A STUDY OF ARACHNOID GRANULATIONS.

BY

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Fig. 1 Pacchioni's illustration of arachnoid granulations in man.
From the "Opera", 4th edition.

Fig. 2 Vesalius' illustration of granulations(K) in man.
From Singer's translation of 'Vesalius on the Human Brain".

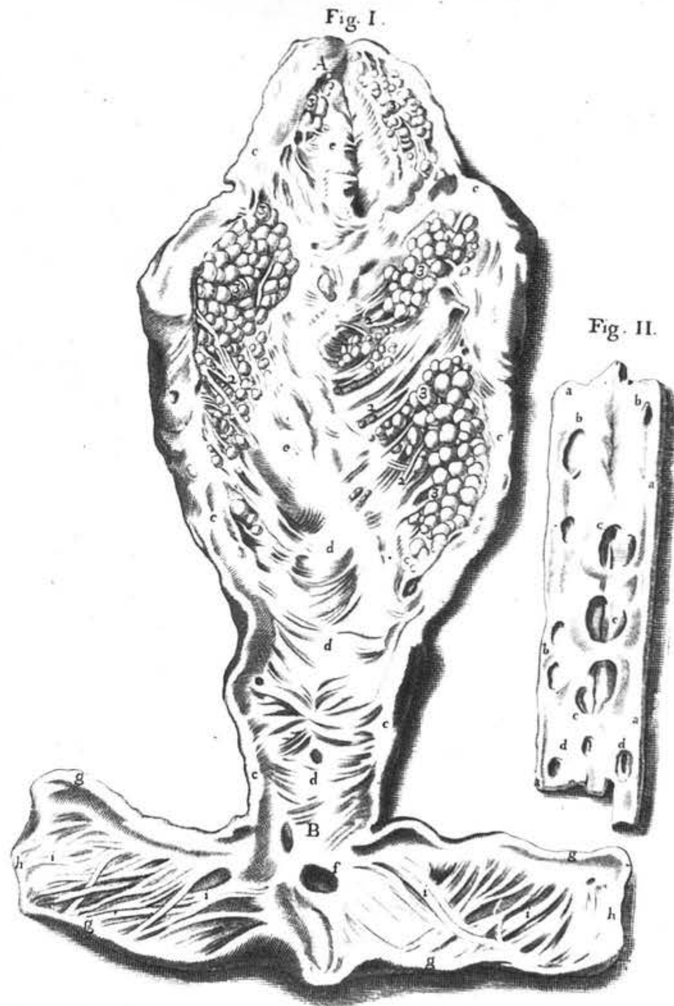

FIG. 1.

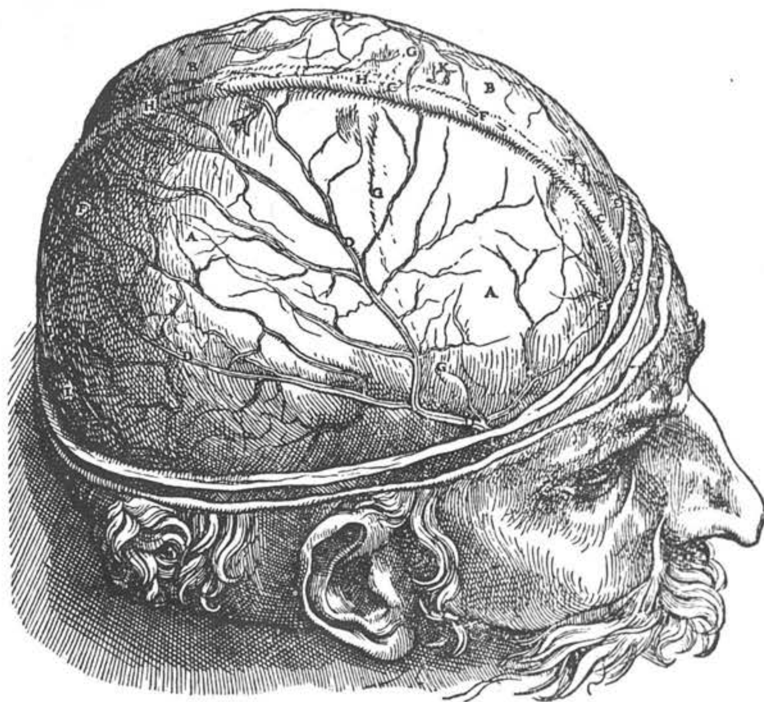

FIG. 2.

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INTRODUCTION.

Arachnoid granulations are found both in relation to intra-cranial venous sinuses and over the surface of the cerebral cortex. They are pearly white cauliflower-like bodies of varying consistency and are either pedunculate or sessile. The granulations related to the venous sinuses penetrate through the dura mater forming the walls of the sinuses and project directly into the sinuses or into lacunae laterales. Those granulations situated over the surface of the cerebral hemispheres are present within the subdural space and they abut against the overlying dura mater. The term 'granulation' has been used to denote structures which are visible by the unaided eye while structures very similar in morphology but only observed with the aid of a microscope are called 'villi'.

HISTORICAL.

The classical description of arachnoid granulations was given by Pacchioni in 1705, who in a letter to Lucae Shrokio wrote about the structures he had observed in the intra-cranial venous sinuses, thus:- "In the longitudinal sinus, immediately beneath the expansion of membrane, in the intervals between the chords of Willis, and indeed above the chords, are situated innumerable rounded granules, enclosed in their own very thin membrane as in a small bag; for the most part they are in clusters, rarely spread out thinly.... They exhibit a roundish shape, which often varies because of their mutual pressure on each other; in meninges that have not been soaked they look like the eggs of silk worms; but if a thicker meninx, first washed in ordinary water is kept for a month or more in vinegar.... granules of this sort swell so much along with the meninx itself that they equal in size and sometimes/

sometimes exceed a grain of coarser millet before it's outer skin has been stripped off. In the aged however, and those wasted by disease, they stand out so conspicuously that they may be seen without the help of soaking or of the microscope.... Each one is surrounded by very thin fibres of flesh, whence it seems to take on a pale flesh colour; in the aged.... the granules are seen as whitish and more distended.... They have small arteries with branches penetrating a short distance from the falx through the interior surface of the dura meninx; some of them are derived from the pia meninx and are not far from, and sometimes joined with, the veins leading into the sinus. It is remarkable and equally worthy of note, that these granules are found on either side of the longitudinal sinus only, as they are either never noticed in the lateral sinuses, or with great rarity just a few traces of them.... It is right then that the granules should be situated in the upper part of the brain, that is above the border of the brain where it lies next to the tract of the longitudinal sinus; for, as there is no pressure of any heavy body above them, they can more freely discharge their own fluid and produce excretory vessels, in any direction without, so to speak, fear of rupture or strangulation. From the above mentioned granules emerge innumerable threads, which are so many small excretory vessels, for these lymphatics, intimately joined and entwined with the blood vessels from the dura meninx, are implanted in the pia meninx.... You will see drops of different and distinct liquids, blood and transparent lymph fall from their ends.... meanwhile it is enough to know that the aforesaid granules are present there, and that there emerge from them very thin lymph vessels, spreading through the pia meninx and joining with the blood vessels, at once pervade the very curves of the brain." (Fig.1)

This/

This was not the first time arachnoid granulations had been observed however, because long before Pacchioni's description Vesalius (1543) writing on the meninges noted,
"the smoothness of the external surface of the membrane is interrupted by the frequent appearance of certain projections (arachnoidal projections) where the sagittal suture is joined to the coronal. These when present, protrude irregularly into recesses in the skull to which they firmly adhere". (Fig.2)

Between the interval of Vesalius' and Pacchioni's descriptions there were several workers who described the presence of arachnoid granulations in man, namely Willis (1664), Littre (1684), Collins (1685), Harder (1687) and Mery (1701), but because Pacchioni was the first to give a detailed description and also the first to illustrate the granulations, these are frequently called Pacchionian bodies. Several authors have discredited Pacchioni by saying that at the time of his account he already knew of the previous descriptions of granulations and a very interesting account of this controversy was given by Turner (1957).

Fantoni (1738) hearing about Pacchioni's work wrote to him describing the structures that he too had observed and remarked,
"Some sort of fluid is separated from the blood in these glandules, and that these small tubules carry lymph.... I think the flow of the liquid is rather directed to the sinus than towards the circumference of the cerebrum.... You add that no ostia of the glandules are visible in relation to the sinus, a fact which I think somewhat favours your hypothesis; but you must remember this, that almost infinite numbers of open passages and pores lie hidden in the bodies of animals...."

In the same letter Fantoni also mentioned that

Littre/

Littre had discovered these structures first and that Littre had seen lymph exuding from them when pressure was applied. He then continued:
"You see Pacchioni, how opportunely we have lighted on this situation which has disclosed to us little fountains previously hidden, now playing on the external surface of the dura mater.... and now on the internal aspect also.... They are situated mainly in the highest part of the dura and this is the position and most appropriate and suitable for men, who lead their life with the head erect".

All of these workers considered that these structures seen both in the intra-cranial venous sinuses and over the surface of the cerebrum were glands or glandules, so that until the end of the 19th century, the structures were referred to as the 'glands of Pacchioni', even though most of the later anatomists were fully aware of their non-glandular nature. Nevertheless, the structure of these 'glands' was very uncertain, so that Ruysch (1827) considered them to be fat bodies, and a somewhat similar opinion was advanced by Wenzel (1806), who postulated that they were pathological structures composed of connective tissue filled with fat. Monro (1827) on the other hand, was confident that they were composed of coagulated lymph.

Nowadays the general consensus of opinion is that Pacchionian bodies, or arachnoid granulations, to use the more modern terminology, are normal structures (Davson, 1956; and Turner, 1957,1958), but most earlier anatomists (e.g. Bell, 1803; Calmeil, 1826; Quain, 1837; Meckel, 1838; Krause, 1843; Kölliker, 1850; and Charpy, 1899), held the view that they were pathological structures and many of them attempted to correlate the occurrence of granulations with human behaviour and disease. This was not at all surprising of/

of course, when it is remembered that most of the anatomists of the 18th and 19th centuries were also medical practitioners, who described the structures that they observed in post-mortem specimens. Consequently, Bell (1803) compared arachnoid granulations with 'granulations of a sore' and about the same time Bichat (1802) for the first time used the term 'cerebral granulation' instead of glands or granules of Pacchioni. About thirty five years later Meckel (1838) remarked that arachnoid granulations were best seen in persons with disease of the head, and Todd (1847) made the very interesting observation that he found the greatest number of granulations in alcoholics, in those with irritable tempers and in persons with violent and readily excitable passions. This correlation with alcohol was also proposed by Rokitansky (1850) and Browning (1882), who found in addition that granulations were common in those who suffered from insanity, epilepsy and brain tumours as well as in senility. How important these observations were cannot be evaluated at present, but it is of interest that more recently Winkelman and Fay (1930) found oedema in granulations from people who had died of acute alcoholism and exudative meningitis, and Le Gros Clark (1920) observed hypertrophy of granulations present in subjects that were known to have suffered from arterio-sclerosis, nephrosclerosis and raised intra-cranial tension during life.

The most important point concerns the nomenclature used to refer to these structures related to the intra-dural sinuses and meninges of the brain, for while some are large and easily seen by the naked eye, others are so small that they can only be seen with the help of a hand lens or microscope. Luschka (1852) defined the macroscopic structures as Pacchionian bodies or/

or granulations, while for the microscopic structures he used the term 'villi', a terminology also used by Turner (1958). It seems in fact that the only difference between these structures is one of size, and Hassin (1930) defined a granulation as a "hypertrophied villus consisting of several lobules, or villi, covered by a common membrane and possessing a common stalk". Le Gros Clark (1920) also regarded the structures seen by a hand lens as granulations whereas Cooper (1958) used the term 'granulation' to cover all of these structures, irrespective of size. In the present account the terms 'arachnoid villi' and 'arachnoid granulations' will be retained to refer to the microscopic and macroscopic structures respectively, for although these terms have no exact meaning in so far as the basic form of the two is the same, they are useful terms to retain when referring to structures of different size.

DISTRIBUTION OF ARACHNOID GRANULATIONS IN MAN.

All authors agree that arachnoid granulations are present in the intra-cranial venous channels, but others have found similar structures on the cerebral surfaces, related to the petrous temporal bone and around spinal nerves, in positions where no venous pathways occur. Similar structures have also been described in the canal of Schlemm, in the middle ear, within the ventricles of the brain, in association with the choroid plexus and around the pineal gland.

The number of granulations present in an adult human is not constant, and Charpy (1899) estimated there are between 200 to 300 granulations in a normal adult.

SUPERIOR LONGITUDINAL SINUS.

Most authors have described arachnoid granulations in relation to the superior longitudinal sinus, where the greatest accummulation appears to occur in the middle and posterior parts (Wenzel, 1806; Cloquet, 1828; Knox, 1853; Meyer, 1860; and Heitzmann, 1887), though Cooper (1958) observed them mainly in the middle third and Bichat (1802) only in the posterior third. Froment (1846), Piersol (1907), Fay (1930) and Winkelman (1930) noted that granulations usually occur at the vertex of the brain, while Le Gros Clark (1920) found that whenever granulations lie free within the sinus itself, they were always in the anterior part. In fact Le Gros Clark (1920) considered that granulations were more common in the lacunae laterales than in the sinus itself. It is worthwhile noting that Key and Retzius (1875) were of the opinion that the lacunae laterales, where arachnoid/

arachnoid granulations have also been noted by other authors (e.g. Bichat, 1802; Meckel, 1838; Krause, 1843; Kölliker, 1850; Trolard, 1870; Browning, 1882; Weed, 1914b; Gladstone and Dunlop, 1927) are formed specifically for the accommodation of the Pacchionian bodies, which as Schäfer and Symington (1908) demonstrated, may sometimes completely obliterate the cavity of the lacunae. This observation of Schäfer and Symington may have led Winkelman and Fay (1930) to suggest that during life, there was no blood in the lacunae laterales, but only cerebro-spinal fluid.

IN OTHER SINUSES AND VEINS.

Though arachnoid granulations have been observed most commonly in relation to the superior longitudinal sinus they have been described in the transverse, straight, superior petrosal, spheno-parietal and cavernous sinuses, and along the course of the middle meningeal veins.

The occurrence of arachnoid granulations in the transverse sinus was observed by Pacchioni (1705), Bichat (1802), Cloquet (1828), Cruveilhier (1842), Krause (1843), Todd (1847), Kölliker (1850), Meyer (1860), Quincke (1872), Key and Retzius (1875), Obersteiner (1890), Piersol (1907), Weed (1914b) and Cooper (1958) and Le Gros Clark (1920) remarked that whenever granulations appear in the transverse sinus, they were found projecting into the floor of the sinus from the superior aspect of the cerebellum.

Description of arachnoid granulations in the straight sinus have been given by Haller (1762), Cloquet (1828), Cruveilhier (1842), Krause (1843), Todd (1847), Meyer (1860), Piersol (1907), and Cooper (1960). Bichat (1802) and Knox (1853) found them/

them at the opening of the great vein of Galen and it was near this same vein that Le Gros Clark (1940) described a supra-pineal body like an arachnoid granulation.

In addition to those found in the lateral and straight sinuses, arachnoid granulations have been observed in the superior petrosal and cavernous sinuses (Krause, 1843; Key and Retzius, 1875; Schäfer and Symington, 1908; and Le Gros Clark, 1920), in the inferior longitudinal sinus (Weed, 1914b) and in the spheno-parietal sinus (Le Gros Clark, 1920).

Not only have granulations been observed in relation to venous sinuses but they have also been found in relation to the middle meningeal veins (Krause, 1843; Key and Retzius, 1875; and Elliot Smith, 1905) where they probably perform the same functions as those projecting into sinuses.

GRANULATIONS NOT RELATED TO VENOUS PATHWAYS.

Despite the theories of Le Gros Clark (1920) that arachnoid granulations are always associated with venous channels, and of Ojala (1951) who considered that the presence of venous channels was the primary requirement for the development of a granulation, Fantoni (1738), Haller (1762), Bichat (1802), Cloquet (1828), Quain (1837), Knox (1853), Heitzmann (1887), Elliot Smith (1905), Winkelman and Fay (1930), Winkelman (1930), and Fay (1930) observed granulations on the cerebral surfaces, quite unrelated to any venous pathways.

It is appropriate at this stage to consider the relationship of granulations on the superior surface of the brain to the overlying dura mater, bone and venous channels. Many anatomists have observed/

observed recesses in the inner surface of the skull. These have been found to occur mainly in the parietal bones and especially at points where granulations protruded through the dura mater to abut onto the overlying bone, and Vesalius (1543), Quain (1828), Meckel (1838), Rokitansky (1850), Pozzi (1879), Heitzmann (1887) and Ojala (1951), concluded that the recesses in the bone were caused by pressure of granulations. A different concept about the recesses was proposed by Obersteiner (1890) who said that they were caused by the impressions of the lacunae laterales and, agreeing with this view, Le Gros Clark (1920) remarked that the depressions in the bone were formed long before arachnoid granulations were large enough to produce them. Brooks (1898), Gladstone and Dunlop (1927) and Pozzi (1879) were of the opinion that the recess formation was brought about by an absorption of bone by the granulations, rather than by pressure of any underlying structure, and it is of interest to note that Charpy (1899) had actually seen granulations completely piercing the whole thickness of the skull.

The relationship of arachnoid granulations to venous sinuses has not been satisfactorily settled and controversy still exists as to whether the granulations actually project into sinuses or are separated from the blood by the dura and the subdural space. Todd (1847) and Schäfer and Symington (1908) argued that granulations penetrated into sinuses by a thinning of the overlying dura mater caused by pressure exerted by granulations. One theory was that every granulation was surrounded by the subdural space (Todd, 1847; Luschka, 1852; Foerster, 1863; Trolard, 1870; Key and Retzius, 1875; Gower, 1876; Obersteiner, 1890; Hill, 1896; Charpy, 1899; and Schäfer and Symington, 1908;) but Piersol (1907) advanced the view that although the subdural/

subdural space was theoretically present, the granulation was separated from the sinus only by dura. Weed(1914b) also suggested that the subdural space was only a potential one and agreeing with him Fay and Winkelman (1930) remarked that the subdural space may nevertheless be present in abnormal conditions. Conversely, Turner (1958) emphatically denied the existence of the subdural space and remarked that the space seen by previous workers was formed as a result of shrinkage during the preparation of the specimen.

Arachnoid granulations have been observed in other situations in the brain where they exist without relation to venous channels or dura mater, for instance, projecting from the choroid plexus into the lateral ventricles (Bichat, 1802; Meckel, 1838; Froment, 1846; Kölliker, 1850; and Knox, 1853) on the walls of the lateral and fourth ventricles (Todd, 1847) and the fourth ventricle (Knox, 1853). Arachnoidal cell collections similar to those in arachnoid granulations were observed by Bichat (1802), Cloquet (1828), Todd (1847) and Knox (1853) around the pineal gland where they were disposed in two rows and united anteriorly to form a triangle.

Arachnoid granulations are not common outside the cranial cavity but where they do occur, are they performing the same function as those granulations found in relation to venous sinuses? For example, the canal of Schlemm in the eye is analogous to a venous sinus and within it Wegefarth (1914) described 'pectinate villi', similar to arachnoid villi of the central nervous system, projecting into definite interruptions in the inner scleral surface of the eyes of dogs, cats and rabbits, and Wolff (1952) saw Pacchionian like bodies projecting into the canal of Schlemm of humans. Gerlings/

Gerlings (1946) also noted the presence of arachnoid granulations projecting into the tegmen of the petrous temporal bone and into the auditory tube, and Mayer (1927, 1928), Nassuphius (1949) and Ojala (1951) described similar structures on the walls of the middle ear cavities.

It is reasonable to assume that wherever arachnoid cells are present in the central nervous system there is a possibility of finding arachnoid cell proliferations in the form of villi or granulations. Thus, arachnoid granulations and villi have been observed along spinal nerve roots by Elman (1923, in the dog), Hassin (1930, in man), Woollam and Millen (1958, in the rat) and Welch and Pollay (1963, in the monkey), and their structure is similar to granulations present in the cranium so that they may have the same function.

DISTRIBUTION WITH AGE.

