ARTERIO-VENOUS ANASTOMOSES IN THE TONGUE OF THE DOG

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The only author who has reported the presence of arterio-venous anastomoses in the dog's tongue is Brown (1937). She, however, was chiefly interested in the nervous elements of these structures and no adequate account of their morphological features is available. The object of this paper is to show the characteristic form of these direct communications between the arterial and venous systems of the dog's tongue and the appearance of these vessels in different types of preparation.

METHODS

The tongues of more than thirty dogs obtained post-mortem were examined. Only the oral portion of the organ was studied in any detail. Some tongues were not injected and were used solely for histological examination, but in most instances an injection mass was introduced via the lingual arteries. Attempts to inject the tongue from the venous side were unsuccessful, owing to the presence of large numbers of valves in the lingual veins.

Radio-opaque injection masses. Tongues injected with suspensions of bismuth carbonate or barium sulphate were X-rayed both whole and in thick sections to demonstrate the distribution of the major vessels. In spite of the relatively large particle size of these masses, the veins were always filled to a greater or lesser degree.

Neoprene latex. Casts of the lingual blood vessels were made by injection of neoprene latex. The method used in preparing the casts was essentially similar to that described in Trueta, Barclay, Daniel, Franklin & Prichard (1947, pp. 46–7), but after maceration of the tongue in concentrated HCl the cast was placed in 10 % KOH to remove the fat. The injections of neoprene were made at pressures ranging from 200 to 300 mm. Hg. The casts were dissected under water and examined with a stereoscopic binocular microscope.

Berlin blue. This was used in a 2 % solution in distilled water, and proved to be a very satisfactory injection mass in this organ.

Grant's method. In two tongues the walls of the blood vessels were stained by perfusion with Ehrlich's acid haematoxylin by the method of Grant (1930).

Frozen and/or paraffin sections were made of all tongues, other than those injected with neoprene latex, after fixation in 10 % formol saline.

Frozen sections. Sections ranging from 25 to 1000 μ in thickness were cut on the freezing microtome either in the coronal plane or in the planes of the dorsal or ventral surfaces of the tongue. The thinner sections were lightly stained with Ehrlich's acid haematoxylin or alum carmine, the remainder were cleared and mounted unstained.

Paraffin sections. Blocks were embedded in paraffin and cut in one or other of the planes mentioned above, at thicknesses of from 7 to 15μ. In many instances serial sections were made. The majority of sections were stained with Ehrlich's acid haematoxylin and eosin. This proved to be very satisfactory for demonstrating some of the most striking features of the arterio-venous anastomoses, and we preferred these preparations to those stained with Masson's trichrome method. Some sections were stained by Weigert's elastin method, and counterstained with haematoxylin or saffranin. Other sections were stained by van Gieson's method.

For the purpose of demonstrating the general morphology of the arterio-venous anastomoses and their related vessels the use of several injection methods has proved extremely valuable since each method has its own advantages. In neoprene casts, for example, the continuity of the anastomotic channels can be established beyond all doubt by dissection and manipulation under the dissecting microscope. Moreover, any irregularities in the shape of the vessels are faithfully represented in the cast. In thick frozen sections of tongues injected with other masses the communicating channels may be obscured either by overlying vessels or because the plane of section is unsuitable. However, it has been possible to identify with certainty many arterio-venous anastomoses in this type of material, and the fact that other portions of the same tongues could be used for histological examination has proved of great value.

The distinction between artery and vein has presented no difficulty, as each of these vessels has its own characteristic features in the types of preparation studied.

RESULTS

Definition and use of term 'arterio-venous anastomosis'

