

THE UNUSUAL MODE OF DEVELOPMENT OF THE BLOOD VESSELS OF THE OPOSSUM'S BRAIN

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FIVE PLATES (SEVEN FIGURES)

In 1937 Wislocki and Campbell demonstrated an unusual manner of vascularization of the brain of the adult opossum. It was shown that the opossum's central nervous system is supplied by blood vessels which enter the brain in pairs, consisting of an artery and vein. These vessels branch in pairs down to and including the capillaries which consist of thread-like terminal loops. Consequently the brain of the opossum is supplied by distinct and separate vascular units, which do not anastomose with one another. These units consist of end arteries in the sense of Cohnheim with the additional feature that the terminal capillaries, instead of anastomosing with one another in a capillary net, are also end vessels. Although the terminal looped capillaries never anastomose, it was observed that the capillary loops of contiguous vascular trees interdigitate freely. These observations were confirmed by Scharrer in 1938.

This peculiar configuration of the blood vessels in the brain of the opossum raises the interesting question as to the manner in which these vascular structures arise embryologically. The present study represents an attempt to investigate this subject. A brief summary of the main findings in this investigation appeared in the Proceedings of the Association for Research in Nervous and Mental Disease (Wislocki, '37).

MATERIAL AND METHOD

For the elucidation of the problem, the vascular system of several pouch young opossums was injected through the heart with a dilute suspension of Higgins' waterproof India ink. For the youngest of the injected specimens, which measured 8.5 mm. from crown to rump, I am indebted to Dr. Chester Heuser of the Carnegie Department of Embryology.

After fixation in 10% formalin, the injected opossums were embedded in paraffin and cut into sagittal sections of 200μ which were cleared. These sections give excellent views of the developing blood vessels in the central nervous system. Several injected embryos were sectioned at 10 and 20μ and stained in various ways. The thin sections yielded disappointingly little information compared to the thick, transparent ones. It is chiefly the latter which form the basis of the following observations.

DESCRIPTION OF MATERIAL

The results of this study are shown in a series of photographs and drawings. Figure 1 illustrates the topography of the injected central nervous system of the 8.5-mm. opossum. The blood vessels are in process of vascularizing the brain. Thus far only the spinal cord and the floor of the medulla and midbrain have been penetrated by vessels, whereas the entire forebrain is vascularized solely by a surface plexus located in the pia mater. The vessels which penetrate the brain all arise from the pial plexus on the ventral surface of the medulla and cord. Figure 5 illustrates the degree of penetration of the cord and medulla in a 14-mm. opossum. In the roof of the mesencephalon at this stage the formation of vascular loops is just beginning.

It is clear from examination of figures 3, 4 and 6 that during the formative period the blood vessels are typically paired, constituting individual loops or very simple branching trees which do not anastomose with neighboring ones.

Thus it is evident that the peculiar vascular units of the opossum's brain do not arise by the reduction or simplification of a primary anastomosing capillary net. From the time of their first appearance the vessels supplying the brain consist of simple, non-anastomotic loops.

In the present material cerebral vessels can be observed ranging from a simple loop, such as is shown in figure 6, b, to more complex trees as seen in figure 6, a, c, d and e. These structures composed of paired capillary threads take origin at this period from the pial plexus on the ventral surface of the brain and cord. The central nervous system is surrounded by a network of pial vessels, but, by the time the capillaries penetrating the brain arise, the pial plexus is beginning on the ventral surface of the cord to become resolved into a plexus possessing distinct afferent and efferent capillaries. It seems clear upon examination of the paired threads of the capillaries penetrating the brain that they do not connect with adjacent points on one and the same pial capillary but arise, as is shown in figure 6, b, c and d, from two separate and distinct pial vessels, one of which is arterial and the other venous.