While most of the early workers agreed about the occurrence of arachnoid granulations in the human adult, there was a great deal of controversy about their presence in infants and children. Thus, Wenzel (1806), Burdach (1822), and Froment (1846) were unable to find any arachnoid granulations in the human foetus and Cloquet (1828), Cruveilhier (1842), Knox (1853), Froment (1846), Cushing and Weed (1915) and Winkelman and Fay (1930) denied their existence in infants. On the other hand, granulations were found in some infants by Burdach (1822), and Foerster(1863), and Kiss and Sattler (1956) observed them consistently in infants from 5 to 6 months, though they were small. Le Gros Clark (1920) maintained that arachnoid granulations were first visible macroscopically about the age of

18/

18 months, Wenzel (1812) at the age of 3 years, Todd (1847) and Meyer (1860) after the age of 6 years, and Cruveilhier (1842), Obersteiner (1890), Charpy (1899) and Schäfer and Symington (1908) never before the 10th year. Luschka (1852), Weed (1914b) and Winkelman (1930) were quite definite that the arachnoid villi of infants were distinctly different from adult arachnoid granulations.

GRANULATIONS IN ANIMALS.

There has been complete disagreement about the presence of arachnoid granulations in animals, and this seems to have arisen from the fact that the various authors have used different criteria to distinguish between arachnoid villi and arachnoid granulations.

The following table summarises the opinions of the various authors about the presence of granulations and villi in animals.

+ indicates presence; - indicates absence

Animal	Author	Granulations	Villi
Rabbit	Quincke (1872)	+	
	Simmonds (1952,53)		+
	Key and Retzius (1875)	-	
	Browning (1882)	-	
	Hill (1896)	-	
	Turner (1961)	-	
Cat	Quincke (1872)	+	
	Weed (1914b)	-	+
	Fankhauser (1962)	-	

Dog/

Animal	Author	Granulations	Villi
Dog	Quincke (1872)	+	
	Key and Retzius (1875)	+	
	Sisson (1910)	+	
	Cooper (1961)	+	
	Alksne (1962)		+
	Pollay and Welch (1962)		+
	Fankhauser (1962)	-	
Oxen and Pig	Sisson (1910)	+	
	Browning (1882)	-	
	Fankhauser (1962)	-	
Sheep	Key and Retzius (1875)	+	
	Browning (1882)	-	
	Fankhauser (1962)	-	
Horses	Luschka (1852)	+	
	Sisson (1910)	+	
	Fankhauser (1962)	+	
Primate	Kolesnikov (1944)	+	
	Turner (1957)		+
	Welch and Friedman (1961)		+
	Cushing (1902)	-	-
Fish	Monro (1783)	+	
	Kiss and Sattler (1956)	+ (Sinus organ)	

Weed (1914b) denied that arachnoid granulations were present in any animal other than in man and stated in 1938, that arachnoid villi were present in all mammals. Kiss and Sattler (1956) and Fankhauser (1962) maintained that arachnoid granulations comparable to those of man were not present/

present in any other animal, with the possible exception of the horse (Fankhauser, 1962). Monro's observation of the presence of arachnoid granulations in fish is interesting, and so is Kiss and Sattler's description of 'sinus organ' in the same species, but whether they are comparable to arachnoid granulations of man is doubtful.

MORPHOLOGY OF THE ARACHNOID GRANULATIONS.

Earlier, granulations were defined as those structures observed by the unaided eye and villi as those structures seen with a microscope, a definition used by most workers, though as previously mentioned, Cooper (1958) classified bodies of both sizes as arachnoid granulations with the exception of the very large ones which she termed Pacchionian bodies.

Pacchioni's classical description conveyed a fairly accurate account of the gross appearance of arachnoid granulations and later workers have varied only in slight degree their accounts of the macroscopic appearance. The granulations have been described as being about 2 mm by 1 mm in diameter, often present in bunches in the venous sinus, lacunae laterales or at the opening of cerebral veins. They have been described as rounded bodies (Pacchioni, 1705), sessile or pedunculate (Weed, 1914b)and communicating with the subarachnoid space through prolongations of subarachnoid tissue which joins the granulation. (Key and Retzius, 1875; Gladstone and Dunlop, 1927).

SURFACE EPITHELIUM OF GRANULATION.

In this work the following terms are used. Endothelial cells, mesothelial cells and arachnoid epithelial cells. Endothelial cells are defined as those cells of granulations which line blood vessels; mesothelial cells as those found in the core of the granulation and subarachnoid tissue, and arachnoid epithelial cells as those of the arachnoid membrane lining the subdural space.

The origin of the surface epithelium of arachnoid granulations has been attributed to a number of/

of sources. Thus, Haller (1762), Bichat (1802), Cloquet (1828), Todd (1845), Obersteiner (1890) and Gerlings (1946) considered it to be formed of endothelium; Key and Retzius (1875), Gower (1876), Hill (1896), Brookes (1898), Schäfer and Symington (1908), Weed (1914b) and Le Gros Clark (1920) by the extension of the arachnoid membrane, while Millen and Woollam (1962), although they considered that arachnoid villi were covered by endothelium, regarded the granulations as being covered by condensations of the pia-arachnoid with a contribution of dural fibres. A similar contribution by the pia-arachnoid and dura mater was also proposed by Turner (1958) who showed, as did Todd (1847), Luschka (1852) and Gerlings (1946), that at some points on the surface, the cells are layered or stratified to form 'epithelial cell caps'. A similar stratification of cells has been observed in the various parts of the arachnoid membrane by Meyer (1859), Cushing and Weed (1915), Essick (1920), Brockbank (1929) and Stolk (1962). In 1920 (b), Weed advanced the interesting theory that the stratification was a phenomenon of advancing age, but Watt (1962) exploded this theory by observing arachnoid cell collections in the foetus as well as in post-natal infants. Chornyak (1948) suggested that cell aggregations of the arachnoid were produced by anoxaemia, but no one has suggested that these aggregations and the epithelial cell caps of the granulations are produced by the same condition, but this is a possibility that should be borne in mind.

CORE OF ARACHNOID GRANULATIONS AND VILLI.

Probably the most detailed account of the core of granulations was given by Key and Retzius (1875)/

(1875) in their famous monograph of nearly ninety years ago. Since then very few authors have added anything new to that description. Key and Retzius described a network of fibrous tissue trabeculae enclosing spaces lined by mesothelial like cells, having oval nuclei surrounded by a narrow zone of cytoplasm. These spaces anastomosed with one another in the core of the granulation, and were similar to the spaces found in the subarachnoid space. They described the trabeculae towards the periphery of the granulations as being more densely packed than those at the centre. Key and Retzius believed that arachnoid villi were similar in structure, both structures being extensions of the arachnoid and its contained subarachnoid space. Similar descriptions of arachnoid granulations were given by Schäfer and Symington (1908), Le Gros Clark (1920), Gladstone and Dunlop (1927) and Cooper (1958) and of villi by Weed (1914b). Schäfer and Symington (1908) believed that the space between the trabeculae was filled with cerebro-spinal fluid and Weed (1914b) stated that the connective tissue forming the trabeculae gave the "staining reactions of myxomatous tissue", though he does not explain what he means by this statement. A somewhat different account of the core was given by Turner (1958) who, while stating his belief in the presence of fibrous tissue in granulations, denied the presence of inter-trabecular spaces. He advanced the theory that the spaces purported to have been observed by other workers were produced artificially by shrinkage during the preparation of tissue. Some authors believed that the core of granulations was solid in young individuals. Among these were Luschka (1852), Kölliker (1850), Foerster (1863), and Meyer (1860). Schaltenbrand (1955) saw cavities lined by endothelium/

endothelium, but was uncertain whether they communicated with the venous sinus. Ducts have been seen by Pacchioni (1705) and Kiss and Sattler (1956), to traverse granulations and pass into the subarachnoid space through the pedicle or stalk at the subarachnoid side of the granulations.

PORES AND OPENINGS IN ARACHNOID GRANULATIONS.

Many workers were puzzled as to how particulate matter passed through granulations into the venous sinus, in view of the contention of Key and Retzius (1875) and of Weed (1914 a,b and c) that only fluid was transported across the surface epithelium. Some workers explained this passage of particles by suggesting an open communication between the subarachnoid space and the venous sinus, whereby both fluids and particulate matter could reach the venous sinus. For instance, Cruveilhier (1842) stated that Pacchioni had seen ducts which opened into the venous system from granulations, whereas Fantoni (1738) referred to Littre having found passages opening on the surface of granulations which on pressure yielded some serum, and Trolard (1870) described cavernous spaces within granulations which communicated with the venous sinus. This suggested a direct communication between granulations and the venous system, and was further confirmed by the experiments of Key and Retzius (1875). In their experiments 'cinnabar ground in water' was injected into the subarachnoid space, and they found cinnabar particles on the surface of arachnoid granulations, concluding that though fluid was filtered through the granulations, particulate material appeared on the surface by passage through 'stomata' situated between the surface/

surface epithelial cells of the granulation.

Within the last few years the idea of open communication between granulations and the venous system has become popular, especially after the work of Welch and Pollay (1961) who demonstrated the passage of colloidal gold particles, polystrene spheres, yeast and erythrocytes through 'coapted tubes' in the villi of monkeys, after these substances had been injected into the subarachnoid space. One end of the tubes was shown to open into the subarachnoid space and the other into the venous sinus, the evidence being more physiological than anatomical. (See p.28).

BLOOD VESSELS AND NERVES.

The presence of blood vessels in granulations has long been established. They were seen as early as 1705 by Pacchioni, and subsequently by Bichat (1802), Bell (1803), Cloquet (1828), Knox (1853), Le Gros Clark (1920), and Kolesnikov (1940), but Turner (1958) criticized these findings and said that the blood vessels seen by previous workers were dural blood vessels, in dural tissue found between adjacent granulations.

Many authors have been unable to demonstrate the presence of nerve fibres in granulations, though using silver techniques Kiss and Sattler (1956) described bulbous endings in nerve fibres and quoted Beleckij (1946) and Baron (1949) as also having found a rich nerve supply to granulations.

DEGENERATIVE CHANGES IN ARACHNOID GRANULATIONS.

Like most organs in the body, arachnoid granulations/

granulations frequently undergo degenerative change, amyloid and calcareous changes being most common. Calcareous changes have been observed more frequently than amyloid changes (Meyer, 1859; Charpy, 1899; Schmidt, 1902; Cushing and Weed, 1915; Le Gros Clark, 1920; Gladstone and Dunlop, 1927; Brockbank, 1929; and Winkelman and Fay, 1930) with actual bone formation (Bichat, 1802; Meyer, 1860 and Pozzi, 1879) and Gerrish (1899) referred to hard structures within granulations as 'brain sand'.

Attention has been drawn to the possibility of the so-called dural 'endotheliomata' arising in arachnoid cells which bear some relationship to corpora amylacea (Cushing and Weed, 1915; Le Gros Clark, 1920; and Gonatus and Besen, 1963) and Schmidt (1902), and Russell (1950) believed that meningiomas arose in nests of arachnoid cells, included in the dura as arachnoid villi.

ORIGIN OF ARACHNOID GRANULATIONS.

No final conclusion has been reached about the source of arachnoid granulations, for some considered them to be developed from the dura, some from the arachnoid and still others from the pia mater. Those who believed in a dural origin were Cruveilhier (1842) and Luschka (1852) although they felt that the arachnoid and subarachnoid tissue also contributed. The latter view being supported by Meyer (1860), Key and Retzius (1875), Gower (1876), Cushing (1914), Weed (1914b) and Villiger (1912). Todd (1847) and Knox (1853) held the view that though the granulations were formed of arachnoid, the primary source was in the pia mater, and that reciprocal movements of the arachnoid and falx cerebri produced elevations of the pia to form arachnoid/

arachnoid granulations (Tafel, 1882). Turner (1958) and Fankhauser (1962) were of the opinion that granulations were herniations of the pia-arachnoid complex extending into dural venous channels, while Rokitansky (1850) thought that granulations were fibroid thickenings of the arachnoid membrane.

FUNCTIONS OF GRANULATIONS AND VILLI.

Finally, mention must be made of the many functions attributed to the granulations and villi.

On the assumption that these structures were glandular, Willis (1664) and Fantoni (1738) considered them to be part of the lymphatic system, pouring their 'humour' into the blood stream, while Pacchioni (1705) concluded that their secretions were responsible for the irrigation of the meninges. An entirely different concept was that of Luschka (1852) and Charpy (1899) and more recently of Scarff (1949), who considered that granulations acted as supports or 'rivets' (Trolard, 1870) to hold the brain in place inside the skull.

The most plausible concept of the function of arachnoid villi is that of Weed (1914b), who as a result of a series of subarachnoid injections of potassium ferrocyanide and iron ammonium citrate in cats, suggested that cerebro-spinal fluid was filtered through the villi into the venous system. It should be pointed out however, that Weed considered that granulations were merely hypertrophied villi, and did not play such an important rôle as villi in this filtration. In support of this he mentioned that granulations were absent in animals and infants. A similar concept of filtration, but through granulations, has been suggested by Key and Retzius (1875), Hill (1896), Villiger (1912) and Dixon and Halliburton (1916), all of whom emphasised the importance of granulations in the return of cerebro-spinal fluid to the blood stream. Quincke (1872) who held similar views about the functions of granulations, stressed that they acted as filters, retaining solid particles which were phagocytosed by macrophages. He injected cinnaber particles into the subarachnoid space and/

and found that most of the particles were taken up by macrophage cells. A similar view regarding the activity of arachnoid cells was proposed by Russell (1950) who concluded that arachnoid cells formed the macrophages of the cerebro-spinal fluid pathways in the meninges. She found that the macrophages were vitally stainable with trypan blue and so considered them to be a component of the reticulo-endothelial system.

Weed's hypothesis about the filtration of cerebro-spinal fluid through the villi has held sway for nearly forty years, but the recent results of Courtice and Simmonds (1951), Simmonds (1952 and 1953), Sweet and Locksley (1953) and Turner (1961) though not in disagreement with that of Weed, suggested that granulations were sites of selective absorption of proteins. Courtice and Simmonds (1951) injected homologous heparinized serum labelled with Evan's blue into the cisterna magna of anaesthetized rabbits. They estimated the concentration of the dye-protein in cerebro-spinal fluid, circulating blood plasma and the cervical and thoracic duct lymph, with or without ligation of the lymphatics, and found that the level of the dye protein was not appreciably altered by ligation. They concluded that protein was mainly absorbed into the blood, presumably through villi, rather than through lymphatics. Simmonds (1952 and 1953) performed a similar procedure with labelled erythrocytes and plasma in rabbits, and arrived at the same conclusion. Sweet and Locksley (1953), injected isotopes of potassium (K-42) and chlorine(Cl-38) into the cerebral ventricles and estimated the clearance of the isotope ions together with the clearance of labelled human serum albumen from the cerebro-spinal fluid in a patient, and came to the same conclusion that/

that arachnoid villi were specifically responsible for protein absorption from cerebro-spinal fluid into blood. Turner (1961) based his belief on clinical evidence that the protein concentration in cerebro-spinal fluid of adults was higher than in infants, and believed that because of this the opacity of the pia-arachnoid membrane over the supero-medial surface of the cerebral hemispheres was more marked in adults than in infants. This he said, suggested a high concentration of protein near sites of protein absorption and was the reason why the opacity of the pia-arachnoid in adults was more marked than in infants. The conclusion that arachnoid villi subserve a special function of protein absorption is based on circumstantial evidence, and none of these workers have demonstrated the actual passage of proteins through the villi. Even before Weed's experiments, the concept that arachnoid granulations filtered cerebro-spinal fluid was put forward by Key and Retzius (1875) following injection of coloured gelatine into the subarachnoid space of cadavers and detecting the gelatine in the venous sinuses, but Schäfer and Symington (1908), were unconvinced that filtration took place under normal conditions, though not based on experimental evidence. Instead they suggested that "villi seem to afford a passage of cerebro-spinal fluid from the subarachnoid space into venous sinuses, when the fluid pressure in the subarachnoid space becomes from any cause increased above normal". Thus they felt that passage of cerebro-spinal fluid occurred through granulations and villi only when the pressure of the fluid exceeded a certain level, and in normal conditions villi did not filter cerebro-spinal fluid. While agreeing with this concept of filtration by granulations and villi, Howe (1928) and Howarth and Cooper (1955) considered that the main filtration took place/

place through blood vessels of the subarachnoid space, the granulations playing only a secondary role. Howe (1928) injected a 1% dextrose solution into the femoral vein of an anaesthetized cat and observed the leptomeninges through a trephine hole in the skull. He found that within a few minutes of injection of the dextrose solution, the arachnoid membrane collapsed while the cortical vessels were well outlined. He concluded that most of the cerebro-spinal fluid was directly absorbed into the cortical vessels, for the dramatic response could not have occurred, if the fluid was filtered only through the villi. The reasoning in this experiment seems weak, since collapse of the arachnoid would undoubtedly outline the cortical vessels. Howarth and Cooper's experiments in 1955 seem to be more reliable. They injected colloidal palladium and silver into the cisterna magna and lumbar regions of anaesthetized cats. The pressure of introduction never exceeded that of cerebro-spinal fluid by more than 20 mm of 0.9% saline. These workers found that there was a higher level of palladium in blood when it was injected into the cranial subarachnoid space than when it was injected into the spinal region. They also repeated Weed's experiments with prussian blue and observed granules in contact with blood vessels of the subarachnoid space as well as in the core of arachnoid villi.

Hassin (1924) on the other hand emphasised that cerebro-spinal fluid was not absorbed into blood through arachnoid granulations and villi but through the choroid plexus of the cerebral ventricles. He had observed lipoid deposits in the tuft cells of the choroid plexus in cases of multiple sclerosis and myelomalacia. In cases of subarachnoid haemorrhage, the presence of haemoglobin deposits in the choroid plexus/

plexus also seemed to support his view. Later, in 1930 and in 1948, he postulated that the spinal arachnoid villi were not responsible for filtration of cerebro-spinal fluid but that the drainage probably occurred through the perineural lymphatics. This was not based on any good experimental evidence nor on any sound reasoning. It is probable that the other pathways of drainage of cerebro-spinal fluid into blood do exist, in addition to the mechanism by way of granulations and villi.

Following the experiments of Weed (1914 a, b and c), Cushing (1914) agreed that granulations and villi played a rôle in the passage of cerebro-spinal fluid into blood by filtration, but in 1902, in common with the more recent results of Welch and Friedman (1959 and 1960) and Welch and Pollay (1961), he postulated that an open communication existed between the subarachnoid space and the blood system. By such a form of open communication, Welch and his co-workers (evidence given earlier) postulated as had Schäfer and Symington in 1908, that when the pressure of the cerebro-spinal fluid increased beyond a critical level, the granulations acting as valves, opened physiologically to allow the passage of fluid into the venous sinus. Welch and Friedman (1960) fixed an arachnoid villus of a monkey between 2 glass tubes, containing Ringer lactate solution. The tube on the subarachnoid side of the villus contained an air bubble, and by increasing the pressure on this side and observing the movement of the air bubble, they found that the bubble moved initially only above a certain critical pressure. The villus was examined histologically, and it was demonstrated that the tubes in the core of the granulation were dilated. Conversely, when the fluid pressure of the sinus increased beyond that in the subarachnoid/

subarachnoid space, they postulated that the valves closed, thereby preventing a backflow into the subarachnoid space, through the tubes opening into the venous sinus. This was shown by having the air bubble now on the venous side and increasing the pressure of this side above that on the subarachnoid side. There was no movement of the air bubble and histologically the granulation was flattened and the tubes in the core of the granulation obliterated.

Two totally different concepts of the functions of arachnoid granulations were advanced by Kiss and Sattler (1956), who were unable to confirm the passage of cerebro-spinal fluid through granulations either by filtration or by direct passage, after injection of India ink into the subarachnoid space of cadavers. Firstly, on the basis of the fact that nerve fibres were present they suggested that it was possible that granulations acted as baroreceptors at the junction of two fluids, the cerebro-spinal fluid and blood. Secondly they suggested that the presence of endothelial cells in the core of the granulation, was in favour of the granulations being a form of an endocrine organ - 'organon arachnoidale', the secretions of which entered the cerebro-spinal fluid and made contact with brain cells, after passage through the perivascular spaces of the brain. The suggestion that granulations are new endocrine glands is a little far fetched considering that the endothelial cells are nothing but the tongue like extensions from the 'epithelial cell caps' (Turner, 1958).