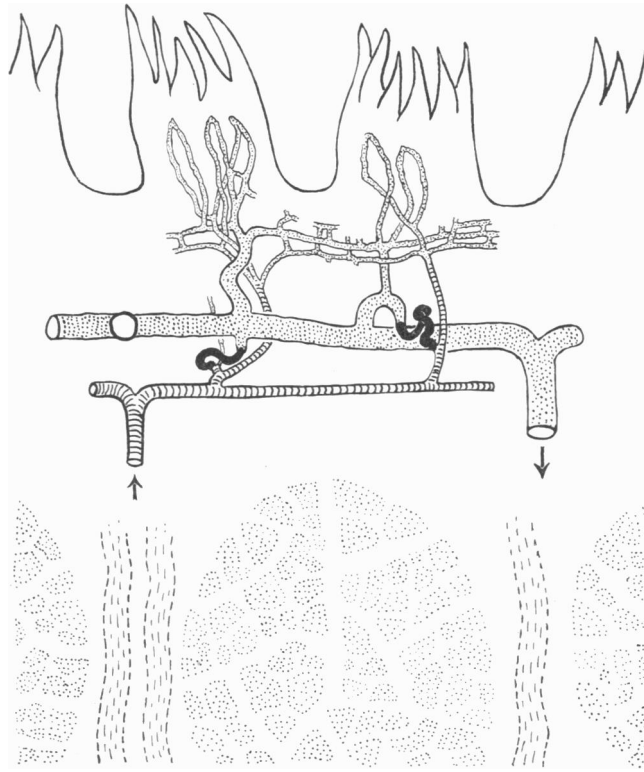
In describing arterio-venous anastomoses one is faced with a problem of terminology. In general terms an arterio-venous anastomosis can be considered as a channel which permits blood to pass from the arterial to the venous system without traversing a capillary bed. In the tongue, as in certain other organs, the vessel which forms the anastomotic channel can be considered as being composed of three parts, an arterial portion, a venous portion, and a junctional segment. This concept of an arterio-venous anastomosis has arguments in its favour, but the use of these terms presents some difficulty. Since the arterial and venous segments of the anastomosis show no morphological features apart from their continuity with the junctional segment which distinguish them from other small arteries and veins, the determination of the points of origin and termination of the anastomosis must be arbitrary.

Because of this difficulty we have preferred to apply the term arterio-venous anastomosis to the 'junctional segment' alone. This is more convenient and it has this merit, that the term arterio-venous anastomosis is confined to the only segment of the communicating vessel which in virtue of its distinctive features can be identified histologically as such.

Situation of the arterio-venous anastomoses

The intrinsic muscles of the tongue are enclosed, as it were, within a capsule of fibrous tissue, the corium, to which they are attached, and by which they are separated from the epithelium. Running through this layer of fibrous tissue, in

a plane parallel to the surface, there are both arteries and veins, the continuations of vessels which, deeper in the tongue, respectively supply and drain the muscles of the organ. The veins lie superficial to the arteries. By the interconnexion of the arteries with one another and by the free anastomosis of the veins, two systems of relatively large vessels are formed in the corium beneath the epithelium, those on the dorsum of the tongue being particularly striking because of their large capacity. The vessels of these arterial and venous systems give off or receive small branches



Text-fig. 1. Diagram to show the site of arterio-venous anastomoses in the corium of the dorsum of the dog's tongue. The papillae are seen above and the more superficial of the intrinsic muscles of the tongue are indicated below. The arterio-venous anastomoses (solid black) form direct communications between arteries (hatched) and veins (the larger stippled vessels) which supply and drain the subepithelial capillary plexus.

which supply or drain the subepithelial capillary plexus (including, on the dorsum, the capillaries of the papillae), and it is as direct communications between these small arteries and veins, or even between their parent vessels, that the arterio-venous anastomoses are to be found (Text-fig. 1). They are more numerous on the dorsal aspect of the tongue than on the ventral aspect. We have not attempted a detailed count of the arterio-venous anastomoses in the dog's tongue, but an indication of their frequency on the dorsal aspect was given by a paraffin section, cut in the plane of the dorsum, which showed parts of ten separate arterio-venous

anastomoses in an area of approximately 0·9 sq.mm. It was of interest to note that elsewhere in the same section an equivalent area was found to contain ten papillae. This finding, taken in conjunction with other observations, suggests the possibility that each papilla may have beneath it an arterio-venous anastomosis.

The anastomotic channels are best seen in their entirety in injected preparations when the vessels of the corium are viewed from the deep aspect. Sometimes a good view can be obtained in tissue cut in the coronal plane. Although arterio-venous anastomoses may occasionally be seen adjacent to, or even partially surrounded by, muscle fibres immediately underlying the corium, we have been unable to find these structures amongst the muscles deeper in the tongue.