How the paired threads which form loops in the brain substance actually grow is difficult to demonstrate. In view of the evidence regarding the connections of the threads with separate capillaries in the pial plexus, the growth of the loops cannot be ascribed to a simple buckling of any single pial capillary which is consequently pushed into the brain substance. It seems more reasonable to assume that the paired threads arise as two independent outgrowths of angioblasts from neighboring pial capillaries. These parallel solid sprouts apparently invade the brain, extending from the pia where they arise through the entire thickness of the neural tube to terminate in the vicinity of the neural canal, where the tips of the two cords of angioblast presumably finally undergo fusion with one another. Subsequent canalization of the angioblastic strands throughout their lengths

would account for the establishment of the circulation through the completed capillary loop.

This concept of the manner of growth of the primary loops is supported by the observation cited above that each capillary limb of one of the loops connects with a different pial capillary. It is also reinforced by the fact that in some instances the loops extend through the entire thickness of the medullary tube as unbranched threads parallel to one another, as can be seen in figures 2 and 5. Simple loops of this type are regarded as the primary stage preceding the formation of secondary branches.

The manner of formation of the secondary loops is also an interesting problem. It is our belief that these originate in much the same way as we have postulated for the primary loops. The secondary branchings do not appear to occur in the main as dichotomous divisions of the tips of the primary loops, but take origin for the most part at intermediate points between the root and the terminal end of a primary loop. Since the simple primary loops appear to follow in the main a straight course through the entire thickness of the wall of the neural tube, the formation of secondary loops leads advantageously to supplying the growing intervening brain tissue with blood vessels. In figure 6 a number of typical branching capillaries in the spinal cord of the 14-mm. stage are presented. It will be observed that, at the points of branching, both of the capillary limbs divide so that a component of each passes into the secondary loop. This is typical of all of the true branchings seen in the opossum's brain. In view of the foregoing considerations we ascribe the growth of the secondary branches to the formation of angioblastic sprouts at two contiguous points on the two threads of an intermediate section of the primary vascular loop. These sprouts grow out, we believe, for a certain distance, which is probably determined by the metabolic needs of the surrounding brain tissue, whereupon the tips of the sprouts presumably fuse to form a secondary loop. By hollowing out of the angioblastic strands forming

the loop, the blood flow is probably initiated. The only direct evidence for this concept of the manner of the formation of the secondary loops is derived from consideration of the structures shown in figure 7. Along the course of established loops one encounters now and then small buds, double in nature, which are connected to the two limbs of a pre-existing loop in the manner shown in figure 7. These structures are interpreted as being angioblastic sprouts arising from contiguous points on a parent loop and destined to form secondary loops in the way outlined above. It is possible, of course, that this appearance may simply be due to the presence of loops which have failed to become filled with ink as a result of incomplete injection. In view of the apparent completeness of the injections in the material before us, we regard this explanation as unlikely. Consequently it seems justifiable to regard the structures showing this appearance as being angiogenic sprouts. Williams ('37) advances the hypothetical view, and cites evidence for its support, that vessels penetrating the brain are attracted to regions where the cells are dividing rapidly and consequently the metabolism is elevated.

In conclusion a minor point deserves to be mentioned. It is clear from study of the adult, as well as the present material, that the vessels penetrating the opossum's brain are independent vascular units. Nevertheless, there are occasional exceptions to this rule, for in studying several hundred capillary loops at the 14-mm. stage, three places were encountered where anastomoses exist between contiguous vascular units. In two places, one of which is shown in figure 6 e at the site of the arrow, a capillary has been seen extending across from one vascular unit to a neighboring one. In another place a situation was encountered where double capillary threads connected two units. These findings are, however, extremely exceptional.

Finally, it should be pointed out that the terminal capillary loops described here in the opossum brain do not occur in any other organs and tissues. It is clear from examination of a number of young opossums, serially cut, that the de-

veloping vascular beds of all other parts of the body, including the pial capillaries enveloping the brain, are of the usual plexiform or network type.