Bell (1803) surmised on morphological grounds, that granulations may act as valves by allowing blood to go through the veins towards the sinus, but prevented blood from taking a retrograde course, a view/

view similar to that of Cloquet (1828) and Cruveilhier (1842) who maintained that since the diameters of granulations are about the same as those of cerebral veins, they were morphological venous valves. Cooper (1958) asserted that because granulations occurred mainly at those sites where major cranial veins joined the longitudinal sinus, they were responsible for the organisation, regulation and stabilization of the venous flow in the skull in accordance with the fluctuations in cerebro-spinal fluid pressure, which itself was dependent on venous pressure. An explanation of the action of granulations as venous valves was made by Todd (1847), who maintained that they acted on a ball-valve principle and a similar mechanism was attributed by Le Gros Clark (1940) to the supra-pineal body (like an arachnoid granulation), which he considered to impede the venous return from the third and lateral ventricles.

Because of the wide disparity of views expressed by previous workers, as outlined above, no definite conclusions could be drawn about the structure and function of arachnoid granulations. It was therefore considered that a new investigation was called for, using light microscopy and the more recent technique of electron microscopy, which had hardly been applied to the problem. Consequently, a survey of the occurrence and structure of granulations and villi was undertaken in a series of mammals to find the most suitable animal. As a result of this survey, sheep were finally chosen for a detailed study. The results obtained with sheep will be presented first and afterwards used as a basis for the description of granulations in other mammals, including man.

INVESTIGATION IN SHEEP.

MATERIAL AND METHODS.

Light microscopy.

Paraffin sections.

Animals used.

(a) 9 adult sheep

(b) Foetal sheep - two of each age

40 day foetus
60 " "
90 " "
110 " "
120 " "
140 " "

Adult sheep were killed by decapitation using a guillotine method. The cranial vault was removed 10 minutes after death and the whole head immersed in 10% formol saline. After 3 days, the brain together with its coverings was carefully removed and re-immersed in the same fixative for a further 24 hours. The venous sinuses were then examined both macroscopically and with a dissecting microscope to determine the sites of the arachnoid granulations. The granulations were removed as small blocks of tissue with the surrounding dura mater, superior longitudinal sinus, the parasagittal membranes and the adjacent portions of cerebral cortex.

Portions of spinal cord together with spinal nerves were also removed from adult sheep immediately after death, and immersed in 10% formol saline.

Like the adult material, foetal material was also obtained from the Animal Research Establishment at Moredun, Edinburgh, where the period of gestation was accurately recorded. The foetus, immediately after removal from the mother, was perfused through the umbilical vessels with 10% formol saline, and later the head was immersed for a further period of 3 days, in a bath/

bath containing the same fixative. The cranial vault was removed and the procedure adopted for the removal of specimen tissues was the same as that for the adult animal.

After removal from the fixative, all tissues were left to wash in tap water overnight and then placed in 70% alcohol for 1 day, in 90% alcohol for 1 hour, 96% for a further 1 hour and finally in 2 changes of absolute alcohol for 2 hours. When large blocks of tissue were used the period of time in absolute alcohol was increased. Tissues were then doubly embedded, using one of two methods.

Method 1. Peterfi's technique (see Carleton and Drury, 1957). After final dehydration, the tissue was placed in 1% celloidin in methyl benzoate for 24 hours. At the end of this period the tissue was placed in fresh 1% celloidin in methyl benzoate for a further 24 hours. Usually at the end of this time the tissue was cleared but if blocks were too large, they were placed in a third solution of celloidin in methyl benzoate for a further period of 24 hours. The tissue was then transferred to benzene, using 3 changes of eight hours each, and then allowed to stand in a mixture of equal parts of benzene and paraffin wax at 37°C overnight. The tissue was then embedded under vacuum in 56°C paraffin wax.

Method 2. This was a modification of the method used by Brain (1949). Following final dehydration in absolute alcohol, the tissue was placed in equal parts of absolute alcohol and methyl salicylate and left for 12 hours overnight, before being placed in a solution of 2% celloidin in methyl salicylate for 7 to 10 days. The tissue was now transparent and was then treated in 3 changes of benzene for an overall period of 10 hours and finally embedded/

embedded in 56°C paraffin wax.

Blocks of tissue were serially sectioned at either 10 or 15μ, mounted on albumenized glass slides and dried in a 37°C oven before staining. Sections were routinely stained by one of the following methods.

(1) Harris' haematoxylin and eosin, as a general stain (see Carleton and Drury, 1957).
(2) Mallory's stain - Lebenden modification for fibrous tissue and collagen (see Cullings, 1957).
(3) Peters (1958) silver-proteinate method, and Glees and Marland modification of the Bielchowski method (see Cullings, 1957).

After staining, the sections were mounted in either D.P.X. or Canada balsam.

Details of structure were not very clear in some of the paraffin sections, since these were too thick and produced quite a lot of distortion and shrinkage. Thus, methacrylate and araldite were also used as embedding media for light microscopy.

Methacrylate sections.

4 adult sheep were used.

Small blocks of tissue containing arachnoid granulations and the dura forming the sinus wall were fixed in 10% formol saline for 1 hour and then placed for 2 hours in fixative containing 2% osmium tetroxide (Dalton, 1955). At the end of this period the tissue was washed in 10% alcohol and left in it for a further 1 hour. It was then treated with three, ½ hourly changes of absolute alcohol (over silica gel) and transferred into a mixture containing equal parts of absolute alcohol and methacrylate for 15 minutes. Next, the tissue was placed in methacrylate for 12 hours overnight, and transferred to fresh methacrylate in gelatine capsules at 60°C until the methacrylate solidified, which usually took about 1 hour. Sections were/

were cut at 1μ on a Porter-Blum microtome and stained with 1% toluidene blue in 1% borax solution or by haematoxylin and eosin by the method of Jennings, Farquhar and Moon (1959).

Araldite sections.

4 adult sheep were used.

The procedure employed was almost the same as for methacrylate sections. Following final dehydration in absolute alcohol, the tissue was transferred to two changes of 1:2 epoxy propane of 10 minutes each and embedded in araldite using the method of Glauert and Glauert (1958). Sections were cut at 1μ on a Porter-Blum microtome and stained with 1% toluidene blue in 1% borax solution or 1:4 pyronin-toluidene blue stain (Ito and Winchester, 1963).

As silver impregnation methods for the demonstration of nerve fibres in tissues containing collagen is difficult, methylene blue techniques were employed in whole mounts of granulations to demonstrate nerve fibres and endings.

Methylene blue technique.

2 adult animals were used.

The animal was sacrificed as before. Each common carotid artery was perfused with 125 mls of 3.8% sodium citrate. 300 mls of 0.05% methylene blue dissolved in 1.N saline at 38°C, were injected in equal quantities into each carotid artery and the procedure of Miller, Ralstone and Kasahara (1958) employed for the rest of the technique. The procedure employed for dehydration and embedding was that recommended by Miller and Kasahara (1959).

Electron microscopy.

4 adult sheep were used.

The animals were sacrified using a guillotine. The cranial vault was immediately removed/

removed and blocks of tissue containing arachnoid granulations and the dura forming the sinus wall were excised for fixation within 5 minutes. In order to obtain maximum penetration of the fixative, the tissue was initially treated with 150 units of hyaluronidase in 10 mls of normal saline (Pallie and Pease, 1958). The fixative used was 2% osmium tetroxide in Dalton's solution and the tissue was fixed for 1½ hours at 4°C. After fixation, the tissue was subjected to three, ½ hourly changes of 10% alcohol followed by three, ½ hourly treatments of absolute alcohol (over silica gel). The rest of the procedure adopted was the same as that employed for light microscopy using araldite. Sections were cut between 75mμ to 150mμ on a Porter-Blum microtome, floated on to uncoated copper grids (type 483) and stained with either,

(1) 1% potassium permanganate (Lawn, 1960).

(2) 5 to 7% uranyl acetate (Wohlfarth-Botterman, 1957).

The sections were examined in an A.E.I., E.M. 6 electron microscope, operated at 40 Kv with a 50μ objective aperture.

RESULTS.

ADULT SHEEP.

Macroscopic.

Arachnoid granulations were present in all the specimens of adult sheep examined. In 23 adult sheep superior longitudinal sinuses, the minimum number of arachnoid granulations present was 3 and the maximum number was 8. Granulations measured about 3 mm in length and about 1.5 mm in width, and they were situated in the caudal part of the superior longitudinal sinus near the commencement of the transverse sinus (fig. 3). These arachnoid granulations are cauliflower-like bodies which project freely into the sinus and are found at or near the opening of cerebral veins into the superior longitudinal sinus. Some isolated granulations are visible along the length of the superior longitudinal sinus and again are situated at or near the openings of veins. Arachnoid granulations may also be present in the transverse sinus but they do not appear to occur in other cranial venous sinuses in the sheep.

An attempt was made to relate the weights of brains and choroid plexuses to the number of arachnoid granulations present in an animal, but no correlation was found, in 10 animals.

TABLE.

Weight of brain	Weight of choroid plexus	Total No. of granulations.
122.4 G	0.47 G	6
118.5 "	0.48 "	8
115.3 "	0.37 "	5
113.6 "	0.35 "	8
110.2 "	0.49 "	5
106.9 "	0.35 "	6
105.1 "	0.45 "	6
103.1 "	0.28 "	7
100.2 "	0.41 "	4
99.3 "	0.38 "	3

The choroid plexuses were carefully dissected out, taking care there was no nervous tissue mixed with it. All weighings were done on a microanalytical weighing balance.

Microscopic.

Arachnoid granulations are seen as herniations of the arachnoid membrane and subarachnoid space, into the venous sinuses, through gaps in the dura mater forming the walls of the sinuses. A granulation possesses a body and a neck, through which the core of the granulation communicates with the main part of the subarachnoid space.

The dimensions of the arachnoid granulations are shown in the following table: (see fig. 4).

Width of granulation .... w
Height of granulation .... h
Length of neck of granulation .... n
Width of neck in dura .... wn
Width of neck at junction with subarachnoid space ws

Measurements	Minimum	Maximum	Mean
w	1.12 mm	1.53 mm	1.42 mm
h	0.47 mm	0.59 mm	0.52 mm
n	0.38 mm	0.43 mm	0.41 mm
wn	0.25 mm	0.31 mm	0.29 mm
ws	0.77 mm	0.92 mm	0.85 mm

The dimensions given in this table are based upon measurements made upon 50 granulations. Measurements were made from paraffin sections, using a calibrated squared 1mm slide. No corrections have been made for shrinkage of tissue produced during specimen preparation, and these convey only the relative dimensions of arachnoid granulations.

At this point it may be useful to restate the definition of the following terms:

The/

The surface epithelial cells, are those covering the arachnoid granulation where they form part of the walls of blood vessels (endothelial), mesothelial cells are those found in the core of the granulations and subarachnoid tissue, incompletely lining the spaces between the collagen bundles. Arachnoid epithelial cells are defined as those of the arachnoid membrane lining the subdural space.

SURFACE EPITHELIUM OF ARACHNOID GRANULATIONS.

The surface epithelium of arachnoid granulations is composed of a layer of cells, continuous with that lining the lumina of intra-dural venous sinuses (figs. 6 and 8). Most commonly these cells of a granulation are flattened or fusiform, containing dense basophilic nuclei, but, sometimes the cells are rounded when they have bulbous nuclei. Frequently on the surface of the granulation, these cells are aggregated on the summits, giving the appearance of stratification, which is similar to that found in the scattered aggregations of cells in the arachnoid membrane lining the subdural space (figs. 9 and 9a).

Electron microscopy.

The cells forming the surface epithelium of arachnoid granulations are illustrated by electron micrographs in figs. 10 and 11. The plasma membrane of the surface epithelial cell measures about 10mμ in thickness and is separated from the plasma membrane of an adjacent cell by 12 to 15 mμ, though often the separation may be as much as 60 mμ (fig. 12). In situations where adjacent cells come into close proximity, their plasma membranes form terminal bars made up of 'quintuple layers' (figs. 12 and 13), and the thickness of such a 'quintuple layered unit' is between 13 to 16 mμ. The junctions between cells are formed by abutting or overlapping of cell peripheries/

peripheries but each cell maintains its own plasma membrane, so that there is no indication of a syncitium. The surface of the plasma membrane facing the venous sinus is thrown into numerous folds (figs. 12 and 14). The nucleus which is irregular in shape, and occupies about 2/3rds of the cross sectional area of the cell, is surrounded by a two layered membrane. The thickness of each layer is 6 to 7 mμ and these are separated by a distance of 20 to 25 mμ. The nucleoplasm is more dense at the periphery than at the centre of the nucleus, with a centrally placed and indefinitely shaped nucleolus. Cytoplasmic organelles such as mitochondria, smooth and rough surfaced vesicles, irregular profiles of endoplasmic reticulum, Golgi apparatus and intra-cytoplasmic fibrils measuring 20 mμ in diameter are present. One characteristic feature in the cytoplasm of the surface cell is the presence of a large number of vesicles both immediately under, or in continuity with, the plasma membrane facing the venous sinus, and on the side of the cell towards the core of the granulation (figs. 11 and 12). These vesicles have a diameter between 50 to 60 mμ.

The surface cells rest upon a basement membrane towards the core of the granulation. The basement membrane is 35 mμ in thickness and separated from the plasma membrane of the surface epithelium by a distance of 30 mμ. It is made of fine fibrils, each having a diameter of 2 to 3 mμ, the fibrils lying in the same plane but crossing each other at right angles.

The features of endothelial cells as described by Palade (1953), Moore and Ruska (1957), Buck (1958) and Muir and Peters (1962) are as follows: They are a single layer of flattened cells which rest on/

on a membrane, with terminal bars between adjacent cells. Each terminal bar is made up of a 'quintuple layered unit'. At junctions between cells there are 'lips' formed by an overlap of cell margins. In the cytoplasm the characteristic features are mitochondria, vesicles, fibrils and irregular profiles of endoplasmic reticulum.

The appearance of the surface epithelial cells is similar to that in the description given above and therefore it is probable these are endothelial cells, as suggested by the fact that they are lining a blood filled space, namely the dural venous sinus.

At this point it is worth describing the appearance of the arachnoid cells lining the subdural space, which are similar to the flattened cells lining the peritoneal cavity as described by Odor in 1956. The arachnoid epithelial cells are also flattened, they rest on a basement membrane and there are terminal bars and 'lips' between adjacent cells (figs. 15 and 16). In the cytoplasm there are vesicles, fibrils and irregular profiles of endoplasmic reticulum. It is therefore impossible to differentiate between the surface epithelial cells of the granulation and the arachnoid epithelial cells lining the subdural space. As would be seen later, the mesothelial cells in the core of the granulation are also similar to endothelial and arachnoid cells. Thus, the terminology used depends only on their situation and not on morphological differences between the cells.

CRYPTS AND TUBULES.

Light microscopy.

The surface epithelium of the granulations in sheep presents in many places invaginations into its/

its core. Such invaginations form crypts (figs. 17 and 18). The cells lining these crypts are similar to those found on the surface epithelium of the granulation and could be classified as endothelial cells. (Refer p.39). Within the crypts a large number of blood cells are frequently observed (figs. 7 and 17). The diameters of the crypts vary from 100 to 300 µ. In serial horizontal (tangential) sections (fig. 5) of specimens embedded in plastic material, the crypts are seen to be continuous with tubules within the body of the granulation. Both tubules and crypts are lined by endothelial cells similar to those of the surface epithelium of the granulations (figs. 19 and 20). These tubules seem to extend through the granulation into the subarachnoid space where they appear to communicate with similar tubules found in that space (see page 59 for evidence). The mean diameter of these tubules is about 60µ, but they vary from 45 to 150 µ. As in the crypts, with which they are in continuity, blood cells are sometimes found in the tubules especially in those situated at the periphery of the granulation (fig. 21).

There are, however, other channels lined by flattened endothelial cells measuring from 6 to 15 µ in diameter which usually have blood cells in their lumina and these are considered to be blood capillaries (fig. 22). Further evidence for this statement will be given later. Differentiation between tubules and the narrower vascular channels is possible when India ink is injected into (a) the subarachnoid space, and, (b) into the carotid arteries. Details of the experiment are described on pages 55 and 56. India ink injected into the subarachnoid space fills the tubules while injection into the carotid arteries fill the narrower vascular channels. Thus, there are two systems/

systems of channels in sheep arachnoid granulations lined with endothelium, the only difference being one of size.

Electron microscopy.

The crypts and the tubules are lined by flattened endothelial like cells (figs. 23,24,25 and 76). These cells also possess the characteristic features of the surface epithelial cells namely, basement membrane on the outside of the cell, terminal bars and 'lips' (fig.24) between adjacent cells, with mitochondria, vesicles and irregular profiles of endoplasmic reticulum in the cytoplasm. These cells do not line channels filled with blood, yet, as they are continuous with the surface epithelial cells, are termed endothelial cells. The tubules have a variable shape in cross section; some are oval, others circular while still others are irregular in outline. The electron micrographs of tubules should be compared with those of capillaries (figs. 28 and 29) to appreciate the difference between them. The differences are as follows: The capillaries are usually more circular or oval, and possessing smaller diameters than the tubules. Capillaries have red blood cells in their lumen, while the tubules do not, except in the case of those tubules at the periphery of the granulation.

CAPILLARIES.

Light microscopy.

There are capillaries in arachnoid granulations of sheep (figs. 22, 26 and 27), these arise from blood vessels of the dura mater, and enter the arachnoid granulations at the points where they pass through openings in the dura mater. These capillaries travel some distance into the beck of the granulation/

granulation and then course vertically upwards into the core. Most of these capillaries form loops by joining with others, while a small number appear to end blindly some distance below the limiting membrane of the granulation (see figs. 66 and 67). The diameters of the capillaries vary, the larger ones resemble the endothelially lined tubules. No communications were found either between the capillaries and the tubules or between the capillaries and the venous sinuses.

Electron microscopy.

The capillaries are circular and are lined by endothelial cells containing in the cytoplasm, vesicles and irregularly shaped tubules of endoplasmic reticulum with Palade granules on their outer surfaces. These cells lie on an external basement membrane (opposite to the lumen) with terminal bars and protruding cell borders ('lips') between adjacent cells (figs. 28 and 29). Usually red blood cells are present and are found to fill the entire lumen of the capillary, with the plasma membranes of the red cells apparently in close contact with the plasma membranes of the endothelial cells.

CORE OF ARACHNOID GRANULATIONS.

The core of arachnoid granulations is formed of collagen bundles between which are spaces often surrounding the tubules, capillaries, nerves and a variety of cells. The bulk of the granulation is composed of a net-work of these collagen bundles, similar to that found in the subarachnoid space (fig.6). The collagen bundles are sometimes covered by mesothelial cells, and are more tightly packed at the periphery of the granulation than at the centre. Spaces are present between the collagen bundles, and these spaces communicate with one another (fig.6), and/

and with similar spaces found in the subarachnoid space. Some of the spaces are lined incompletely by mesothelial cells and on section, these spaces give the appearance of tubes (fig. 22), an appearance which probably led Welch and Friedman (1959) to term them 'coapted tubes'. The widths of the spaces vary, the smaller spaces being between 10 to 30 μ and the larger ones between 40 to 100 μ. Scattered at random in the collagen network are cells (macrophages, blood cells, fibroblasts etc) which may have entered the granulations by extravasation from the venous sinus, during excision of tissue from the animal. The collagen bundles have no definite or regular orientation.

At the point of emergence of the granulation through the dura mater there is intimate blending between arachnoid and dural tissue, and it is impossible to define and separate the two structures (figs. 8 and 30). Dural fibres pass into the neck of the granulation and the subarachnoid tissue seems to invade the adjoining dura mater.

Electron microscopy.

In electron micrographs, the detailed structure of the collagen bundles and the spaces between them are well shown (fig. 31). The spaces between the collagen bundles are quite distinct from the tubules described earlier. The cytoplasmic processes of mesothelial cells partially cover the collagen bundles surrounding the spaces, and form incomplete linings of the spaces. The tubules on the other hand are completely lined by endothelial cells. Sometimes, the cytoplasmic processes of mesothelial cells lie on a definite basement membrane but in other situations the basement membrane is absent. They interdigitate with the margins of adjacent cells, but terminal bars are only observed occasionally (fig.32). In/

In some areas in the core of the granulation, cells are tightly packed with little collagen separating them, and between the cells only small inter-cellular spaces are found (fig. 31), probably similar to those observed by Alksne (1962) in the villi of dog. Pease and Schultz (1958) describe similar spaces in the subarachnoid tissue of rats and these they term 'cerebro-spinal fluid cisterns'.

CELLS FOUND IN ARACHNOID GRANULATIONS.

MESOTHELIAL CELLS.

Light microscopy.

