environment and mode of life of the two creatures are so great that this contrast cannot be regarded as significant.

Discussion of quantitative observations

The observation that during development the degree of vascularity (in terms of length of vessel per unit volume of spinal cord) is increased while the number of vascular segments per mm.^{3} remains almost constant, leads to the finding that the average length of the vascular segments is increased. It is possible that this increase in average length occurred in one of three ways, or as the result of



TEXT-FIG. 4. Diagram showing three ways in which the vascularity of the spinal cord of *X. laevis* might be increased if the number of vascular segments per unit volume is constant and the average length of the vascular segments is increased. A, primary condition. B, vascularity increased by tortuosity. C, vascularity increased by decreasing the angle of divergence from parent stem with no increase in tortuosity. D, vascularity increased by passing other vessels with no increase in tortuosity.

the combination of several. If the capillary network is arranged so that any given vascular segment branches from one vessel and runs to join a neighbouring vessel without passing another vessel, then its length may be increased by increasing its tortuosity as in Text-fig. 4 A, B, or by decreasing the angle at which

it diverges from its parent stem (Text-fig. 4 A, C). Alternatively, if the capillary network is arranged so that any given vascular segment may arise from one vessel and pass other vessels to join a third vessel, then the increase in average length can occur without increasing the tortuosity of the vascular segment or decreasing the angle at which it diverges from its parent stem (Text-fig. 4 A, D).

On re-examination of the sections it was found impossible to assess whether increased tortuosity or decreased angle of divergence occurred. However, it was found that vascular segments which passed other vessels without joining them made their appearance at stage 55 and were present in all subsequent stages.

The increase in vascularity, in terms of length of vessel per unit volume of spinal cord, took place during the period of development in which the primary motor and sensory systems of the cord are replaced by their adult equivalent (Nieuwkoop & Faber, 1956; Hughes, 1957; Hughes & Tschumi, 1958; Hughes, 1959). A similar increase in vascularity has been shown to occur during the period in which the cerebral cortex differentiates in mammals by Craigie (1925), Petren (1938), and Gyllensten (1959).