DISCUSSION

The generally accepted view of the development of blood vessels attributes the formation of almost all vessels to a primordial net (Müller, '03, '04; Evans, '09, '12; Sabin, '17; Woollard, '22). Woollard ('22) summarizes the prevailing concept by concluding that the primordium of the vascular system lies in the vascular net, that the character of the primitive plexuses depends upon the inherent properties of certain cells to form blood vessels, and that by reduction of the primitive net particular paths become established, in accordance with the postulates of Thoma.

It is clear from examination of the present material that the intracerebral vessels of the opossum's brain do not conform to the above concept. They arise instead as individual offshoots or sprouts from the pial plexus which subsequently maintain their individuality and never anastomose to form a vascular net. The intracerebral vessels of the opossum constitute a clear exception to the theory of the development of all vascular beds as initial nets from which the afferent and efferent trunks are ultimately derived by a reduction of the vascular plexus.

This nonconformity of the opossum's intracerebral vessels to the mode of development of blood vessels in general is difficult to understand. Streeter ('18, '27) has enunciated the view that of all the structures of the body the vascular system is one of the most adaptive. Endothelium is highly responsive to its environment. For example, in studying the development of the blood vessels of the human brain, Streeter observes that the vascular apparatus is continuously adequate and complete for the growing brain as it exists at any particular stage. Furthermore, as the environmental structures progressively change, the vascular apparatus also changes and thereby is always adapted to the new conditions. In

short, he concludes that embryonic blood vessels have no ground plan of their own, independent of the structures around them.

Streeter's hypothesis is admirably suited to cover the situation encountered in the opossum. Here one must assume that the chemical or physical constitution of the opossum brain imposes peculiar conditions upon the labile angioblastic tissue, resulting in the formation of terminal vascular loops instead of an endothelial network. Thus the formation of the nonanastomotic vascular pattern appears to be an adaptive one dependent on unknown but peculiar factors resident in the brain substance of the opossum. This explanation is materially supported by the observation that in all of the other tissues of the young opossum the capillary beds grow as ordinary networks. Consequently the mode of growth by sprouting and subsequent loop formation is not an inherent or obligatory characteristic of the angioblastic tissue of the opossum.

In this connection Scharrer ('39) has recently shown that under conditions of regeneration the end arteries of the opossum's brain do not anastomose. Moreover he observed the growth of capillary loops into dead tissue of a brain graft. From the latter observation Scharrer deduces that the formation of loops in the opossum brain is independent of living nerve tissue. Moreover, he concludes that the nervous tissue of the opossum brain does not determine the capillary pattern in its basic principle; this is determined rather, he continues, by the inherent principles of development of the cerebral vascular system itself. There is no reason to doubt the above observation but the conclusion derived from it seems to us to be open to a better interpretation. The evidence cited by Streeter that the growth of vascular beds is determined by the needs of the individual tissues and organs, instead of by an inherent principle in the angioblastic tissue itself, is quite convincing. Consequently we believe that Scharrer's observation is more easily explained by postulating that opossum brain tissue—

whether alive or dead—imposes a special mode of growth on angioblastic tissue which depends upon some as yet unrecognized structural arrangement or chemical organization of the opossum's brain.

The evidence adduced in the present study regarding the growth of the intracerebral blood vessels in the opossum indicates that the blood vessels invade or penetrate the neural tube from the outside. Vascularization of the neural tube by a process of ingrowth of angioblastic sprouts has been observed in the sheep (Sterzi, '04), pig (Hoskins, '14) and chick (Williams, '37). Solid sprouts are described in the cord of the 5.5-mm. sheep by Sterzi and hollow capillaries by the 6.6-mm. stage; however, Hoskins describes the first vessels in the cord of the pig as being hollow when first seen at the 7.5-mm. stage. Contrary to the above group of investigators, Tilney and Casamajor ('17) describe the blood vessels of the brain of the rat as arising from two independent plexuses, a perineural and an entoneural one. The entoneural plexus, according to them, arises, in situ, by local transformation of mesenchymal cells, and only later establishes communications with the perineural plexus on the surface of the brain leading to the establishment of the actual circulation. If their observations are correct, the manner of vascularization of the rat's brain differs from that observed in sheep, pig, chick and opossum.