The dimensions of mesothelial cells are the same as those of endothelial cells. They have long processes containing a light acidophilic cytoplasm and possess a darkly basophilic nucleus. As stated earlier, mesothelial cells partially line the spaces between the collagen bundles in the core of the granulation.

Electron microscopy.

In electron micrographs the long cytoplasmic processes of mesothelial cells are seen to contain vesicles about 60 mμ in diameter and electron dense granules (fig. 33). In the cytoplasm surrounding the nucleus the vesicles are larger, between 200 to 300 mμ in diameter. Other cytoplasmic organelles such as mitochondria, Golgi apparatus and endoplasmic reticulum are present. The nuclear membrane is formed of two layers separated by an interval of 30 mμ, and within the nucleus an eccentrically placed nucleolus is observed. It must be emphasised here that it is impossible to differentiate morphologically between endothelial and mesothelial cells.

FIBROBLASTS./

FIBROBLASTS.

Light microscopy.

It is difficult to identify fibroblasts by light microscopy, and differentiate them from mesothelial cells.

Electron microscopy.

In electron micrographs fibroblasts have some similarity to endothelial cells (fig. 34). They also possess vesicles and intracytoplasmic fibrils. However, many more mitochondria are present together with Golgi apparatus and a well developed endoplasmic reticulum.

MACROPHAGE CELLS.

Light microscopy.

Macrophages are larger than mesothelial cells and measure between 12 to 15 μ in diameter and possess basophilic nuclei. The staining properties of the nuclei vary, for some cells possess nuclei with intensely staining properties while in others the staining is light (fig. 35). The cytoplasm is acidophilic with basophilic granules and vesicles.

Electron microscopy.

Two types of macrophages are observed in electron micrographs. Type 1 is present as a normal inhabitant of arachnoid granulations, and type 2 is observed only following subarachnoid injection of foreign material (vide page 57). The type 2 macrophage probably arises from primitive mesenchymal cells or from monocytes which have left the capillaries in reaction to 'foreign body invasion' of the arachnoid granulation.

In the type 1 macrophage, there is a well defined nucleus and nucleolus. The cytoplasm contains granules/

granules, vesicles and electron dense bodies (fig. 36). The characteristic feature of such a cell, found free in the collagen meshwork of the granulation, is the presence of a large number of cytoplasmic processes resembling pseudopodia (fig. 36), which probably serve in engulfing particulate matter (Palade, 1956). In the cytoplasm there are large vesicles measuring 300 to 500 mμ and smaller vesicles 60 to 80 mμ in diameter. These vesicles are surrounded by a membrane about the same thickness as that of the plasma membrane and contain particles of foreign material when this is injected into the subarachnoid space. (vide experiments on page 57 and fig. 37). In the cytoplasm there are mitochondria and granules scattered sparsely, together with some Golgi apparatus and smooth surfaced endoplasmic reticulum (figs 36 and 38). The electron dense bodies (E.D. bodies) are usually surrounded by a membrane, though in some the membrane appears to be absent, especially in those where the electron density of the matrix is reduced (fig. 38). There is considerable variation in the shape and size of the E.D. bodies diameter (between 1 to 3 μ), with fluctuation in the density of the matrix. The smaller E.D. bodies resemble mitochondria (fig. 37), the latter, however, have cristae within them. In electron micrographs at higher ranges of magnification, the matrix of the E.D. bodies show the presence of regularly orientated parallel fibrils extending across them, and the fibrils have a mean diameter of 6 mμ separated by 5 mμ intervals (fig. 37). Within the E.D. bodies there are small circular bodies (S.C. bodies) arranged mostly at the periphery (figs 36 and 37). The S.C. bodies have a diameter of 15 to 150 mμ. The matrix of the S.C. bodies is more dense at the periphery (fig. 38), and they have a sharp margin. There are E.D. bodies which/

which look like mitochondria; they have a well defined membrane with S.C. bodies and some suggestion of cristae within their matrix (fig. 37).

The nucleus possesses one or more nucleoli within it, and the nucleoplasm is denser at the periphery than at the centre.

Macrophage type 2 resembles a mesothelial cell though, like type 1, it has vesicles containing any particulate matter which may have been injected into the subarachnoid space (fig. 39). The cytoplasm is also thrown into pseudopodia like processes as in type 1, but the main difference between the two is that in type 2, there are no E.D. bodies. There are also other minor differences between the two types, for in type 2 the large vesicles vary from 400 to 1300 mμ and there are bodies resembling S.C. bodies of type 1, lying free in the cytoplasm (fig. 40). In addition to these features, there are electron dense granules and organelles in the cytoplasm of these cells. The nucleus is large and occupies more than half the cross sectional area of the cell, it is usually indented on one side, contains evenly distributed nucleoplasm and is bounded by a double layered membrane.

OTHER CELLS.

UNCLASSIFIED CELL.

Light microscopy.

There are large cells resembling mast cells but they do not show evidence of the characteristic granules found in mast cells. These cells are not common in sheep arachnoid granulations.

In electron micrographs the cytoplasm of the cell is observed to be thrown into pseudopodia like processes/

processes and contains dense polygonal bodies (fig.41). These bodies do not contain granules but a homogeneous electron dense material. They measure 0.5 to 1μ from one flat surface to another and the majority have a central clear space, while others have several clear spaces arranged radially (figs. 42 and 42a). Several of the polygonal structures may be enveloped in a single membrane sac (about the thickness of the plasma membrane) in which the polygonal bodies are separated by electron dense granules (fig. 42). Some of the sacs are only partially filled by the polygonal bodies the rest of the sac being filled by electron dense granules (fig. 41). The nucleus is bounded by a double layered membrane, the outer layer is crenated, and fused with the inner layer at some points giving rise to thickenings in the membrane (fig. 42). The nature of these cells is uncertain; they are unlikely to be mast cells since the typical granules are absent. On the other hand, they do not show particulate matter in the cytoplasm after injections into the sub-arachnoid space and thus are unlikely to be another type of macrophage cell. Yet, it must be borne in mind that these could well be a type of resting macrophage cell with selective properties.

BLOOD CELLS.

Light and electron microscopy.

There are a large number of lymphocytes, polymorphs and red blood cells in the collagen meshwork of arachnoid granulations (figs. 43 and 44). The red blood cells may be due to extravasation of blood into the granulation from the venous sinus during tissue excision. The polymorphs may have migrated out of capillaries.

NERVE FIBRES.

Light/

Light and electron microscopy.

In silver impregnated sections, it is difficult to differentiate between nerve fibres and collagen fibres, but by increasing the pH of the silver-proteinate mixture to 8.5 in Peters' method, the nerve fibres stand out more prominently (fig. 45). In osmium fixed specimens, however, medullated and non-medullated nerve fibres can be seen both by light and electron microscopy (figs. 46 and 48). The diameters of the axons of medullated fibres in arachnoid granulations vary from 8 to 10 μ with myelin sheaths with thicknesses varying from 0.8 to 1.2 μ. The diameter of axons of non-medullated fibres vary from 1 to 2 μ. On tracing nerve fibres in serial sections, they are found in bundles with little branching, except towards their terminations (see fig. 66).

On viewing whole mounts of granulations after employment of methylene blue techniques, nerve fibres are observed in the granulation which seem to arise from dural nerves (fig. 47). The density of innervation of the granulation is less than in the surrounding dura. Fine nerve fibres have been observed in electron micrographs to be adjacent to the basement membrane of the surface epithelial cells of the granulation (fig. 49), but no specialized nerve endings were seen either by light or electron microscopy.

SPINAL CORD.

Light microscopy.

Aggregations of arachnoid cells are observed at points where the spinal nerves leave the spinal canal, through the inter-vertebral foramina (figs. 50 and 51). These aggregations resemble arachnoid villi as described by previous workers, and are found near the/

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the spinal dura. They have no relation to venous channels, though they have some spaces in the tissue, as in the cranial arachnoid granulations. Whether or not these aggregations are equivalent to intracranial villi is uncertain.

FOETAL SHEEP.

Macroscopic.

Arachnoid granulations were visible in the superior longitudinal sinus of 90, 110 and 120 day foetuses by means of a dissecting microscope. In a 140 day foetus it was possible to see the granulations with the naked eye (fig. 52). These granulations were again situated at the openings of cerebral veins into the superior longitudinal sinus.

Light microscopy.

In the 40 day foetus, there is very little demarcation of the three meninges as distinct structures. The dura mater, however, is somewhat better defined than the other two, and the space between the dura and the cerebral cortex is filled by loose mesenchymal tissue.

The 60 day foetus shows the developing superior longitudinal sinus in a well formed dura mater, and the tissue between the dura and cerebral cortex shows some organisation into arachnoid and pia membranes, with the formation of blood channels in the subarachnoid space (fig. 53).

The dura, arachnoid and pia are clearly separate structures in the 90 day foetus; and there is considerable amount of arachnoid tissue surrounding the cerebral veins leaving the subarachnoid space to join the superior longitudinal sinus (fig. 54). At the point where cerebral veins join the superior longitudinal sinus, proliferations of arachnoid cells are/

are visible.

In the 110 day foetus the proliferating arachnoid cells at the openings of cerebral veins, are heaped on one or both sides of the veins at this confluence with the superior longitudinal sinus. These heaped up masses of arachnoid tissue resemble villi (figs. 55 and 56), with the core of these arachnoidal cell masses continuous with the subarachnoid space, which is well developed by this stage.

The 120 day foetus shows a narrowing of the cerebral veins with the definite appearance of arachnoid granulations in the superior longitudinal sinus. The granulations approach the adult form in appearance, for they have a well formed body and neck, through which there is communication with the subarachnoid space (figs. 57 and 58). The surface cells of the developing granulation are continuous with the endothelial cells lining the venous sinus, and the core of the developing granulation is very cellular with collagen bundles and spaces between them. The tubules found in the adult have not been seen at this stage.

Well formed arachnoid granulations are observed in the 140 day foetus, with a well defined body, neck and core (fig. 59). The core is formed of collagen bundles and spaces, and the surface of the granulation shows an endothelial covering with the formation of crypts and tubules.(fig. 60). At this stage of development there are cell proliferations resembling arachnoid cells in the dura mater near intra-dural veins. These proliferations are assumed to be precursors of arachnoid granulations. Stages in the development of arachnoid granulations in foetal sheep are summarised in fig. 61.

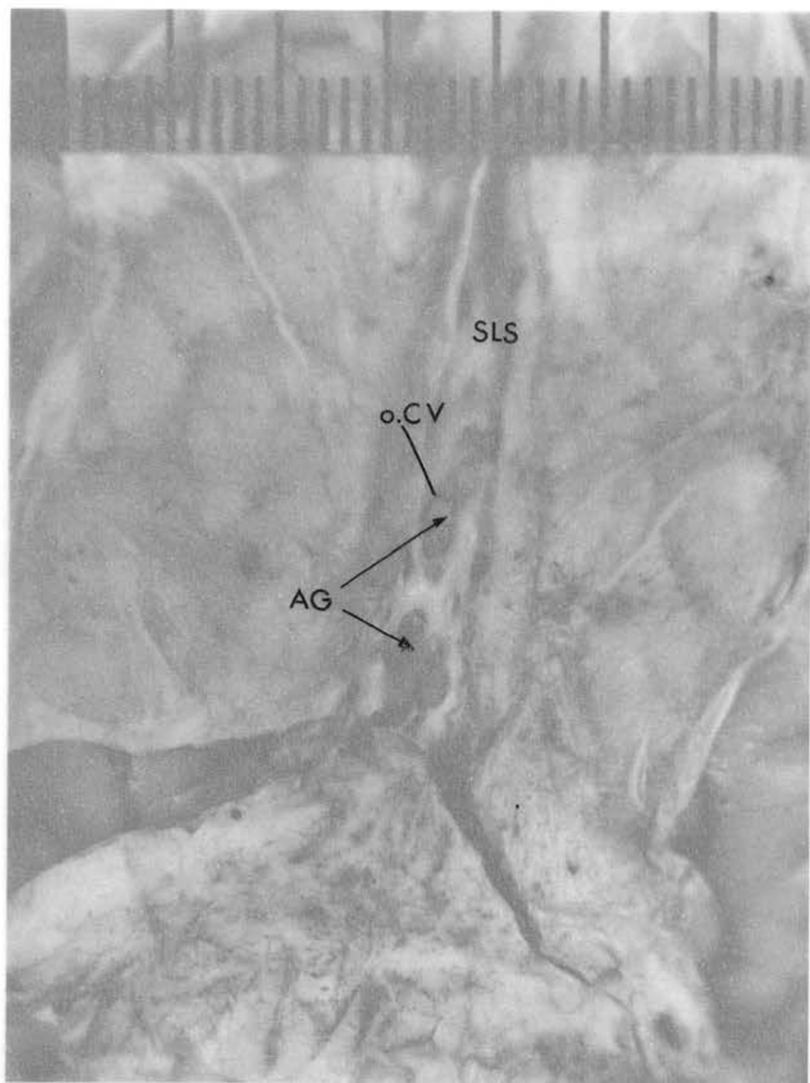


FIG. 3. Photograph of a sheep superior longitudinal sinus, showing arachnoid granulations. (x 1.5)

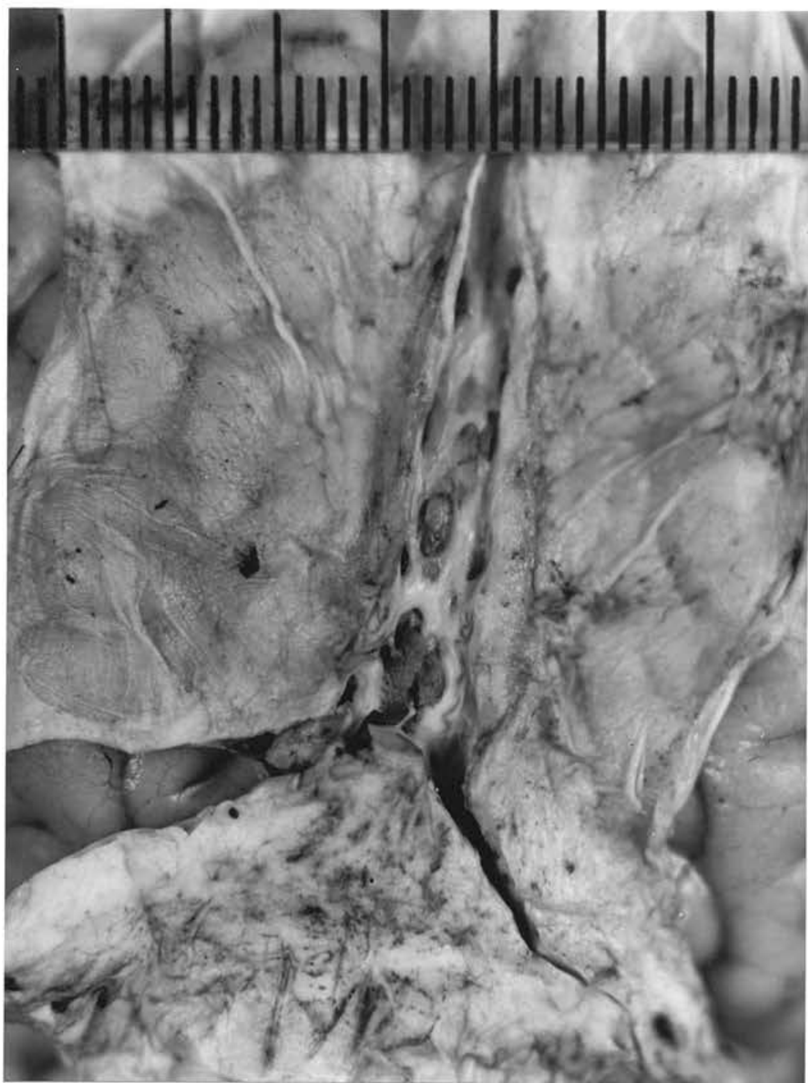


FIG. 3. Photograph of a sheep superior longitudinal sinus, showing arachnoid granulations. (x 1.5)

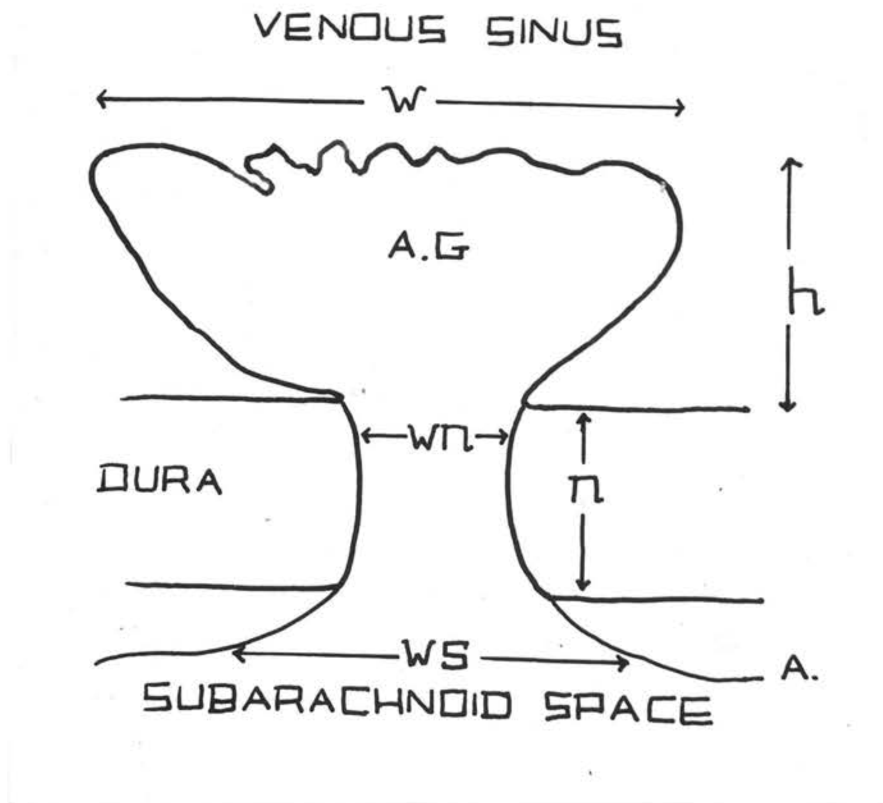


FIG. 4. Diagram showing abbreviations of dimensions of an arachnoid granulation.

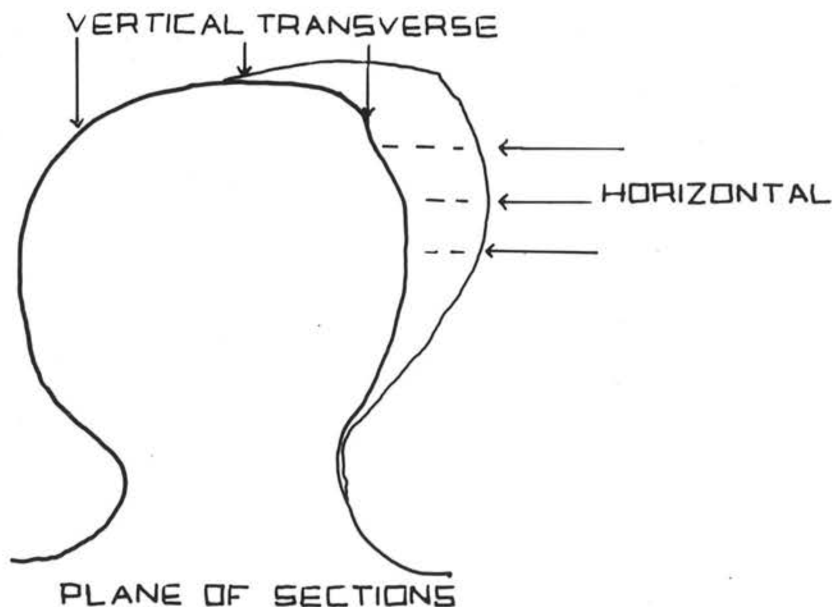


FIG. 5. Diagram showing planes of section. Vertical transverse plane indicated by vertical arrows, and horizontal(tangential) plane by horizontal arrows.