Morphological features

To appreciate adequately the picture of an arterio-venous anastomosis and its related vessels as seen in histological sections, it is helpful to have examined preparations which show these structures in their entirety and so be familiar with their general morphological characteristics. For such a study, neoprene casts of the vessels of the tongue (Pl. 1, figs. 1, 2) and thick frozen sections of tongues injected with Berlin blue (Pl. 1, figs. 3, 4) have proved extremely valuable, and it is from this type of material that the following brief observations on the general morphological features of the arterio-venous anastomoses and their related vessels have been derived.

The arterio-venous anastomoses form direct communications between arteries and veins which respectively supply and drain the subepithelial capillary plexus, but the precise level at which the connexion is made is variable both on the arterial and the venous sides.

In injected preparations the arterio-venous anastomosis is most commonly seen as a tortuous vessel connecting a small branch of an artery with a small tributary of a vein. The vessels which supply or drain the capillary bed immediately distal to the anastomosis may or may not be demonstrated by the injection mass. The origin of the arterio-venous anastomosis is usually indicated by an abrupt decrease in the lumen of the channel (Pl. 1, figs. 3, 4), and its termination by an increase in the lumen which may be either sudden (Pl. 1, figs. 1, 2) or gradual (Pl. 1, fig. 3). During its course the arterio-venous anastomosis frequently makes an S-bend (Pl. 1, fig. 2), and occasionally two arterio-venous anastomoses, arising from a single artery and opening into one or two veins, are intertwined and form a complex, knot-like structure. Irregularities in the contours of injection masses filling arterio-venous anastomoses show that the lining of the vessel is frequently far from smooth (Pl. 1, figs. 3, 4).

The diameter of the lumen varies, not only from arterio-venous anastomosis to arterio-venous anastomosis, but also in the course of a single arterio-venous anastomosis. Measurements of neoprene casts of these vessels gave diameters of the order of 30–50 μ, while cleared preparations of tongues injected with Berlin blue showed diameters ranging from about 10 to 30 μ. The lower values obtained in the latter type of material are probably due to the less compact nature of the injection mass and to the fact that this material was subjected to the effects of dehydration. In length the arterio-venous anastomoses were found to range from about 100 to 500 μ, the most frequent length being of the order of 200–300 μ.

Histological characteristics

One of the most striking features of arterio-venous anastomoses in the dog's tongue is the thickness of their walls (Pl. 2, figs. 5, 7 and 8); this exceeds, by two or three times, that of the average small artery. The thick wall of the vessel is due to the large number of cells which compose the media. These cells consist essentially of two types, smooth muscle cells and 'epithelioid' cells, which are present in variable relative numbers and are irregularly disposed in the vessel wall (see Pls. 2 and 3).

The appearance of the cells varies considerably not only from arterio-venous anastomosis to arterio-venous anastomosis but also from tongue to tongue. Some cells can be readily identified as typical smooth muscle cells, and others as epithelioid cells because of their resemblance to epithelial cells; other cells again, however, appear to be modified versions of one or other of these two types. The typical smooth muscle cells appear to be the exact counterparts of the smooth muscle cells found in the media of muscular arteries. The epithelioid cell, when present in its most highly developed form, is seen to contain a large, pallid nucleus, polyhedral or oval in shape, with a fine stippling of chromatin (Pl. 2, figs. 5, 6; Pl. 3, figs. 10–12). A well-marked nucleolus is not often seen. The borders of the cell are ill defined, and the cytoplasm stains rather poorly with acidophilic dyes.

The wall of the arterio-venous anastomosis contains no internal elastic lamina, although a few elastic fibres may be seen scattered amongst the cells of the media. Endothelial cells are present on the inner surface of the vessel wall, but it has not been possible to establish with certainty whether or not these form a continuous lining to the wall.

The lumen of the arterio-venous anastomosis is frequently very small (Pl. 2, fig. 7), and it is generally irregular in outline (Pl. 2, figs. 5–8). This irregularity, which is an aid in the identification of an arterio-venous anastomosis in sections, is caused by cells of the vessel wall projecting into the lumen (Pl. 3, fig. 9). In specimens in which an injection mass has been used, the arterio-venous anastomosis may show traces of the mass penetrating between the cells of the wall.