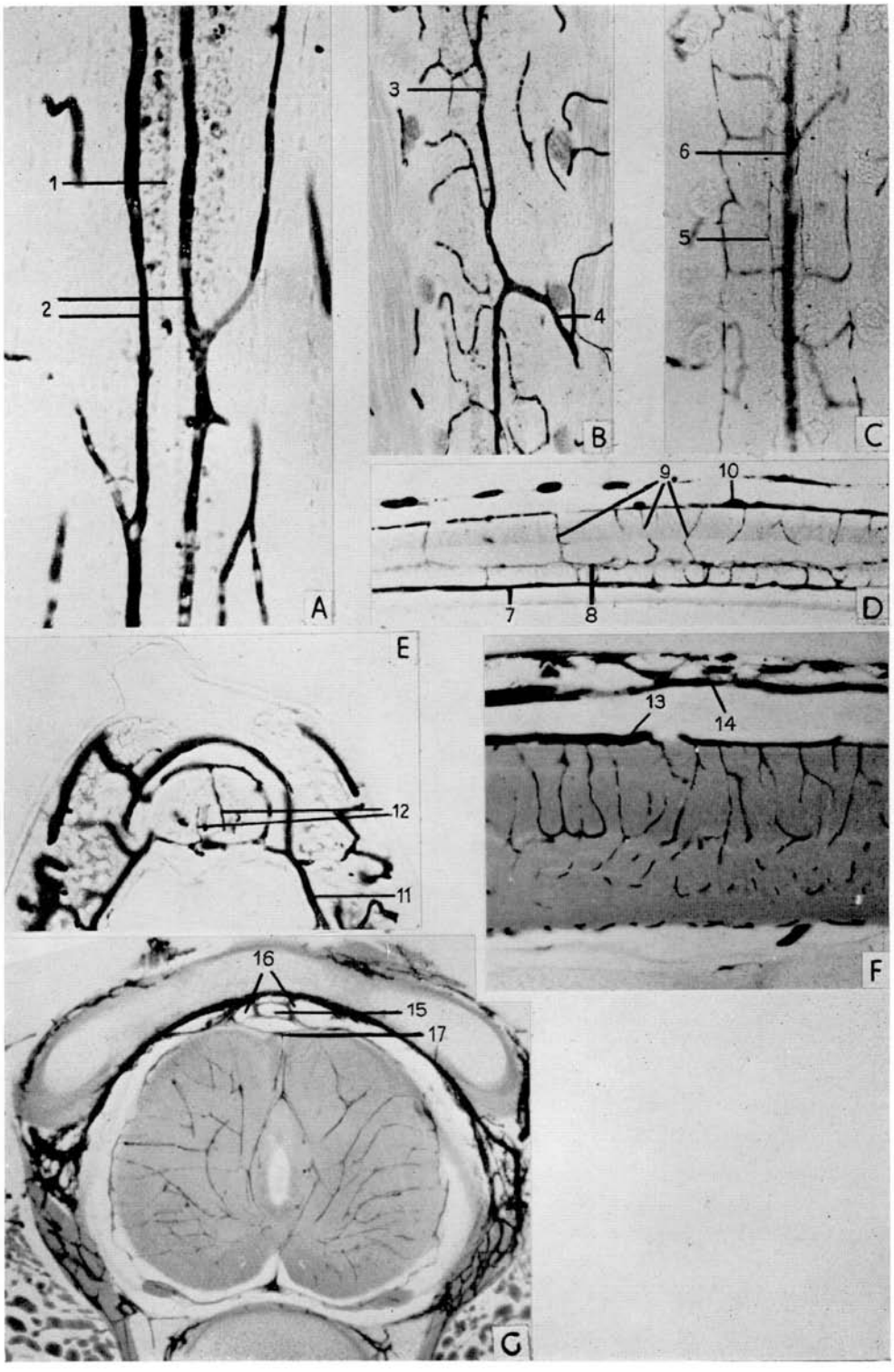
SUMMARY

The blood-vessels of the developing spinal cord of *X. laevis* have been studied from stage 45 to stage 66 by the injection technique. Within the substance of the cord the first vessels injected were found at stage 46, and individual vessels were not constant in position or time of appearance. The vascular network is formed by dichotomous branching and joining of vessels. Estimates of the number of vascular segments (i.e. between branches) per mm.^{3} of spinal cord and the total length of vessel per mm.^{3} showed that the latter increased during development while the former did not. It is concluded that the vascularity of the spinal cord increases as differentiation of the cord proceeds and is brought about by increasing the average length of the vascular segments.

RÉSUMÉ

Les vaisseaux sanguins de la moelle épinière au cours du développement de Xenopus laevis

Les vaisseaux sanguins de la moelle épinière de *Xenopus laevis* en voie de développement ont été étudiés entre les stades 45 et 66 par la technique des injections. A l'intérieur de la substance de la moelle, les premiers vaisseaux injectés ont été trouvés au stade 46; les vaisseaux individuels ne sont constants ni par leur position ni par leur ordre d'apparition. Le réseau vasculaire est formé par des branches dichotomes et des anastomoses. L'estimation du nombre de segments vasculaires (c'est-à-dire entre les branches) par mm.^{3} de moelle et de la longueur totale des vaisseaux par mm.^{3} montre que celle-ci augmente pendant le développement, mais non celui-là. On en conclut que la vascularisation de la moelle augmente en même temps que la différenciation progresse et qu'elle est causée par l'augmentation de la longueur moyenne des segments vasculaires.



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EXPLANATION OF PLATE

FIG. A. Coronal section of tadpole of *X. laevis* at stage 46 showing spinal cord (1) and paired longitudinal anastomotic vessels (2) in groove between spinal cord and notochord. ×250.

FIG. B. Coronal section of tadpole of *X. laevis* at stage 47 showing the ventral spinal artery (3) and branch from the dorsal intersegmental artery (4). ×80.

FIG. C. Coronal section of tadpole of *X. laevis* at stage 49 showing paired longitudinal anastomotic vessels (5) within walls of spinal cord and ventral spinal artery (6) out of focus. ×80.

FIG. D. Sagittal section of tadpole of *X. laevis* at stage 48 showing ventral spinal artery (7), paired longitudinal anastomotic vessels (8) within walls of spinal cord (one is out of focus), and the variable course of the first vessels to enter the cord (9) passing round the central canal to the dorsal spinal vein (10). ×80.

FIG. E. Transverse section of tadpole of *X. laevis* at stage 46 showing dorsal intersegmental arteries (11) passing round notochord and giving branch to superficial vessels of spinal cord from which the first vessels to penetrate the spinal cord (12) arise. ×150.

FIG. F. Slightly oblique sagittal section of *X. laevis* at stage 54 showing the dorsal spinal vein (13) and dorsal dural vein (14). ×90.

FIG. G. Transverse section of *X. laevis* at stage 60 showing dorsal dural vein (15) flanked by sacci endolymphatici (16) with the dorsal spinal vein (17) below it. ×45.

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