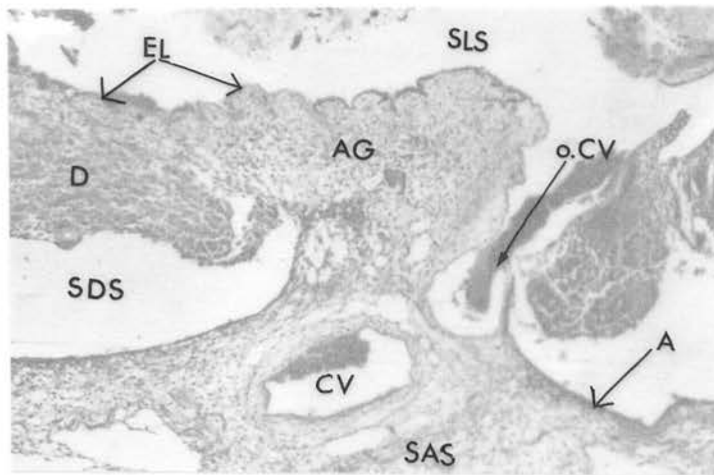


FIG. 6. Photomicrograph of sheep arachnoid granulation in superior longitudinal sinus. EL = endothelial layer.
(paraffin embedded, H & E, x 54)

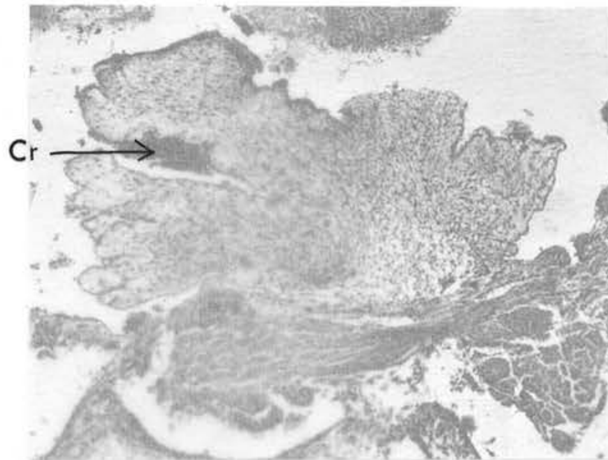


FIG. 7. Photomicrograph of sheep arachnoid granulation in superior longitudinal sinus.
(paraffin embedded, H & E, x 54)

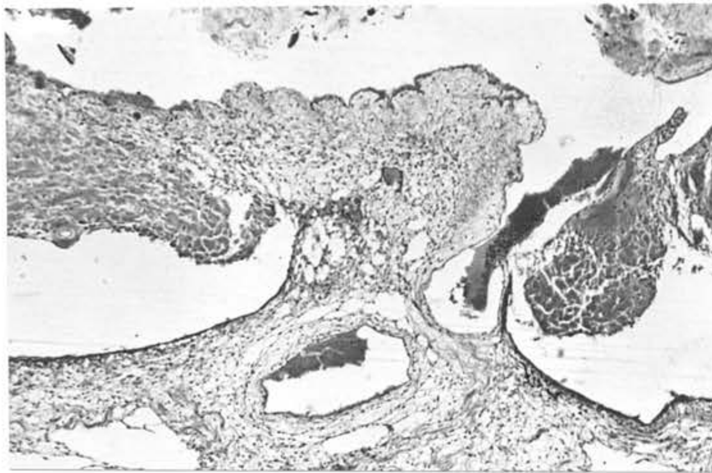


FIG. 6. Photomicrograph of sheep arachnoid granulation in superior longitudinal sinus. EL = endothelial layer.
(paraffin embedded, H & E, x 54)

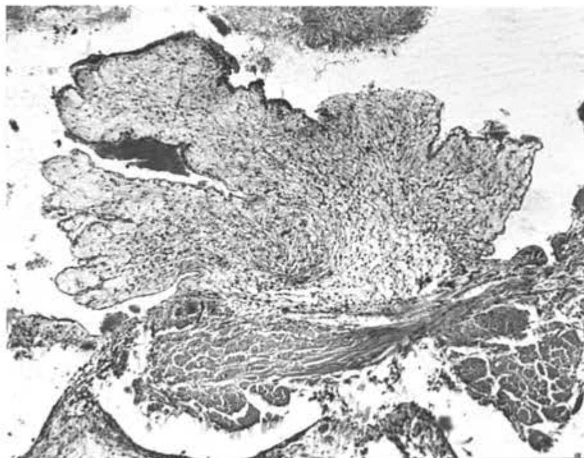


FIG. 7. Photomicrograph of sheep arachnoid granulation in superior longitudinal sinus.
(paraffin embedded, H & E, x 54)

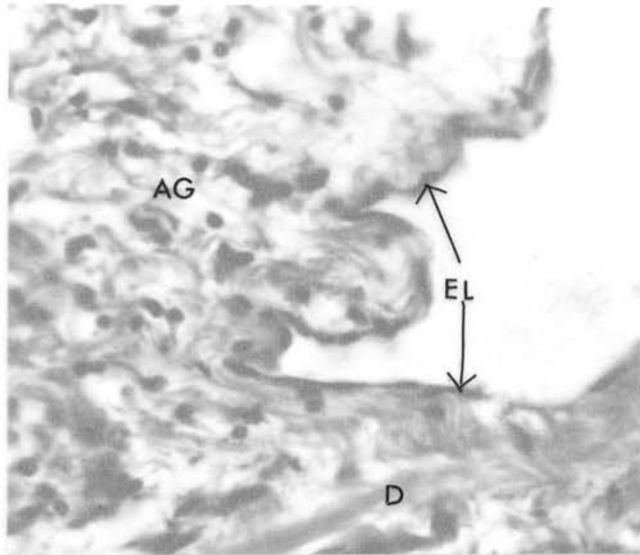


FIG. 8. Photomicrograph of a transverse section showing continuity of cells lining the dural venous sinus and those of the surface epithelium of a granulation. EL = endothelial layer. (paraffin embedded, H & E, x 530)

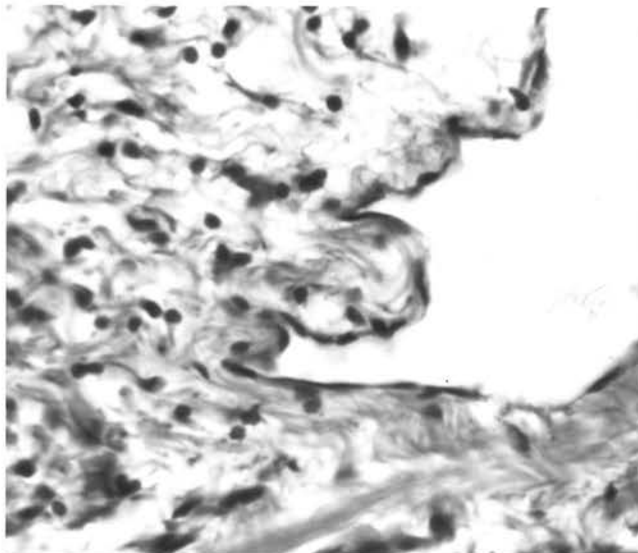


FIG. 8. Photomicrograph of a transverse section showing continuity of cells lining the dural venous sinus and those of the surface epithelium of a granulation. EL = endothelial layer. (paraffin embedded, H & E, x 530)

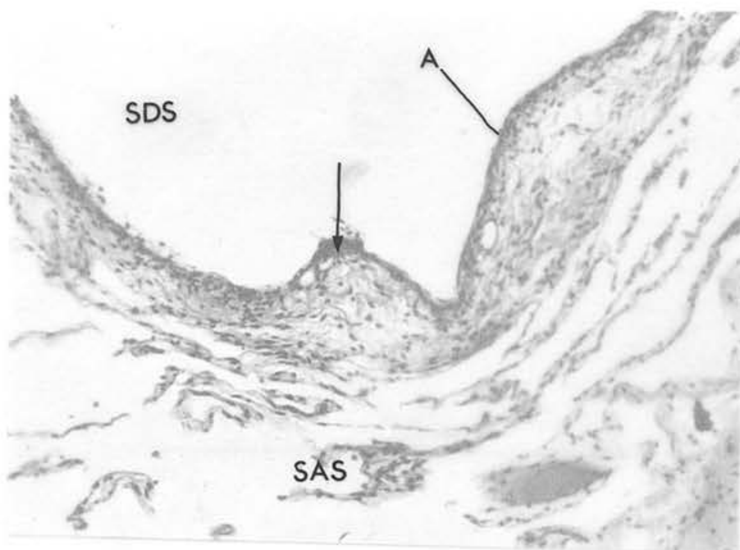


FIG. 9. Cell aggregations in arachnoid membrane of sheep, indicated by arrow.
(paraffin embedded, H & E, x 140)

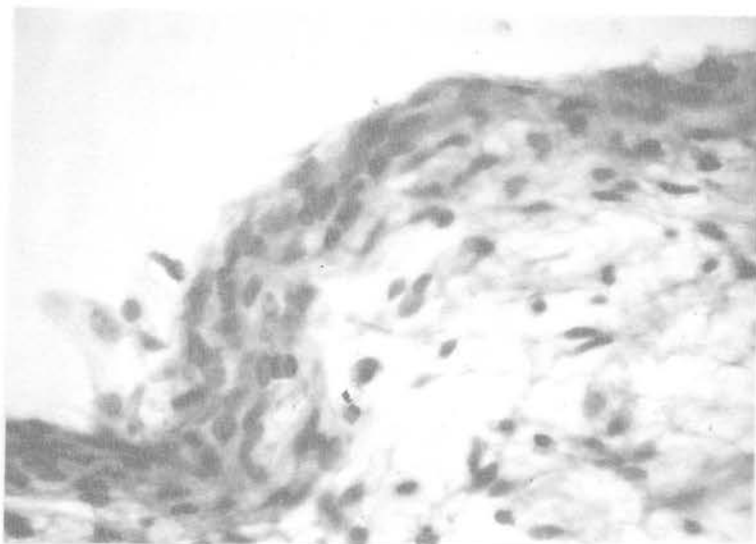


FIG. 9 a Cell aggregations in arachnoid membrane of sheep at a higher magnification.
(paraffin embedded, H & E, x 530)

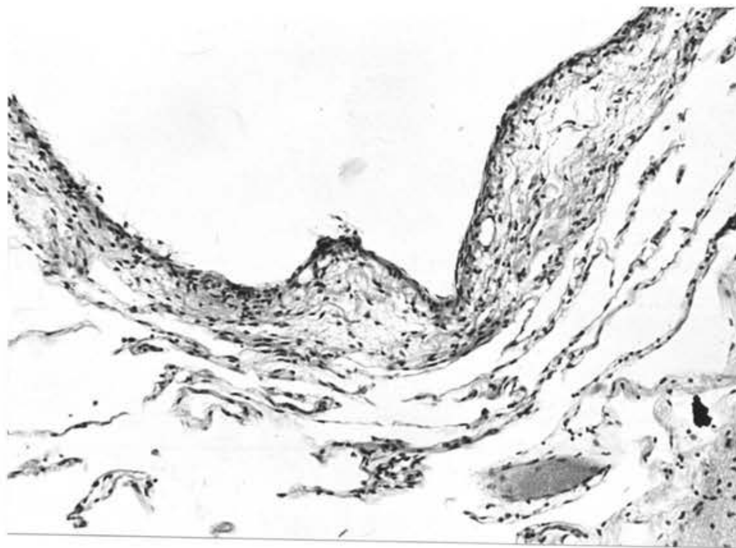


FIG. 9. Cell aggregations in arachnoid membrane of sheep, indicated by arrow.
(paraffin embedded, H & E, x 140)

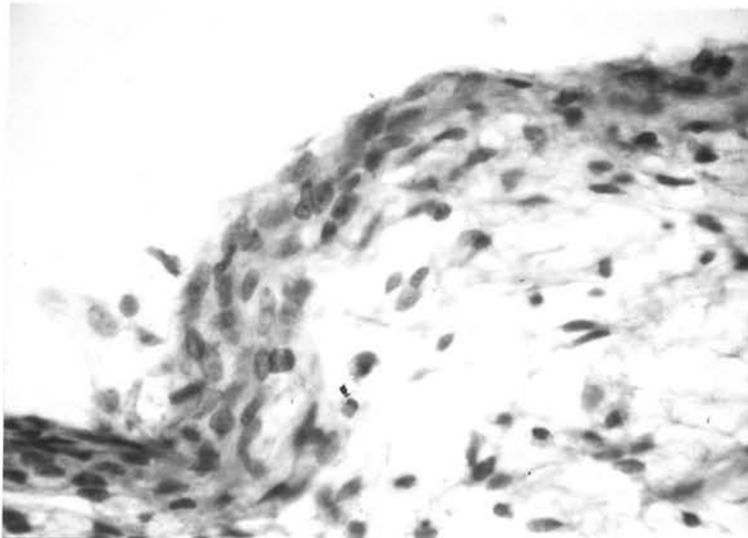


FIG. 9 a Cell aggregations in arachnoid membrane of sheep at a higher magnification.
(paraffin embedded, H & E, x 530)

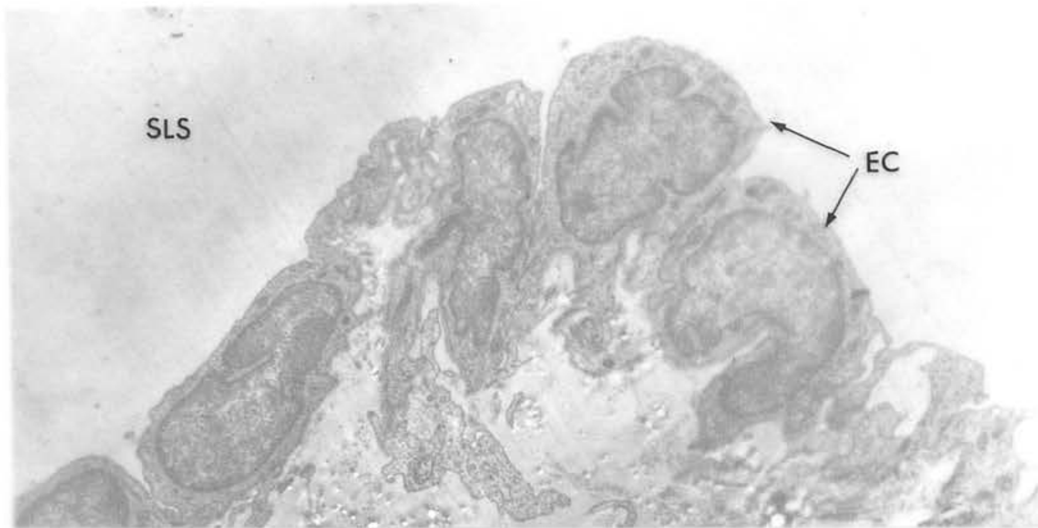
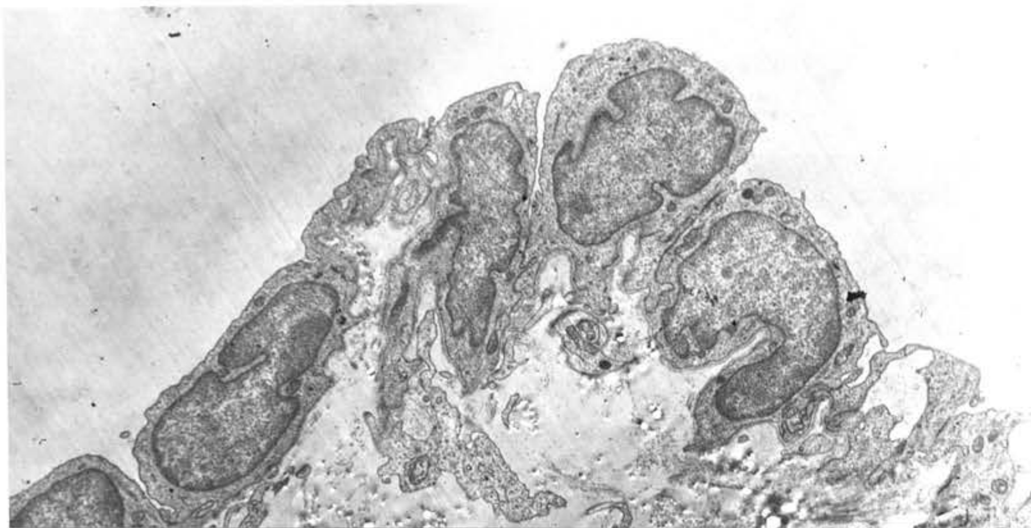


FIG. 10. Electron micrograph of surface epithelial cells of a sheep arachnoid granulation. (x 6,000)

FIG. 11. Electron micrograph of a surface epithelial cell of a sheep granulation. (x 21,000)



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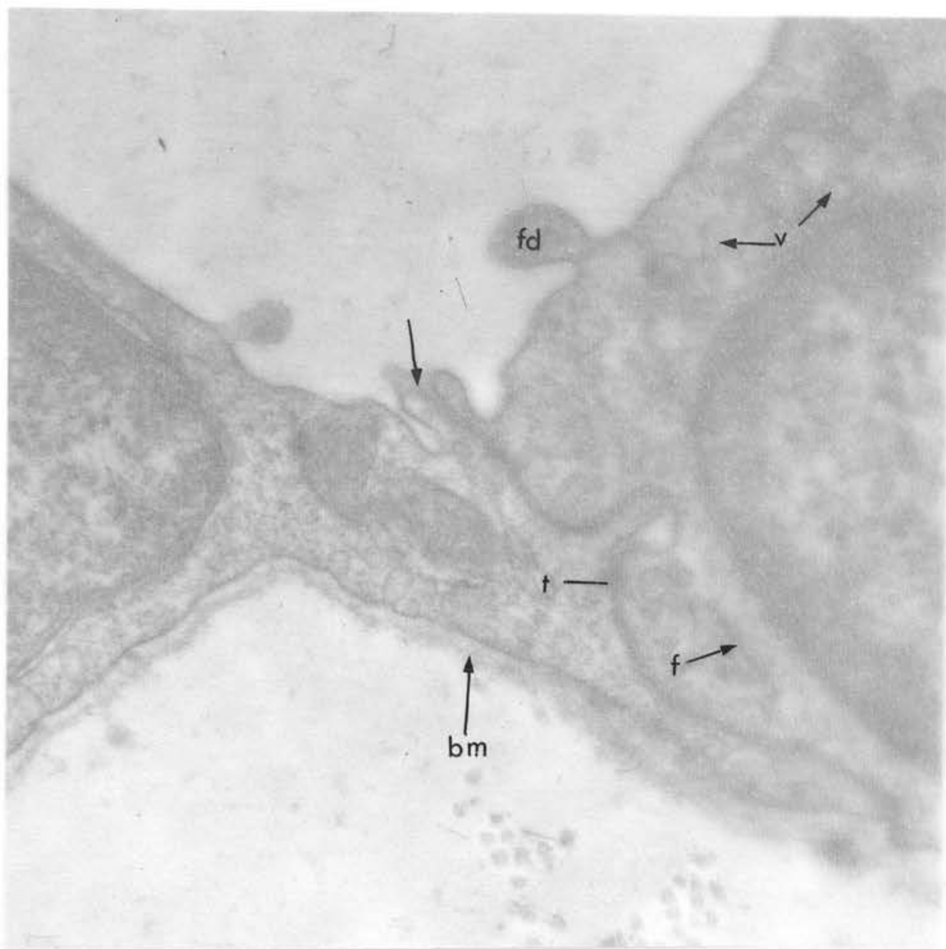

FIG. 12. Electron micrograph showing junction between two surface epithelial cells of a granulation. Unlettered arrow shows 'lipping' between two cells. fd = fold of plasma membrane. (x 105,000)

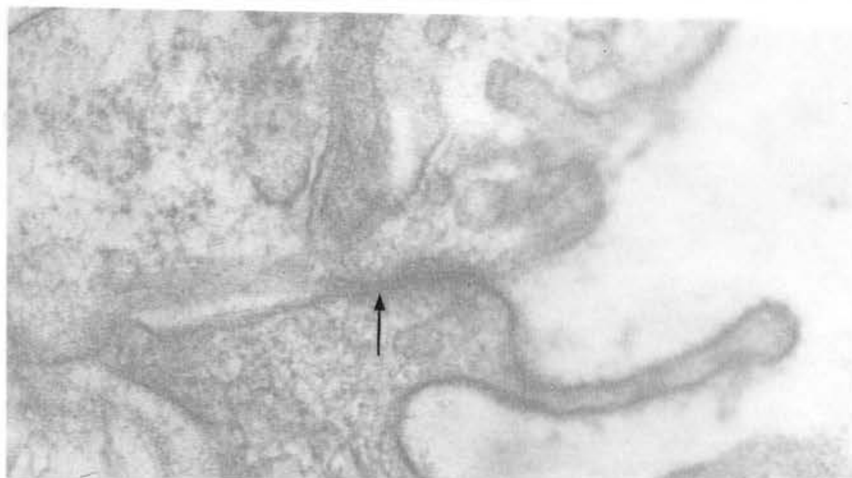

FIG. 13. Electron micrograph of a 'quintuple layered unit ' (arrow), separating two surface epithelial cells. (x 175,000)