Naturally, the precise appearance of an arterio-venous anastomosis in a histological section depends to a great extent on the plane of the section in relation to the vessel and on how much of the anastomosis is shown. The section may include only one part of the vessel (Pl. 2, fig. 7; Pl. 3, fig. 12), or it may show two or more segments of a single arterio-venous anastomosis which, owing to its tortuous course, has been cut at more than one level (Pl. 2, figs. 5, 6 and 8; Pl. 3, figs. 9, 10 and 13). One may see the origin of an arterio-venous anastomosis from its artery, or its termination in a vein, but a section of 7 or 10μ very rarely demonstrates an arterio-venous anastomosis in continuity from artery to vein. Much more frequently an arterio-venous anastomosis and its related vessels are seen as a small group of structures cut in various planes (Pl. 2, fig. 6). In such a complex, capillaries may be seen and a small nerve trunk may also be visible (Pl. 3, fig. 10). The arterio-venous anastomosis itself is enveloped in a sheath of adventitia which often demarcates it from the surrounding fibrous tissue (Pl. 3, fig. 10).

In this investigation we have not made a detailed study of the structure of the vessel wall in the regions of transition between the arterio-venous anastomosis and its afferent and efferent vessels.

In conclusion, one further observation should be mentioned. We found that the histological identification of arterio-venous anastomoses in paraffin sections was easier in some tongues than in others. Fixation of the tissue was in no case unduly delayed so it would appear that this finding was not due merely to differences in the degree of post-mortem changes.

DISCUSSION

It is not our intention here to survey the literature on vessels in various organs which have been described as arterio-venous anastomoses. This has been done by several workers, including Clark (1938), Clara (1927, 1939) and Boyd (1939), each of whom has made a personal study of these structures in one or more organs. Among the tissues, however, which contain vessels that can be accepted as unequivocal arterio-venous anastomoses are the rabbit's ear (Hoyer, 1877; Vastarini-Cresi, 1903; Grant, 1930; Schumacher, 1938), and the human finger and toe (Hoyer, 1877; Grosser, 1902; Vastarini-Cresi, 1902, 1903; Grant & Bland, 1931; Popoff, 1934; Masson, 1937). The anastomoses in these organs, although showing variations in points of detail, have certain characteristic hallmarks. The vessels run a more or less tortuous course; they have a very thick wall, the media containing smooth muscle cells and epithelioid cells in large numbers but in variable relative proportions (according to Clara (1927), and Schumacher (1938), some of the anastomoses in the rabbit's ear are relatively straight and have no epithelioid cells in their walls); in fixed preparations the lumen is usually very small; the vessel has no internal elastic lamina. The arterio-venous anastomoses which are present in the dog's tongue show all these features, and it would appear from our own comparison of these vessels with the arterio-venous anastomoses in the rabbit's ear and in the human finger that the structures are essentially similar in each of these organs. In the tongue of the dog, however, the anastomotic channel is as a rule much simpler in form than it is in the human finger, where the complexity of the unit is one of its most striking features.

As in the case of the arterio-venous anastomoses in the human finger the arterio-venous anastomoses in the dog's tongue are situated in the corium beneath the epithelium. Their position and frequency are such as to suggest that these direct arterio-venous communications, by opening or closing, play an important part in regulating the amount of blood which passes to the subepithelial capillary plexus, including, on the dorsal surface, the capillaries of the papillae. Moreover, the presence of these structures in large numbers suggests that they are devices which, when open, permit a great volume of blood to pass through the superficial parts of the tongue without overburdening the superficial capillary bed. The rich nerve supply of the arterio-venous anastomoses (Brown, 1937) indicates that they are under the control of the nervous system.

The behaviour of arterio-venous anastomoses has been studied in the ears of living rabbits by Grant (1930) and Clark & Clark (1934), who found that these vessels not only responded to various forms of stimulus, but also showed spontaneous rhythmic variations in calibre. Schumacher, who made an extensive anatomical study of the coccygeal body (1908), and also investigated arterio-venous anastomoses in certain other organs, suggested (1938) that the opening and closing

of arterio-venous anastomoses which have epithelioid cells is effected by changes in the volume of these cells. He postulated that the lumen was closed by a swelling of the epithelioid cells, due to imbibition of fluid, and that it was opened by a reversal of this process. He also suggested that during their shrinkage the cells might liberate a substance resembling acetylcholine, and that thus the arterio-venous anastomoses would have more than a purely local circulatory effect.