Resembling the opossum, Sterzi states that in the sheep each dorsal ramus of the primitive arterial tract initially grows into the cord and forms a loop giving rise to a vein which grows back along the artery. Hoskins, however, cannot confirm this for the pig in which the dorsal rami give rise to an undifferentiated capillary plexus in which differentiation into arteries and veins only takes place later.

Whether the result be the formation of individual loops as in the opossum, or a capillary net as in the majority of animals, the development of either type appears to be initiated by the ingrowth of angioblastic sprouts from the pial plexus.

Yet these sprouts appear to be definitely paired in the opossum whereas they are irregularly spaced in the chick (Williams, pl. 2); moreover, in the opossum the paired sprouts remain permanently associated producing individual terminal loops, whereas in the chick and pig the various sprouts anastomose immediately forming a capillary net.

SUMMARY

The intracerebral capillaries of the opossum's brain apparently arise as paired outgrowths from the periaxial (pial) vascular plexus. These paired sprouts unite at their tips creating individual capillary loops which do not anastomose with other loops. Consequently the capillaries of the brain of the opossum are end-capillaries from the time of inception and are not formed as the result of the reduction of a capillary net. The secondary capillary loops in the opossum's brain appear to arise similarly as paired sprouts from the primary loops.

According to the prevailing concept, all vascular beds hitherto investigated develop from an embryonic capillary net in which by gradual remodeling and a variable degree of reduction of the primordial plexus the definitive vascular channels are established. The intracerebral vessels of the opossum develop in an entirely different way; they arise from the pial plexus as independent sprouts which penetrate the brain and create separate branching trees ending in terminal capillary loops which never anastomose with one another.

Evidence is adduced which indicates that the blood vessels of the opossum's brain grow in the manner described as a result of chemical or structural factors inherent in the brain tissue.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Photograph of a 200μ , cleared, sagittal section through the brain of an 8.5-mm. opossum. The blood vessels are filled with India ink. $\times 330$.

2 A part of the floor of the medulla and midbrain of an 8.5-mm. opossum at higher magnification, showing the vascular units traversing the neural tube. $\times 70$.