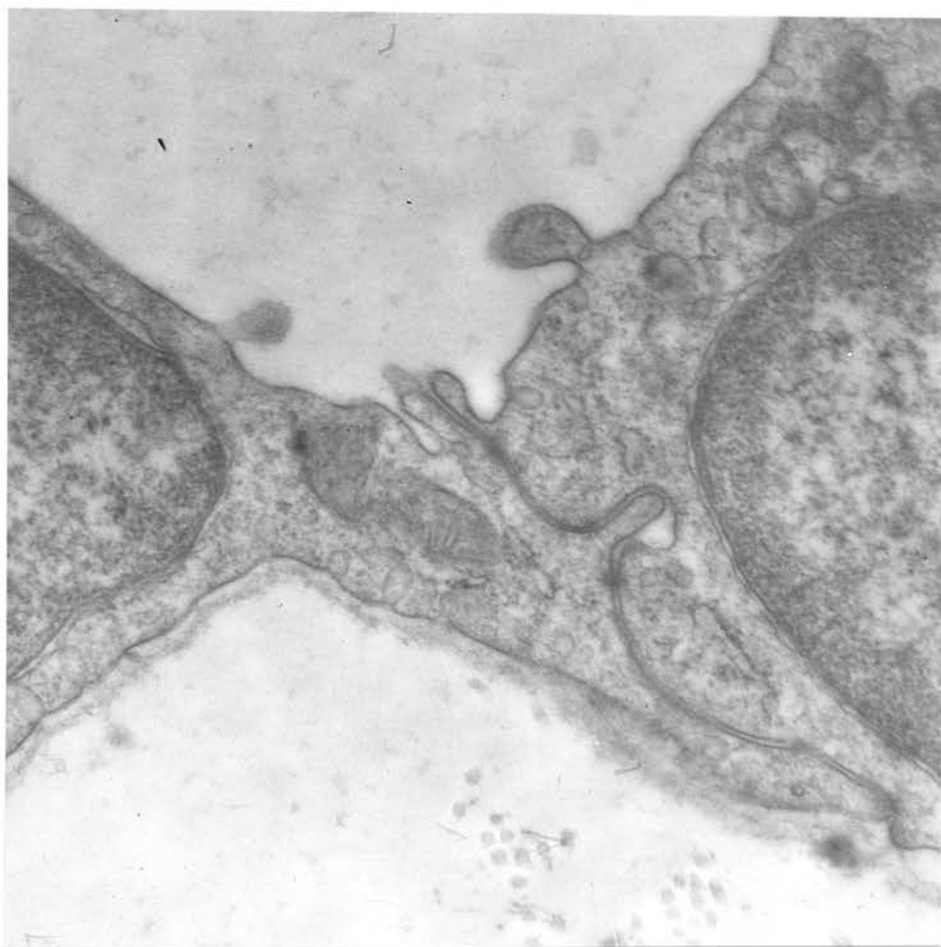

FIG. 12. Electron micrograph showing junction between two surface epithelial cells of a granulation. Unlettered arrow shows 'lipping' between two cells. fd = fold of plasma membrane. (x 105,000)

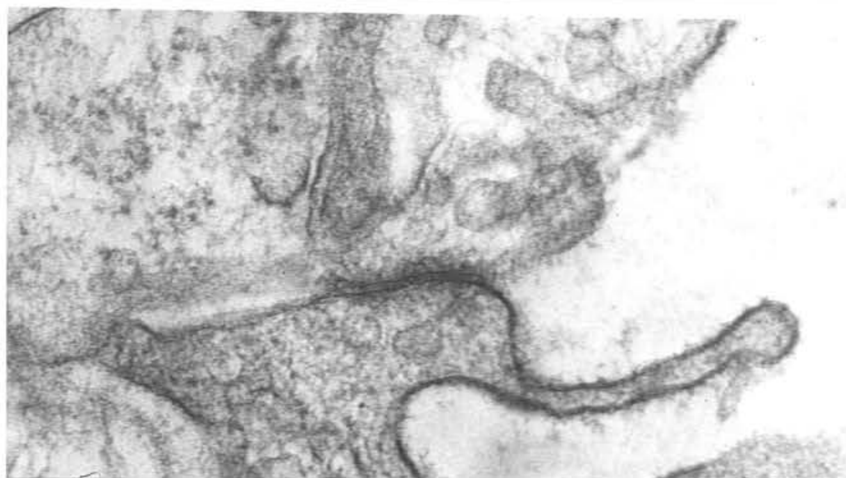

FIG. 13. Electron micrograph of a 'quintuple layered unit ' (arrow), separating two surface epithelial cells. (x 175,000)

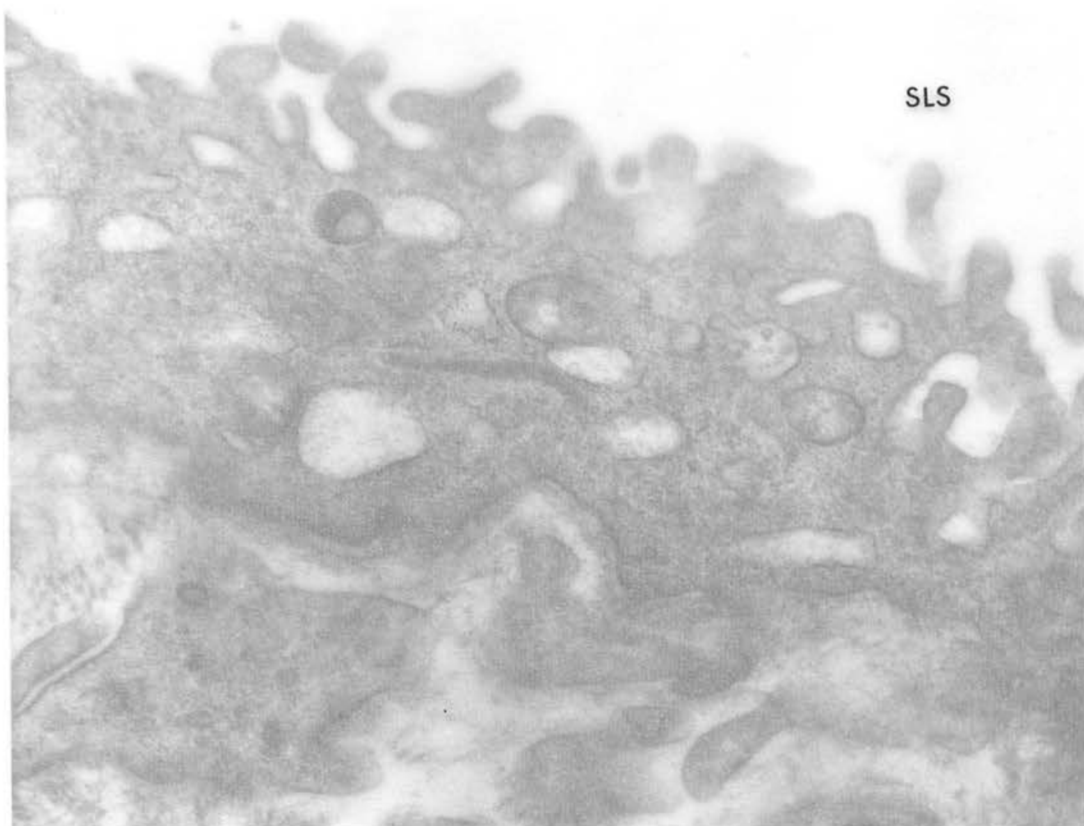

FIG. 14. Electron micrograph of a surface epithelial cell showing folds of the plasma membrane towards the venous sinus.
(x 56,000)

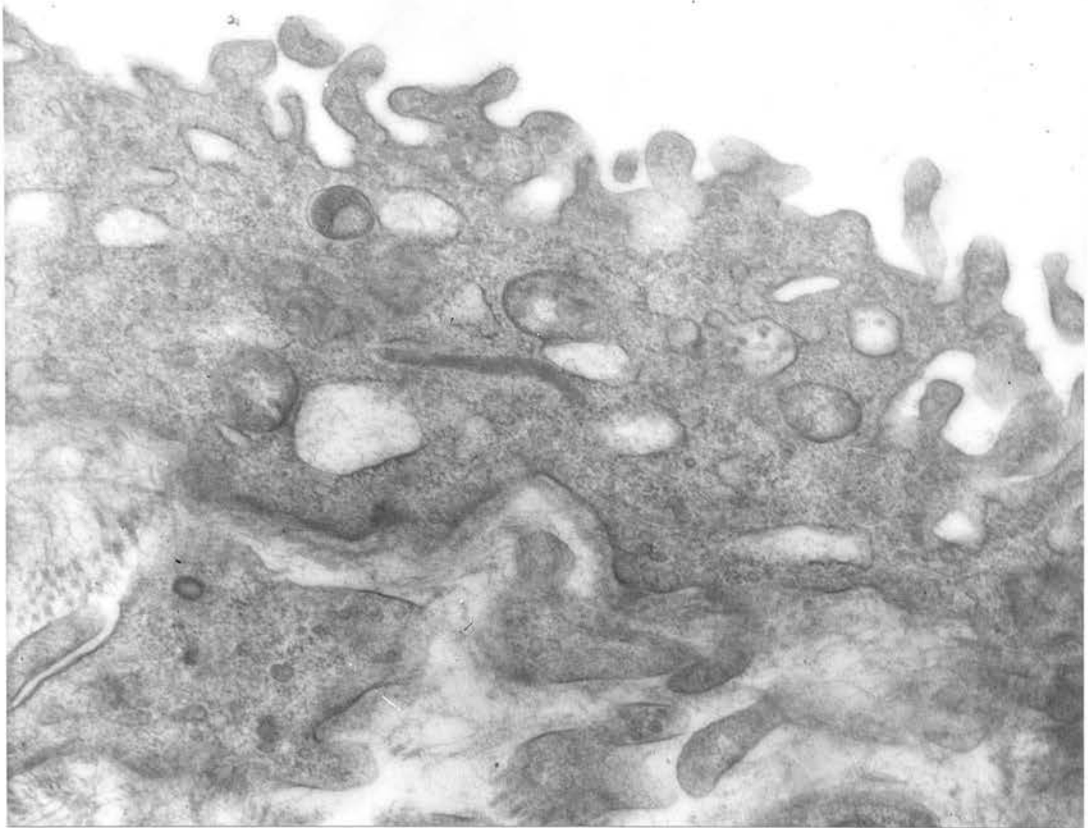

FIG. 14. Electron micrograph of a surface epithelial cell showing folds of the plasma membrane towards the venous sinus.
(x 56,000)

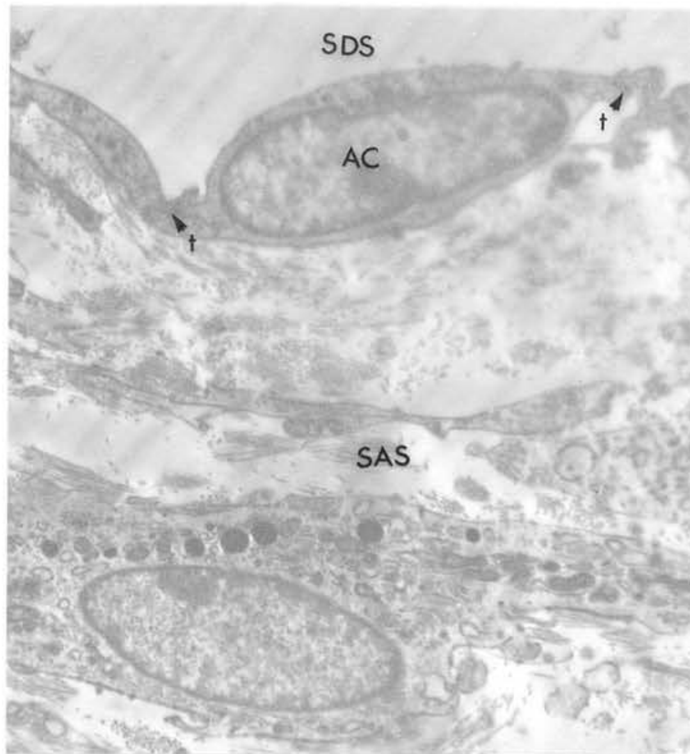

FIG. 15. Electron micrograph of arachnoid epithelial cells showing showing terminal bars(arrows) between adjacent cells.
(x 6,000)

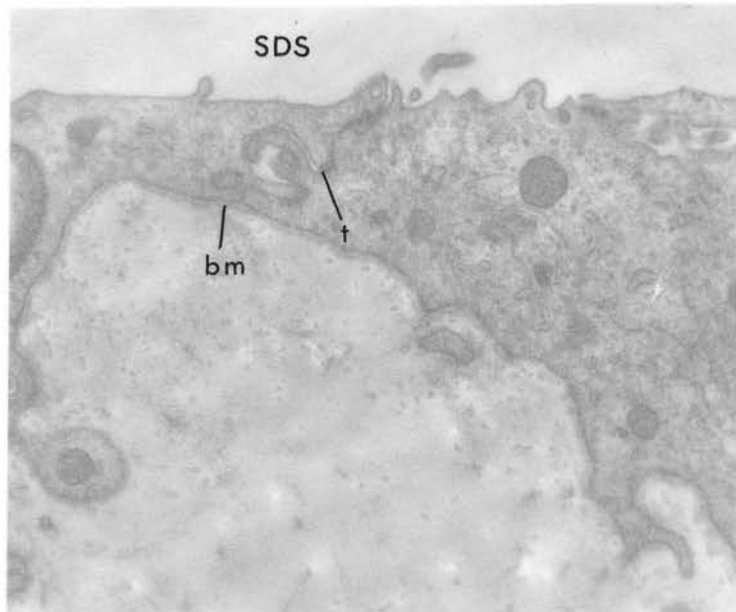

FIG. 16. Electron micrograph of junction between two arachnoid epithelial cells.
(x 28,000)

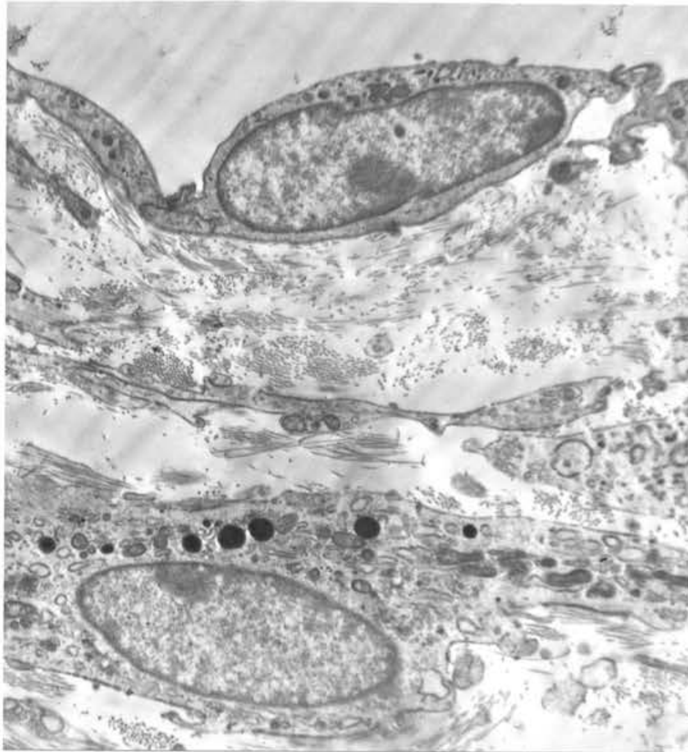

FIG. 15. Electron micrograph of arachnoid epithelial cells showing showing terminal bars(arrows) between adjacent cells.
(x 6,000)

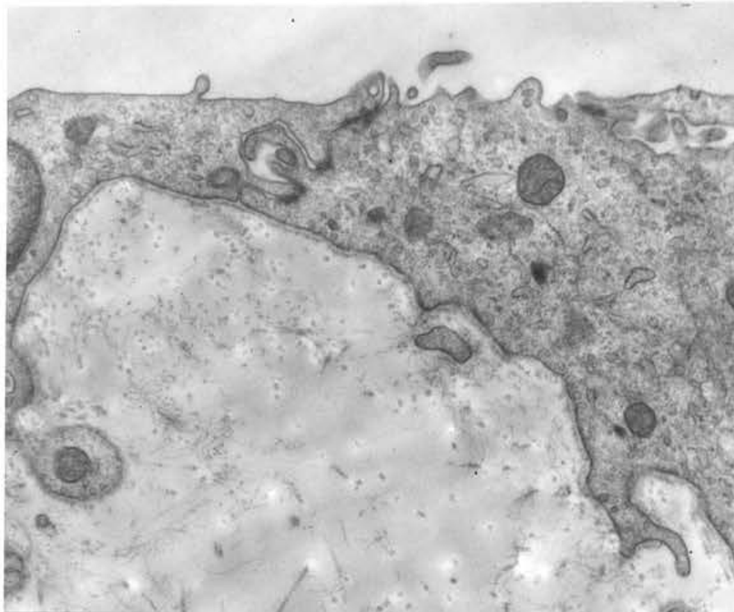

FIG. 16. Electron micrograph of junction between two arachnoid epithelial cells.
(x 28,000)

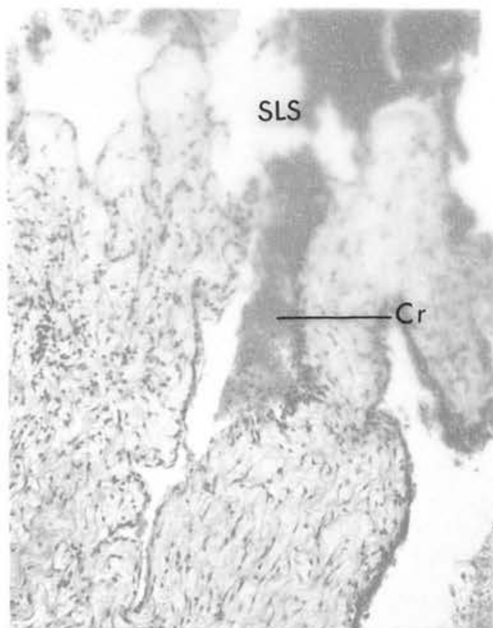

FIG. 17. Photomicrograph of a crypt in sheep arachnoid granulation, showing red cells within crypt. (paraffin embedded, H & E, x 140)

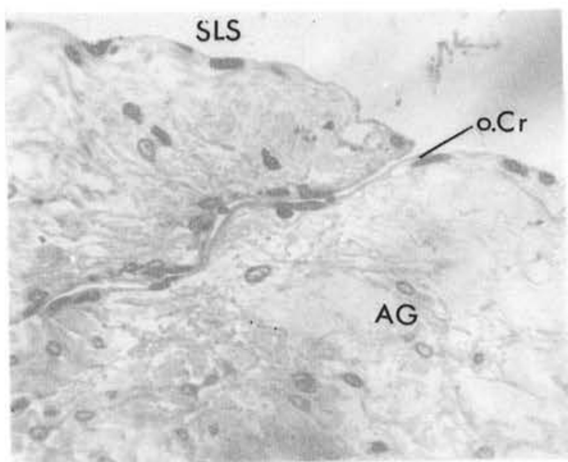

FIG. 18. Photomicrograph of opening of a narrow crypt on to the surface of the granulation. (araldite embedded, toluidene blue, x 580)

FIG. 17. Photomicrograph of a crypt in sheep arachnoid granulation, showing red cells within crypt. (paraffin embedded, H & E, x 140)

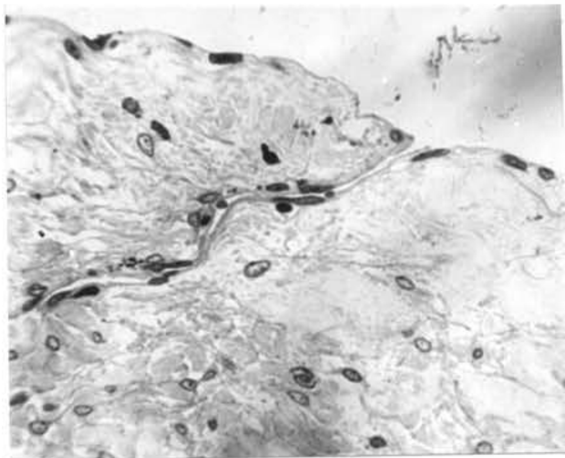

FIG. 18. Photomicrograph of opening of a narrow crypt on to the surface of the granulation. (araldite embedded, toluidene blue, x 580)