Many theories have been advanced as to the function of arterio-venous anastomoses in general (see, for example, Clark, 1938, and Clara, 1939). It has been suggested that they regulate body temperature, blood pressure, and hydration of the tissues, and also that they provide a mechanism for improving the venous return to the heart. The fact that on the whole they occur more commonly in the superficial and peripheral parts of the body rather than in deeper sites has been adduced as evidence for some of these theories. That the arterio-venous anastomoses in the rabbit's ear respond to temperature changes has been shown in the living animal by Grant (1930), Grant, Bland & Camp (1932) and Clark & Clark (1934). As a result of other studies, Grant & Bland (1931) concluded that the arterio-venous anastomoses in the human finger were the vessels chiefly responsible for the increased blood flow which they observed as a reaction to cooling.

Brown (1937) has suggested that the arterio-venous anastomoses in the dog's tongue are concerned with the elimination of heat, a theory which is attractive since the dog has few sweat glands in the skin and protrudes its tongue when panting. On the other hand, we have recently discovered that the tongues of sheep and goats have arterio-venous anastomoses of similar type and in the same situation, an observation which is of interest in view of the different habits of these animals. In none of these animals are salivary glands present in the corium of the anterior portion of the tongue, so the arterio-venous anastomoses situated here cannot be directly concerned with glandular secretion.

SUMMARY

The arterio-venous anastomoses in the corium of the dog's tongue have been studied in injected preparations and histologically. These vessels form direct communications between arteries and veins which respectively supply and drain the subepithelial capillary plexus. They run a more or less tortuous course. They have a very thick wall, the media of which contains smooth muscle cells and epithelioid cells in large numbers but in variable relative proportions. The vessels have no internal elastic lamina, and in fixed preparations the lumen is usually very small. Their situation and great numbers, particularly on the dorsum, suggest that these vessels when open allow a large volume of blood to pass through the superficial parts of the tongue without overburdening the subepithelial capillary bed.

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EXPLANATION OF PLATES

PLATE 1

Figs. 1 and 2. Neoprene latex casts of vessels in corium of dorsal surface of dog's tongue, viewed from the deep aspect. The arteries (*A*) and the veins (*V*) form a capacious vascular bed lying beneath the epithelium, the capillaries of which they supply and drain. The two systems are connected by numerous arterio-venous anastomoses, some of which are here indicated by arrows. Note that in these examples the calibre of the vessel increases abruptly where the arterio-venous anastomosis joins the vein. In fig. 2, close to the origin of the arterio-venous anastomosis, an arterio-arterial junction is seen, this being indicated by a marked bend and a narrowing of the continuous vessel.

Fig. 3. Dog's tongue injected with Berlin blue. Arterio-venous anastomosis (at arrow) in the corium, as seen in a thick frozen section cut in the plane of the dorsum. Note the tortuosity of the anastomosis and the narrow irregular lumen. *A*, artery; *V*, vein.

Fig. 4. Frozen section of similar preparation, lightly stained with alum carmine, showing an arterio-venous anastomosis (at arrow). Note the narrow irregular lumen and also the thick wall of the anastomosing vessel, the outline of which can just be seen along its proximal portion. *A*, artery; *V*, vein.

PLATE 2

Fig. 5. An arterio-venous anastomosis, cut at two levels, is seen lying above one of the large arteries of the corium (*A*). Note the irregular outline of the injection mass in the lumen of the anastomosis, and the large pale nuclei of the epithelioid cells in the thick wall of the vessel. Injected preparation: haematoxylin and eosin.

Fig. 6. Group of vessels in corium of dog's tongue. To the left and above are three segments of an arterio-venous anastomosis cut at different levels. To the right of and below these are two small arteries and a capillary. (The identity of the vessels was confirmed by study of the adjacent section which was stained for elastic fibres, an internal elastic lamina being absent in arterio-venous anastomoses.) Injected preparation: haematoxylin and eosin.

Fig. 7. An arterio-venous anastomosis is seen cut in cross-section and lying between an artery (*A*) and a vein (*V*). The appearance of the anastomosis, with its thick, cellular wall and narrow lumen, is typical. Haematoxylin and eosin.

Fig. 8. Arterio-venous anastomosis cut at two levels. Note the modified smooth muscle cells and epithelioid cells irregularly disposed in the wall of the vessel. Haematoxylin and eosin.