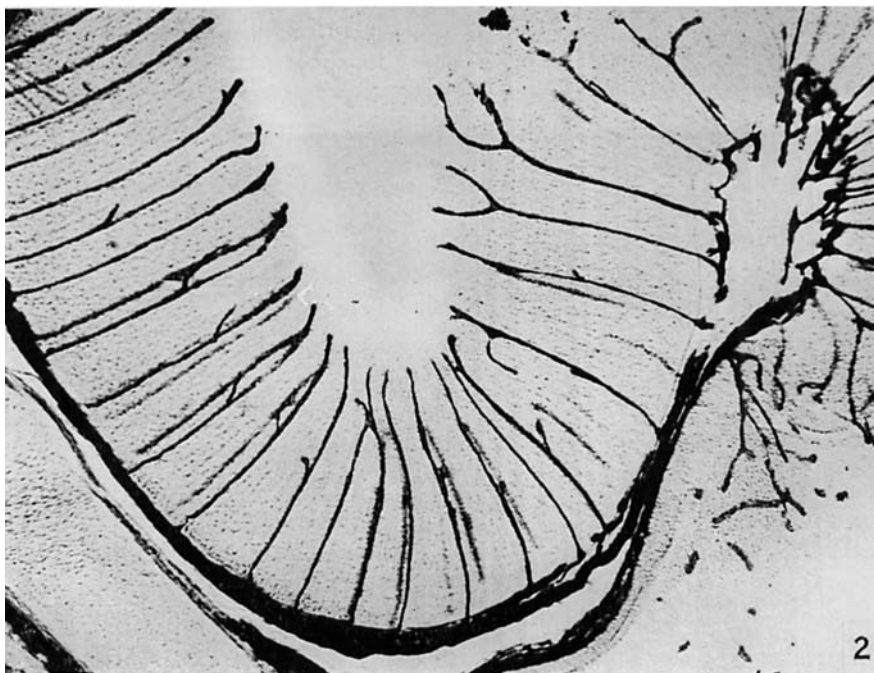
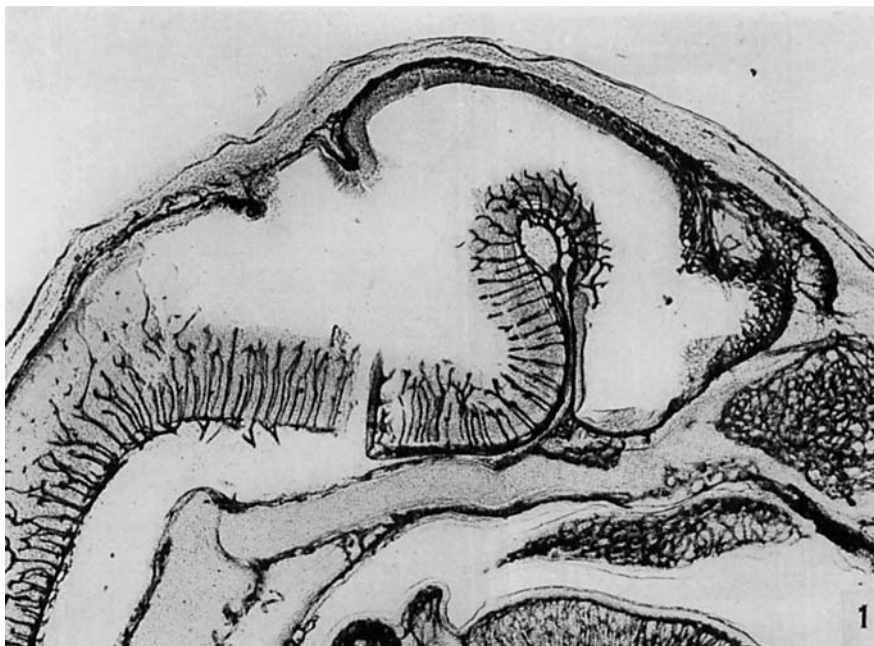