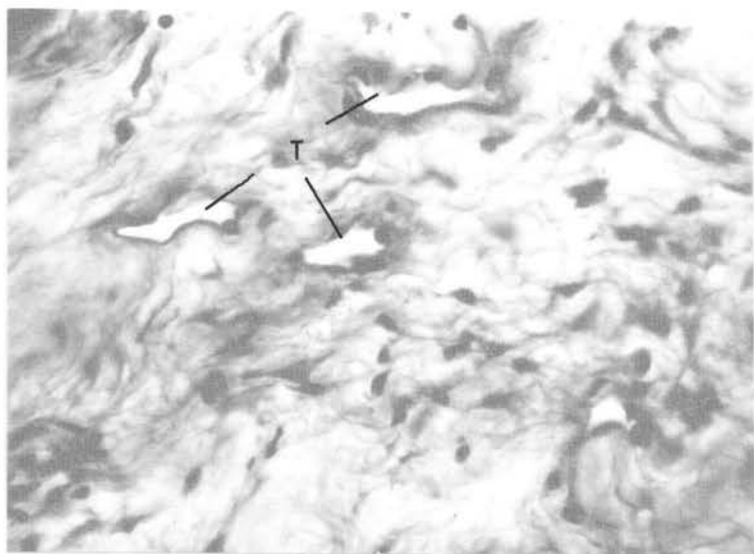

FIG. 19. Horizontal(tangential) section of sheep arachnoid granulation showing tubules in it's core.
(methacrylate embedded, H & E, x 350)

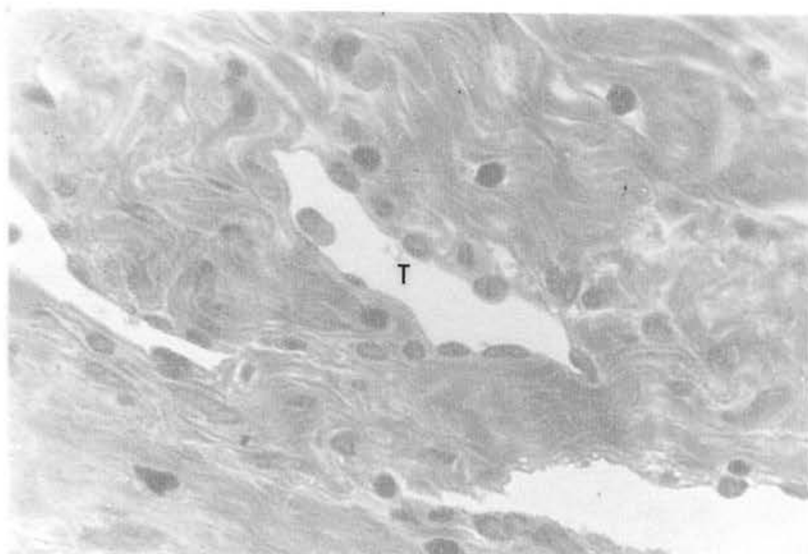

FIG. 20. Horizontal(tangential) section of tubule.
(methacrylate embedded, H & E, x 580)

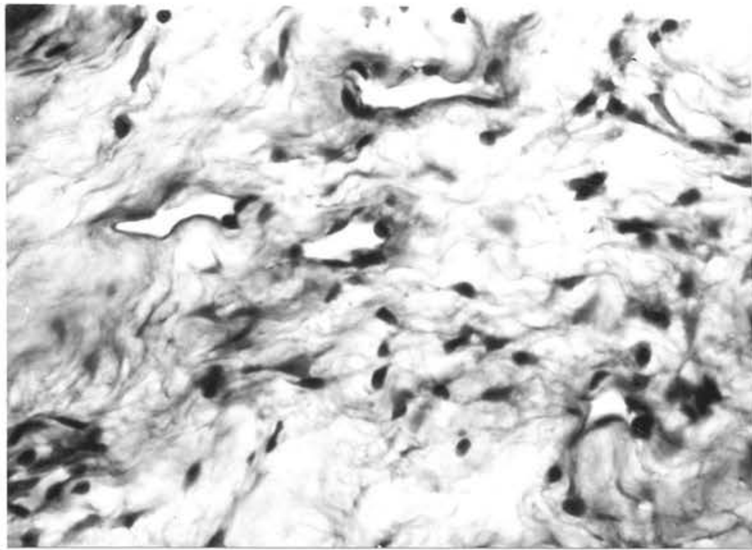

FIG. 19. Horizontal(tangential) section of sheep arachnoid granulation showing tubules in it's core.
(methacrylate embedded, H & E, x 350)

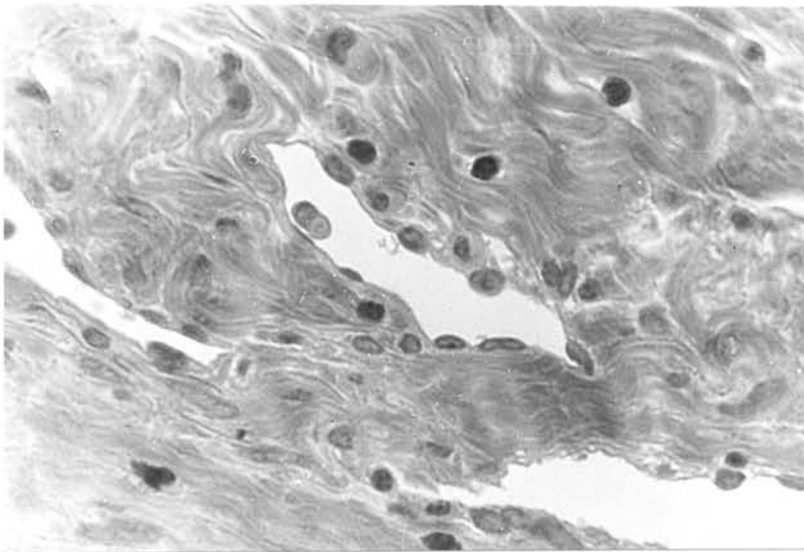

FIG. 20. Horizontal(tangential) section of tubule.
(methacrylate embedded, H & E, x 580)

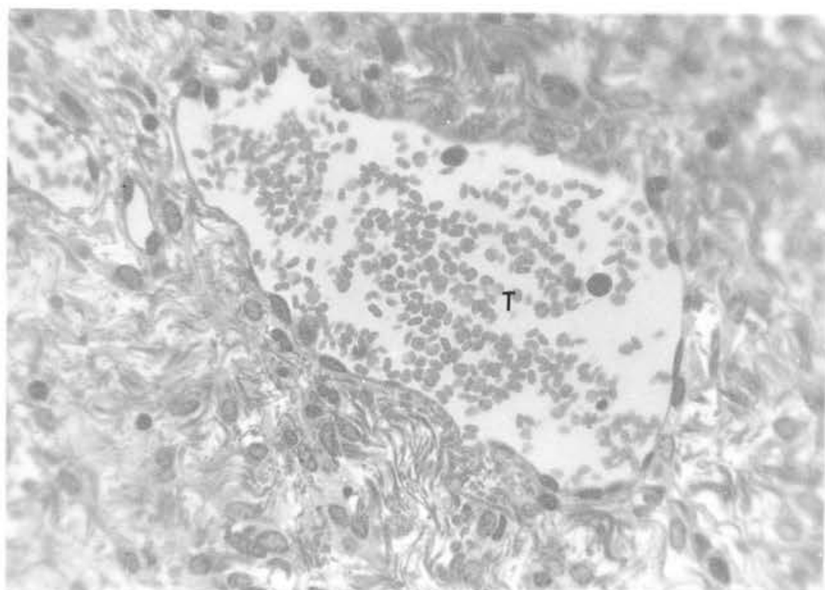

FIG. 21. Photomicrograph of a horizontal(tangential) section of a tubule towards the periphery of the granulation, showing red blood cells in the lumen. (methacrylate embedded, H & E, x 530)

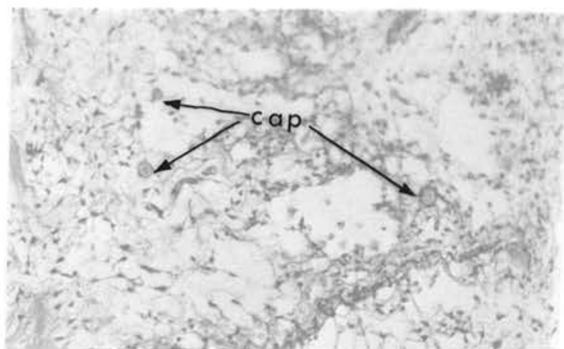

FIG. 22. Photomicrograph of core of arachnoid granulation showing capillaries. (paraffin embedded, H & E, x 140)

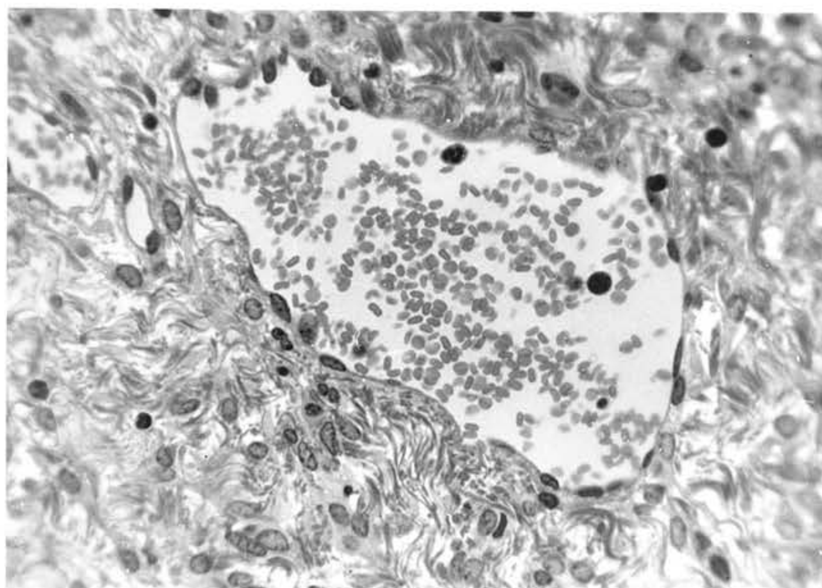

FIG. 21. Photomicrograph of a horizontal(tangential) section of a tubule towards the periphery of the granulation, showing red blood cells in the lumen. (methacrylate embedded, H & E, x 530)

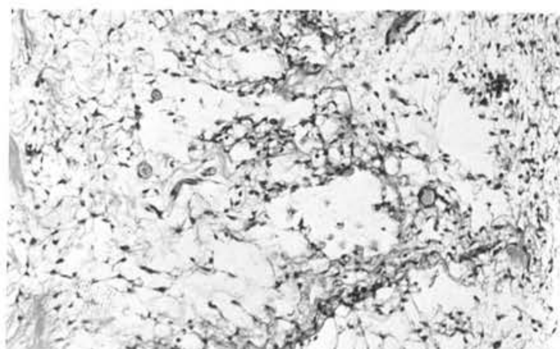

FIG. 22. Photomicrograph of core of arachnoid granulation showing capillaries. (paraffin embedded, H & E, x 140)

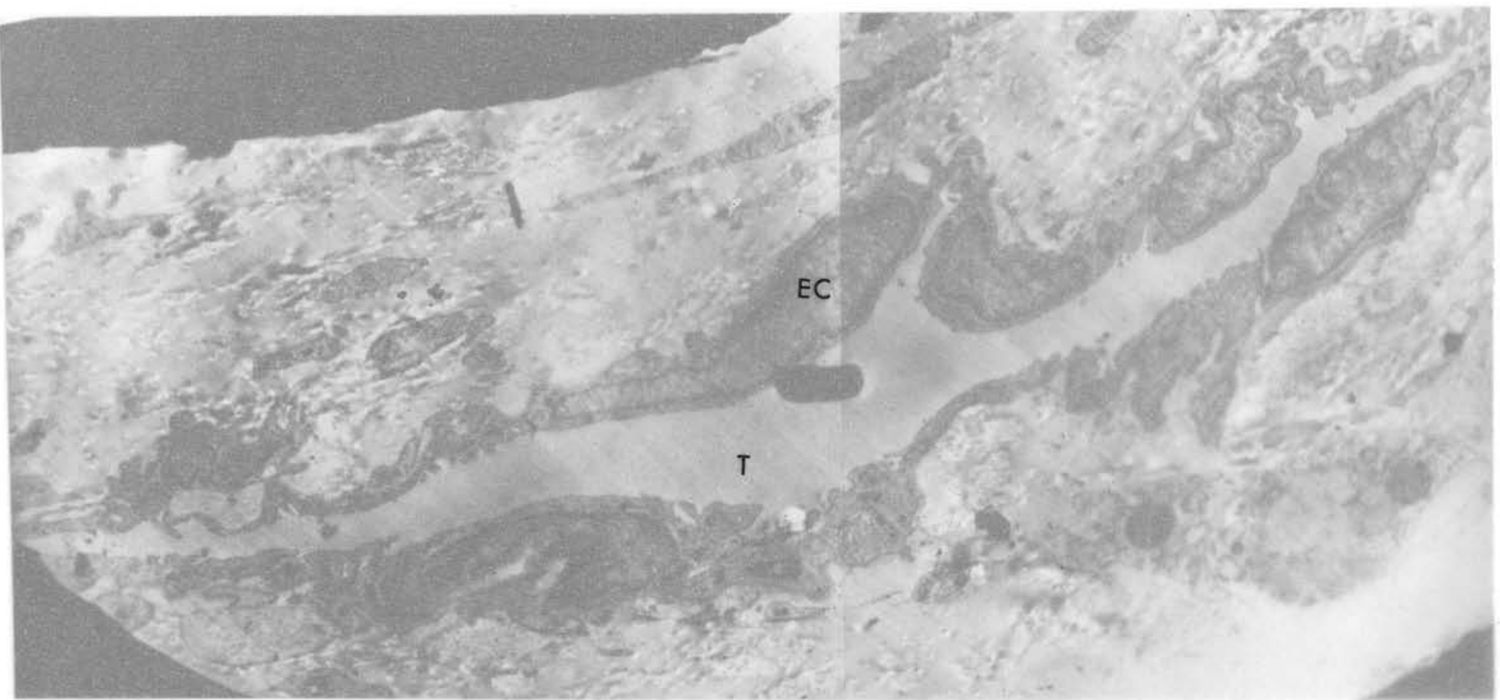

FIG. 23. Electron micrograph of a transverse(tangential) section of a tubule in sheep arachnoid granulation, showing bacterium in the lumen. Montage. (x 4,500)

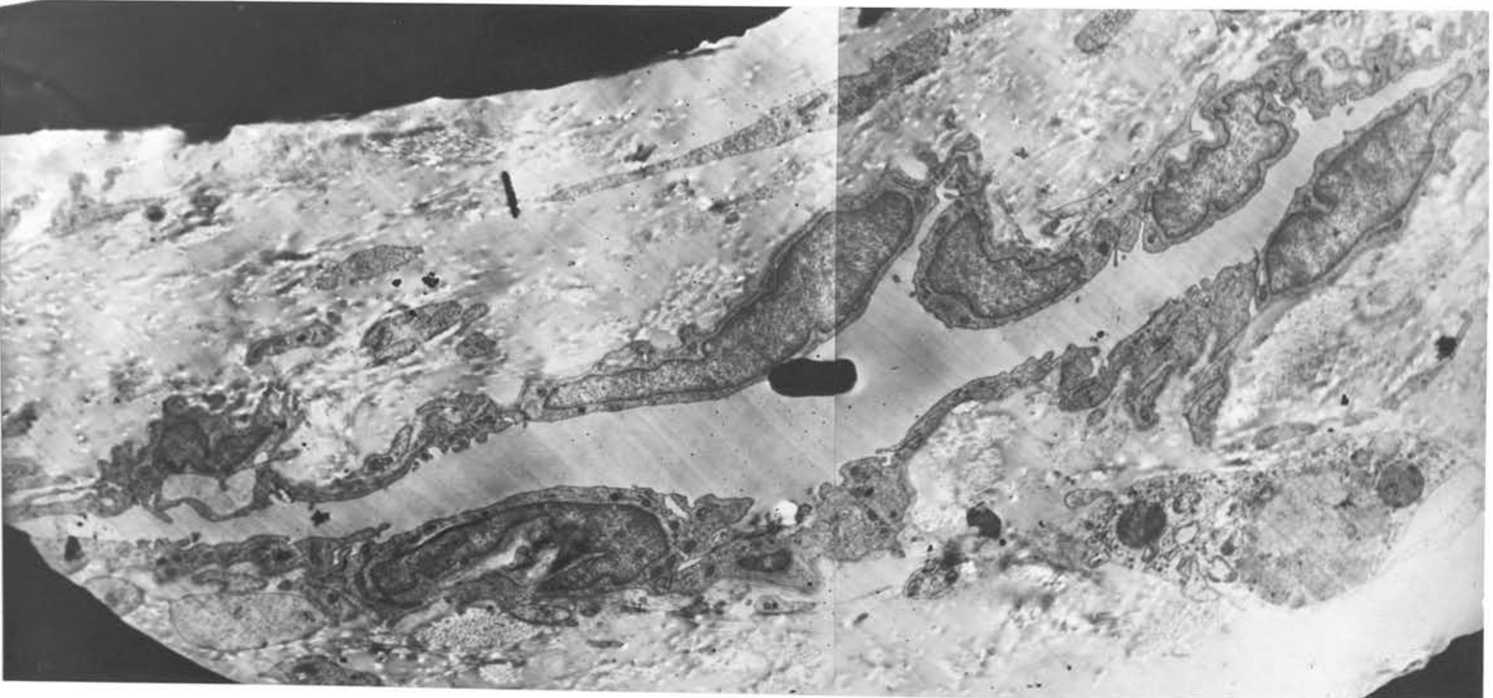

FIG. 23. Electron micrograph of a transverse(tangential) section of a tubule in sheep arachnoid granulation, showing bacterium in the lumen. Montage. (x 4,500)

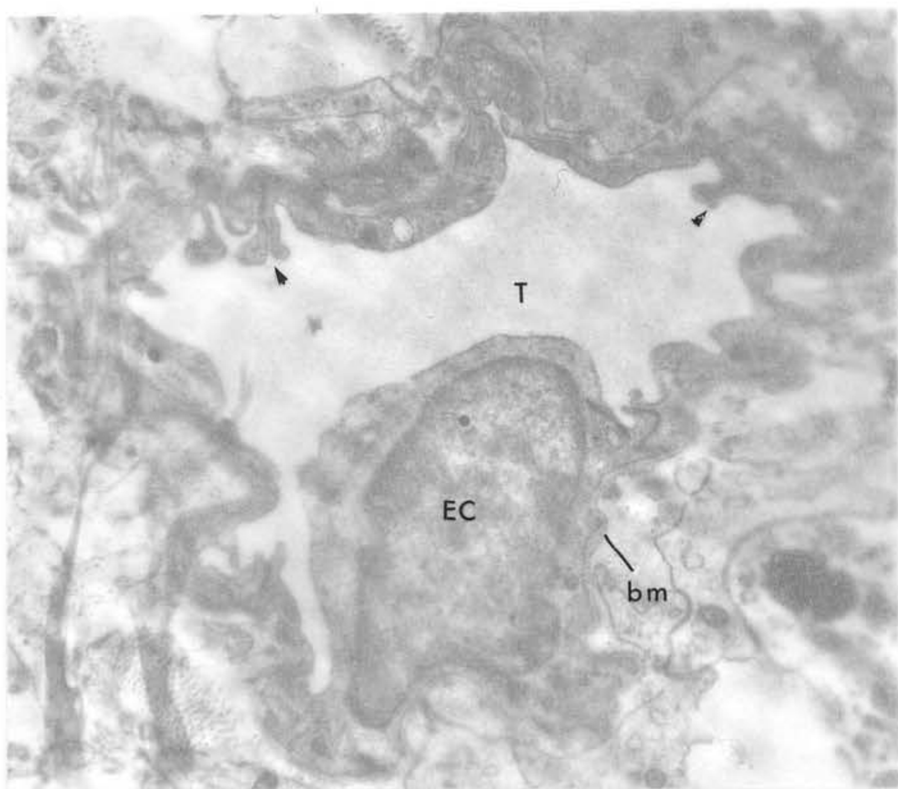

FIG. 24. Electron micrograph of a tubule in sheep arachnoid granulation. Arrow head shows terminal bars and 'lipping' between adjacent cells.
(x 16,000)