PLATE 3

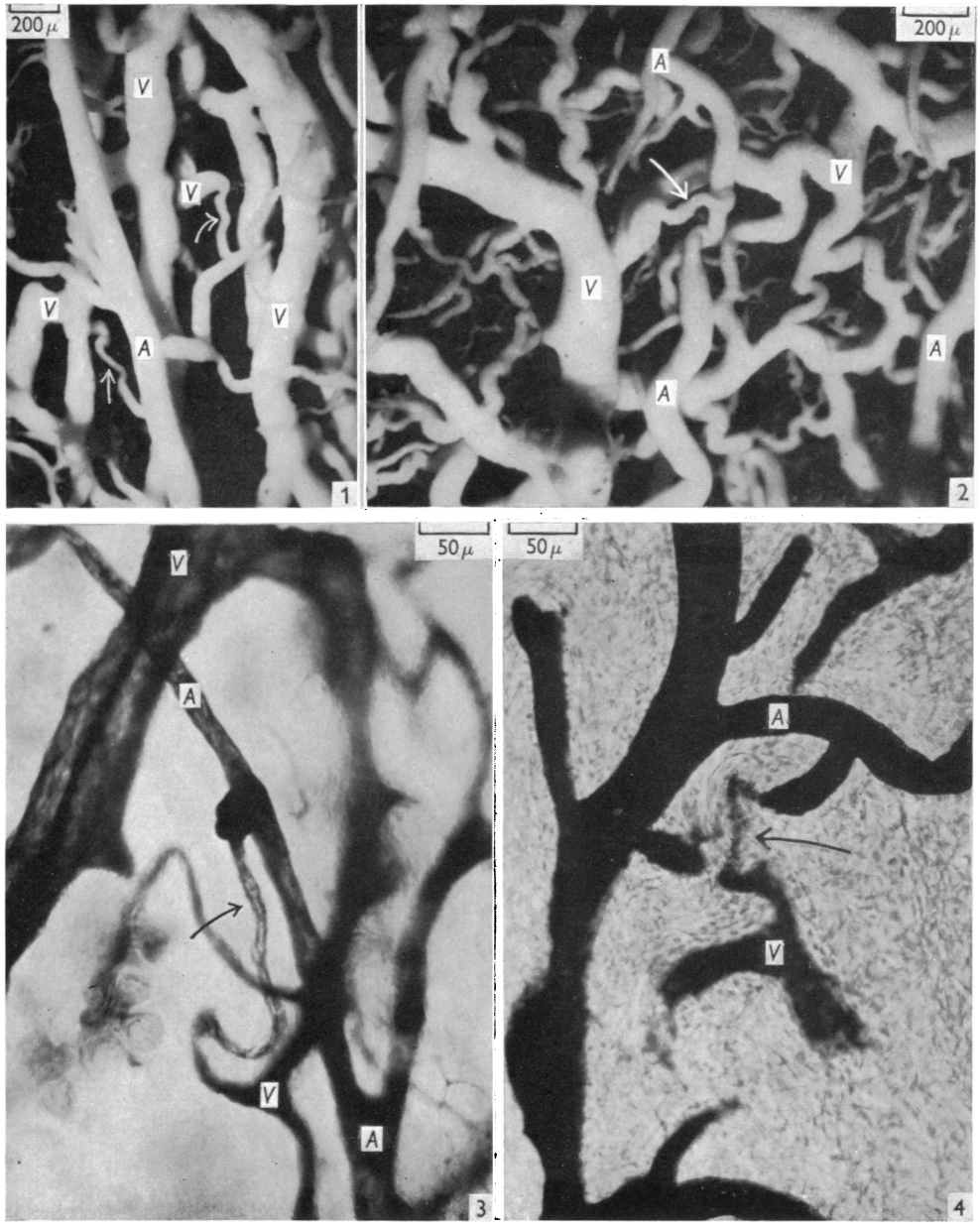
Fig. 9. Part of an arterio-venous anastomosis, the venous end of which is seen below. An artery (containing injection mass) is seen in cross-section on the right. In the portion of the anastomosis seen above, note the cells projecting into the lumen of the vessel, giving the outline of the latter its characteristic irregularity. In this instance most of the cells of the vessel wall appear to be of modified smooth muscle type. Injected preparation: haematoxylin and eosin.