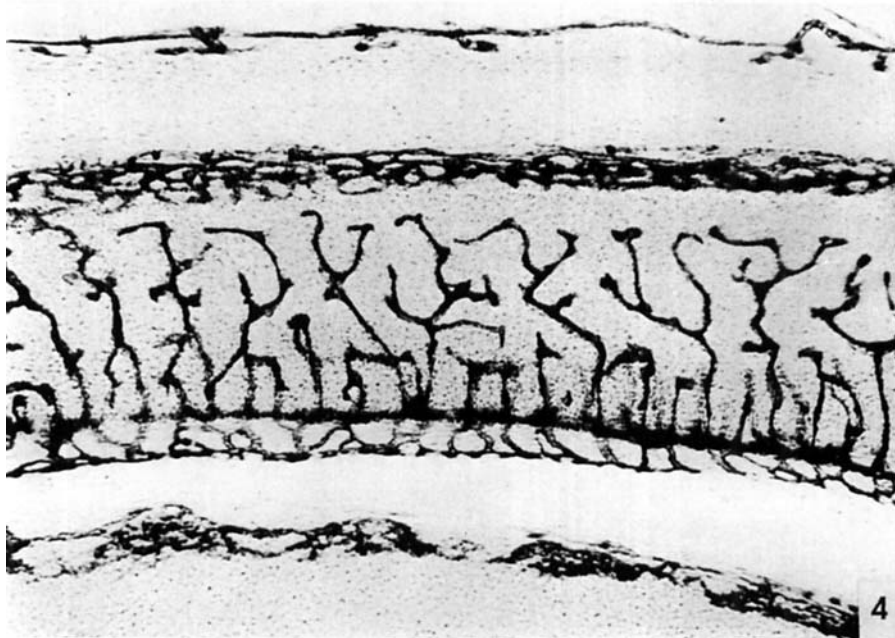
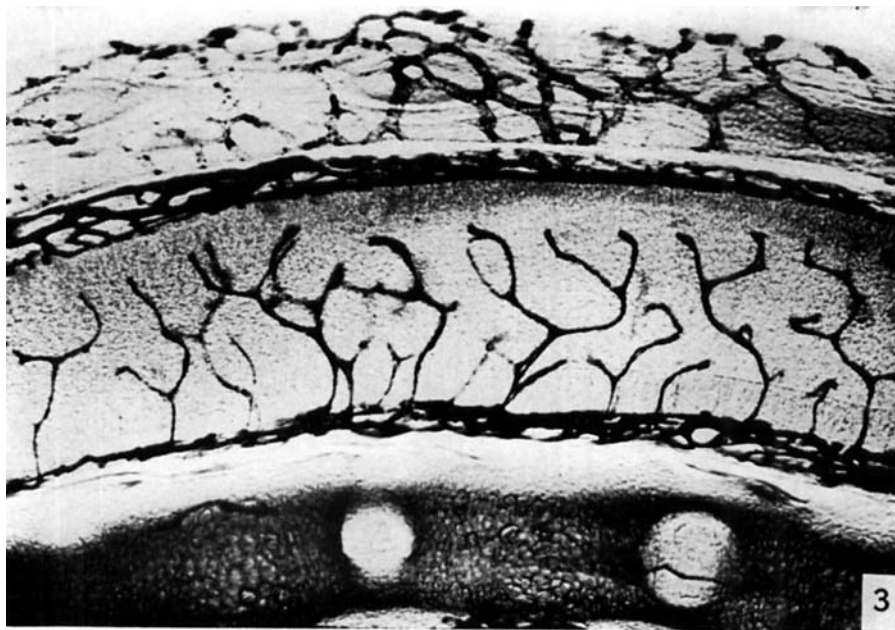