Fig. 10. Arterio-venous anastomosis seen as a compact unit, well demarcated from the surrounding tissue. The section shows two segments of the anastomosis cut transversely, and also part of the wall of the intermediate segment cut obliquely. Epithelioid cells are present in the wall of the vessel. A small nerve trunk is seen on the left. Injected preparation: haematoxylin and eosin.

Fig. 11. Part of an arterio-venous anastomosis seen in longitudinal section. Note the marked bend of the vessel and the many large pallid nuclei of the epithelioid cells. Injected preparation: haematoxylin and eosin.

Fig. 12. Oblique section through an arterio-venous anastomosis. Many of the cells in the vessel wall are of epithelioid type, with large pale nuclei. Haematoxylin and eosin.

Fig. 13. Arterio-venous anastomosis cut obliquely, with its venous end below on the left, and a small artery on the right. A unit such as this is frequently seen (cf. fig. 9). Both epithelioid and modified smooth muscle cells are seen in the wall of the anastomosis. Haematoxylin and eosin.



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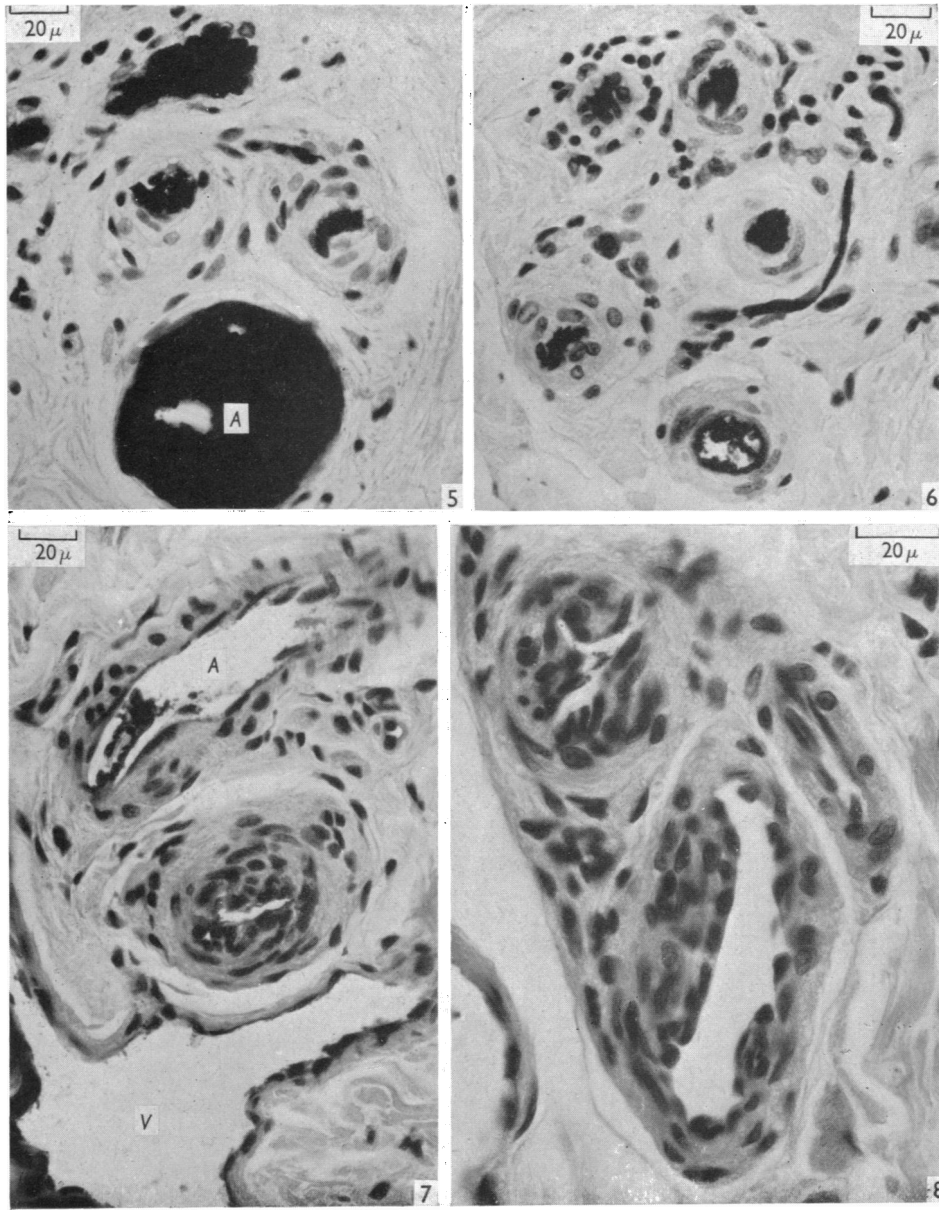

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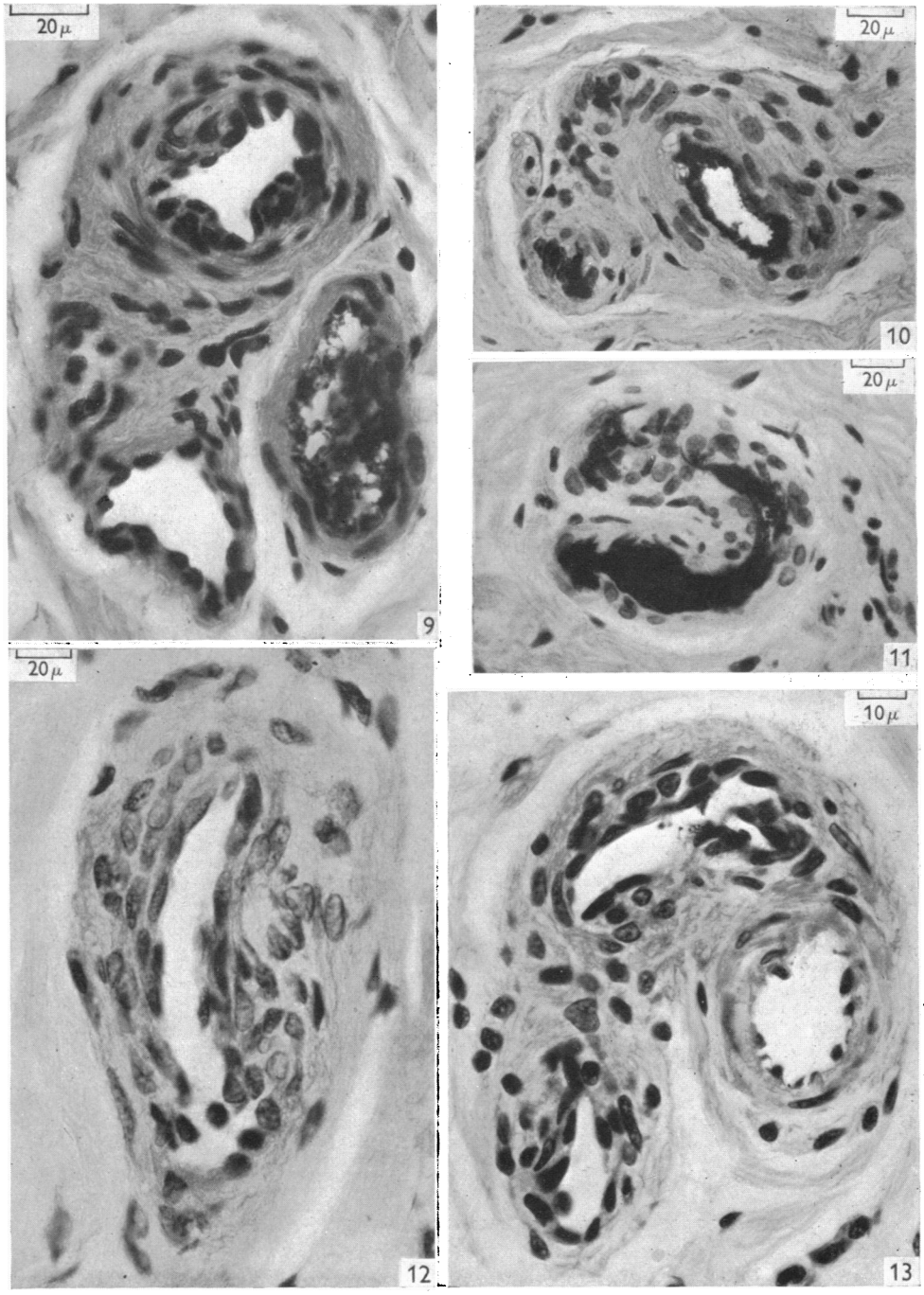

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