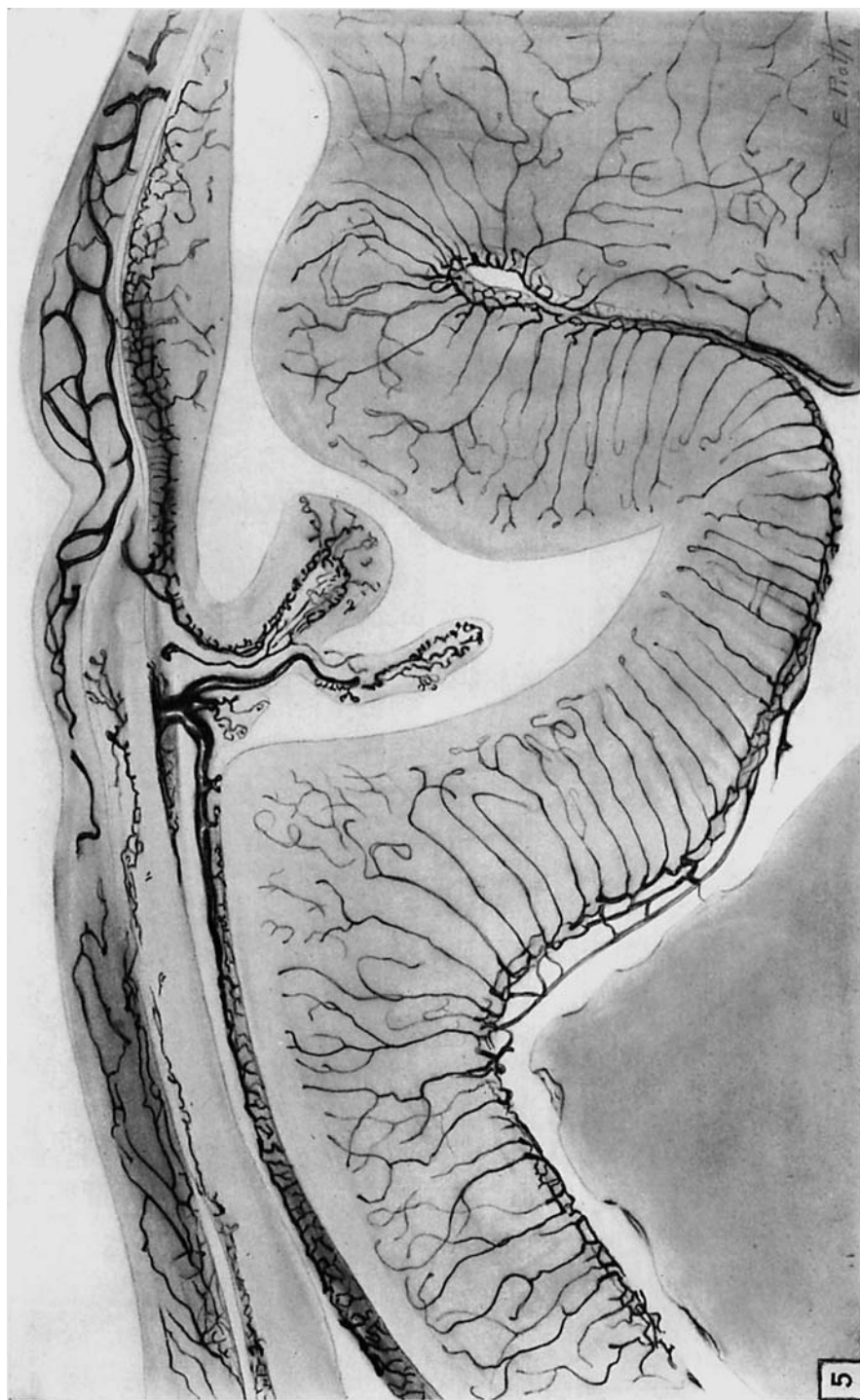
PLATE 2

EXPLANATION OF FIGURES

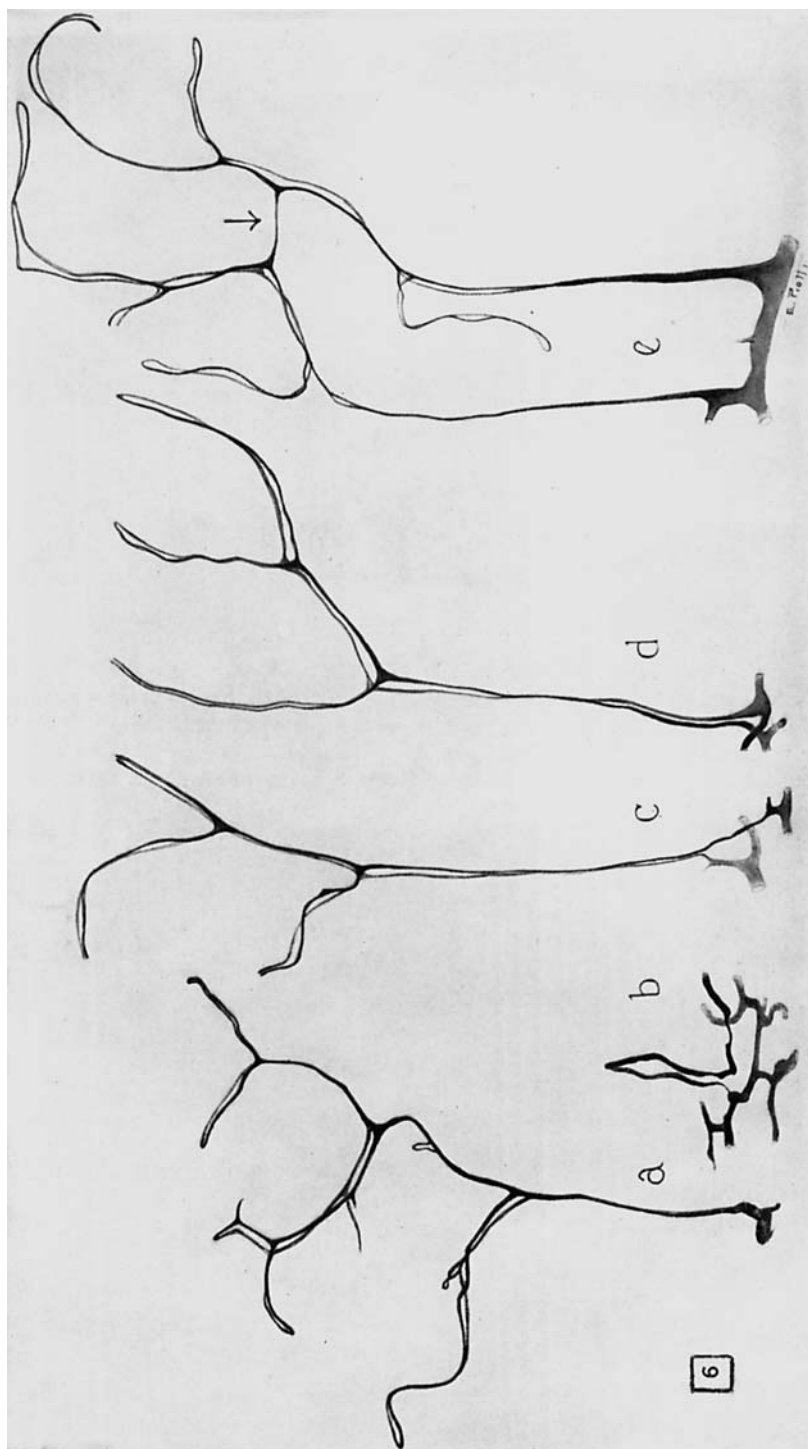
3 A section of the spinal cord of a 14-mm. opossum, showing branching looped capillaries forming small tree-like terminal vascular units. $\times 85$.

4 A section of the spinal cord of an 8.5-mm. opossum, showing the terminal branched looped capillaries. Note, in this and the previous photographs, that although some of the vascular units appear as though they might anastomose with neighboring units, actually the branches are all terminal and do not unite with neighboring ones. $\times 85$.





5 Drawing of the medulla of a 14-mm. opossum, showing the character and extent of the vascular units.



6 Drawings of individual units from the previous sections, showing typical capillary loops. *b*, *c* and *d* show origins of the two limbs of the paired capillaries from separate vessels in the pial plexus. In *a* and *e*, in the region of connection of the vascular units with the pial plexus, the constituent paired capillaries lie beneath one another and consequently appear as one thread. In sketch *c*, at the point indicated by the arrow, is one of the very exceptional places at which a capillary connects two adjacent vascular units.

PLATE 5

EXPLANATION OF FIGURE

7 Drawing 'a' shows a vascular unit in which, at the points indicated by the arrows, it is believed, for reasons given in the text, that angiogenesis of secondary loops is occurring. *b, c, d, e* and *f* show sprouts of the kind indicated in *a*, at higher magnification. These paired buds seen on occasional loops are interpreted as being secondary loops in process of being formed. These sketches were all made from capillaries seen in 200μ cleared sections.