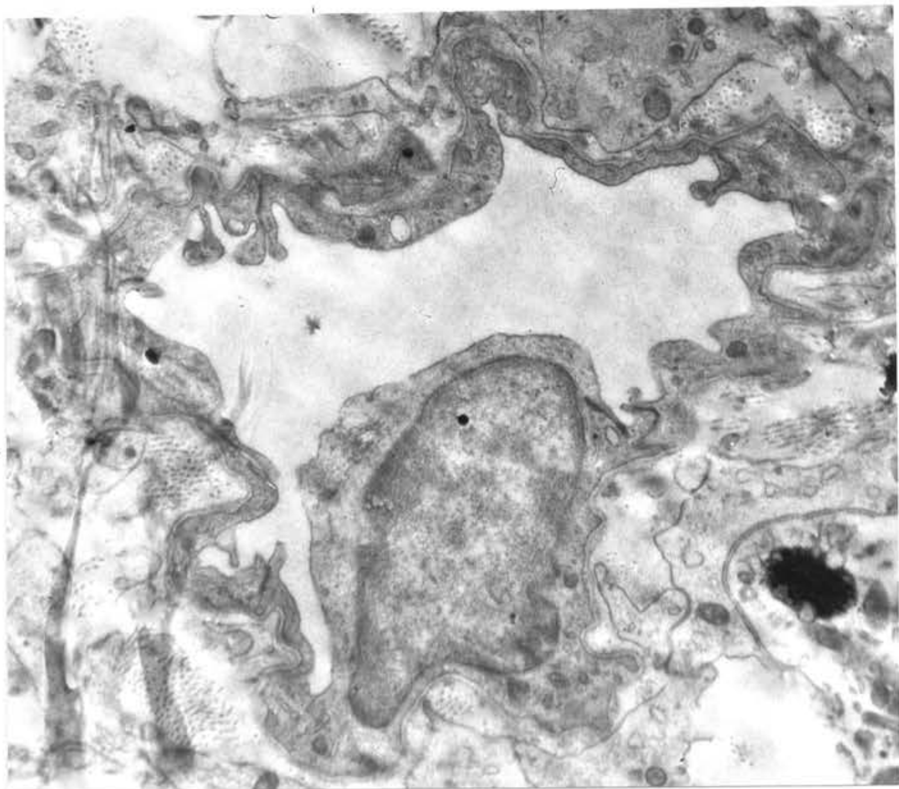

FIG. 24. Electron micrograph of a tubule in sheep arachnoid granulation. Arrow head shows terminal bars and 'lipping' between adjacent cells.
(x 16,000)

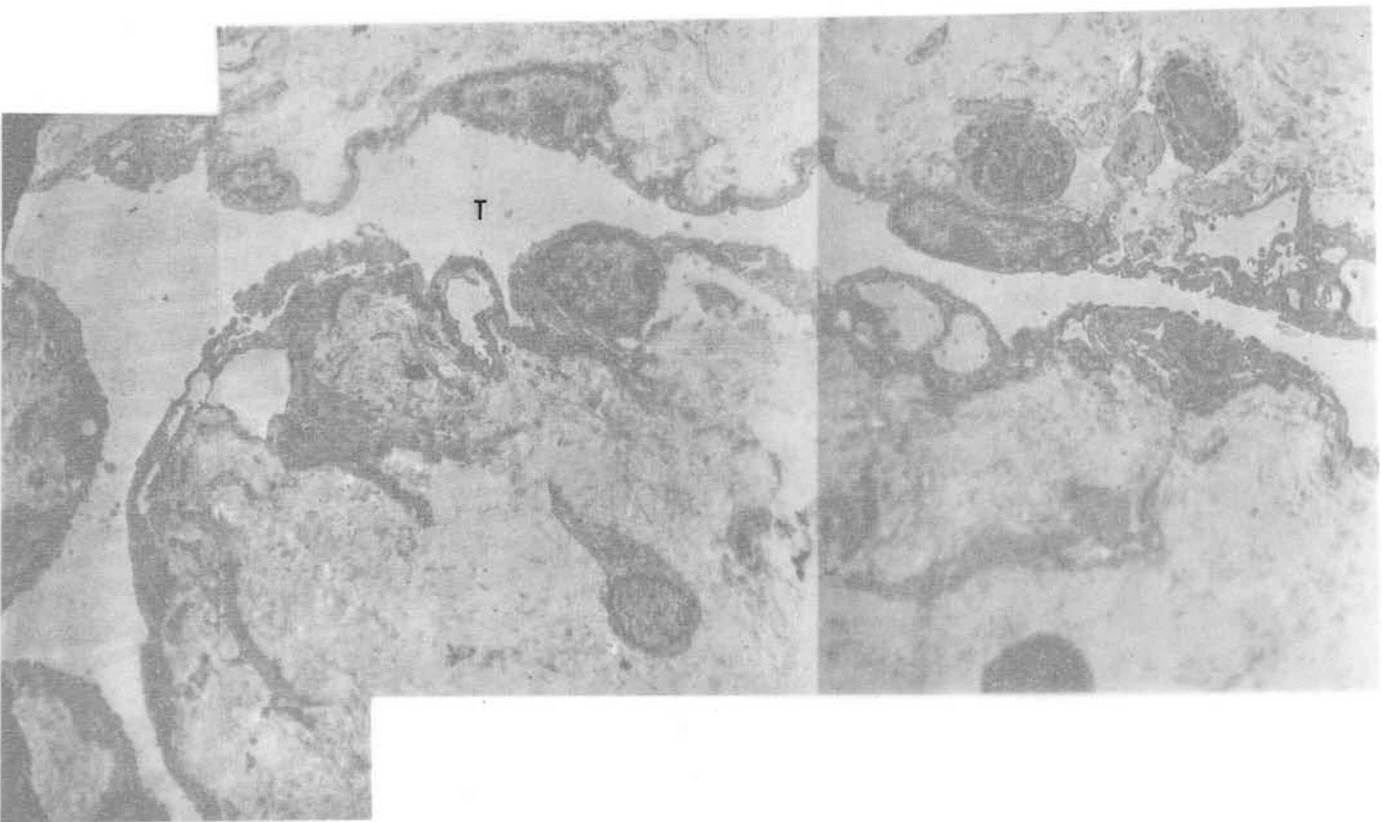

FIG. 25. Electron micrograph montage of a longitudinal section of a tubule in sheep arachnoid granulation. (x 4,000)

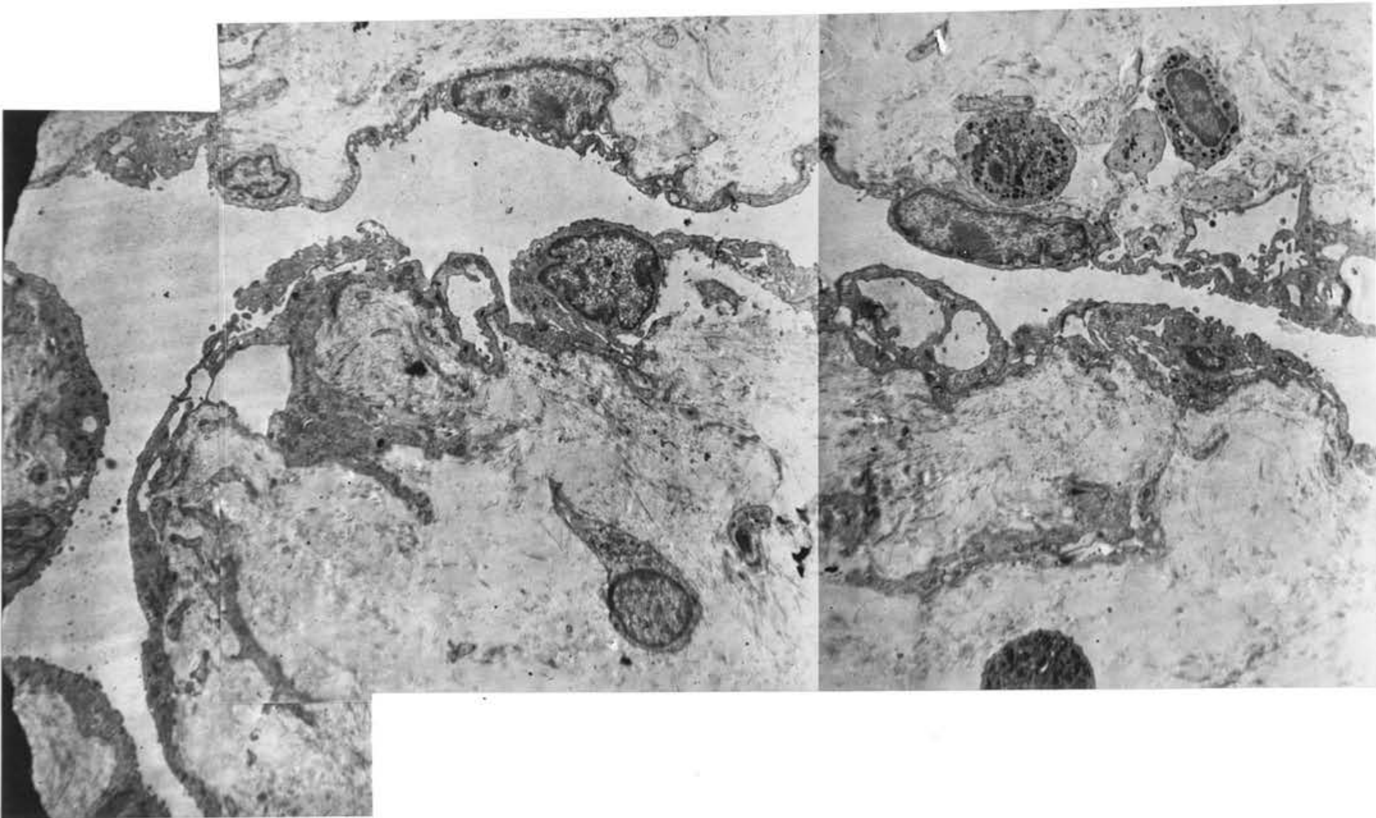

FIG. 25. Electron micrograph montage of a longitudinal section of a tubule in sheep arachnoid granulation. (x 4,000)

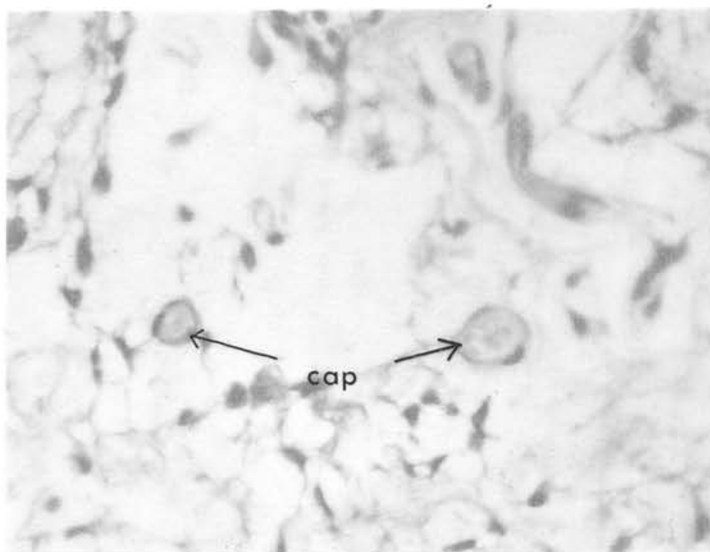

FIG. 26. Photomicrograph showing transverse section of capillaries in the core of the granulation indicated by arrows.
(paraffin embedded, H & E, x 530)

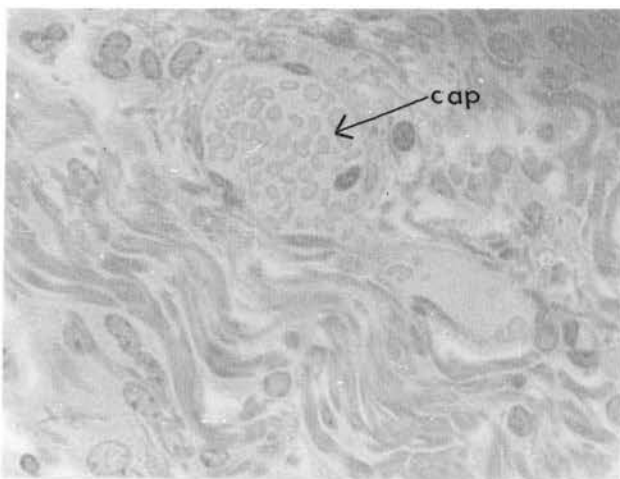

FIG. 27. Photomicrograph of a large capillary in transverse section, found in sheep arachnoid granulation.
(methacrylate embedded, H & E, x 600)

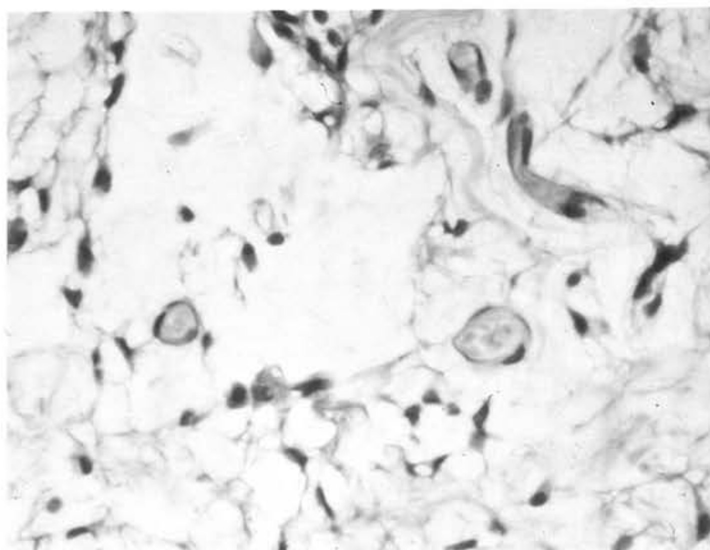

FIG. 26. Photomicrograph showing transverse section of capillaries in the core of the granulation indicated by arrows.
(paraffin embedded, H & E, x 530)

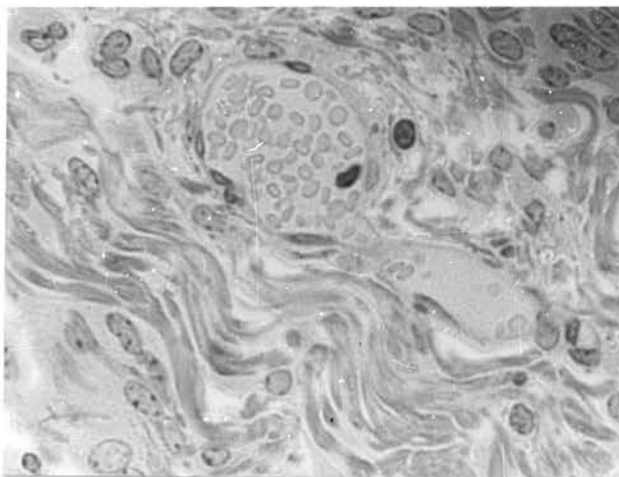

FIG. 27. Photomicrograph of a large capillary in transverse section, found in sheep arachnoid granulation.
(methacrylate embedded, H & E, x 600)

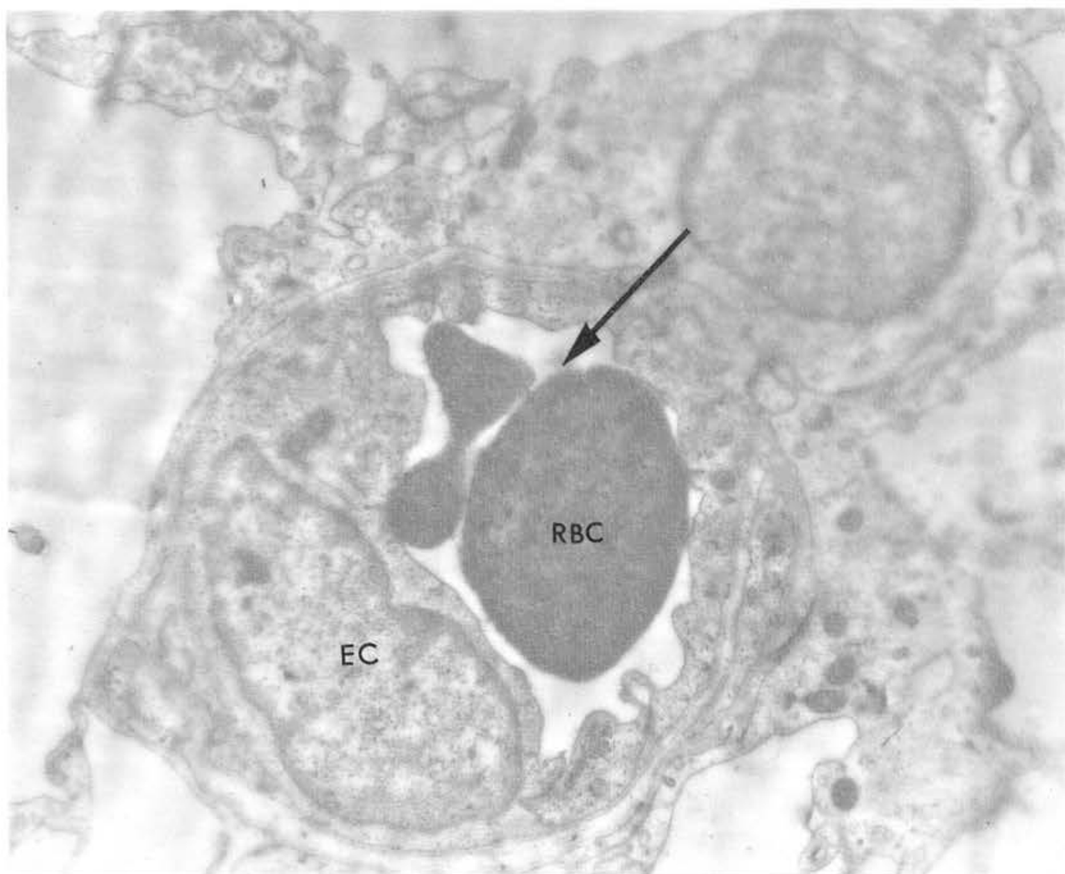

FIG. 28. Electron micrograph of a capillary with two red cells(RBC) in the lumen(arrow); sheep arachnoid granulation.
(x 14,000)

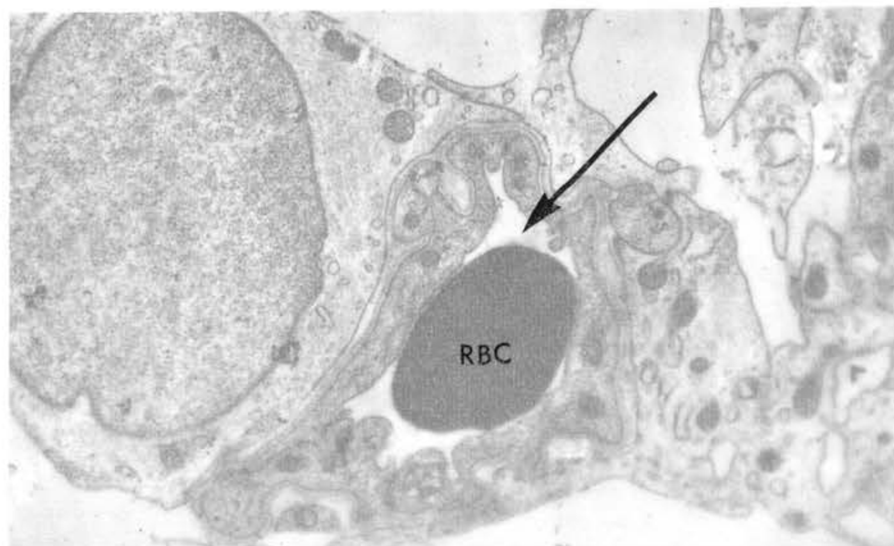

FIG. 29. Electron micrograph of a capillary with a red blood cell(RBC) in the lumen, indicated by arrow.
(x 10,000)

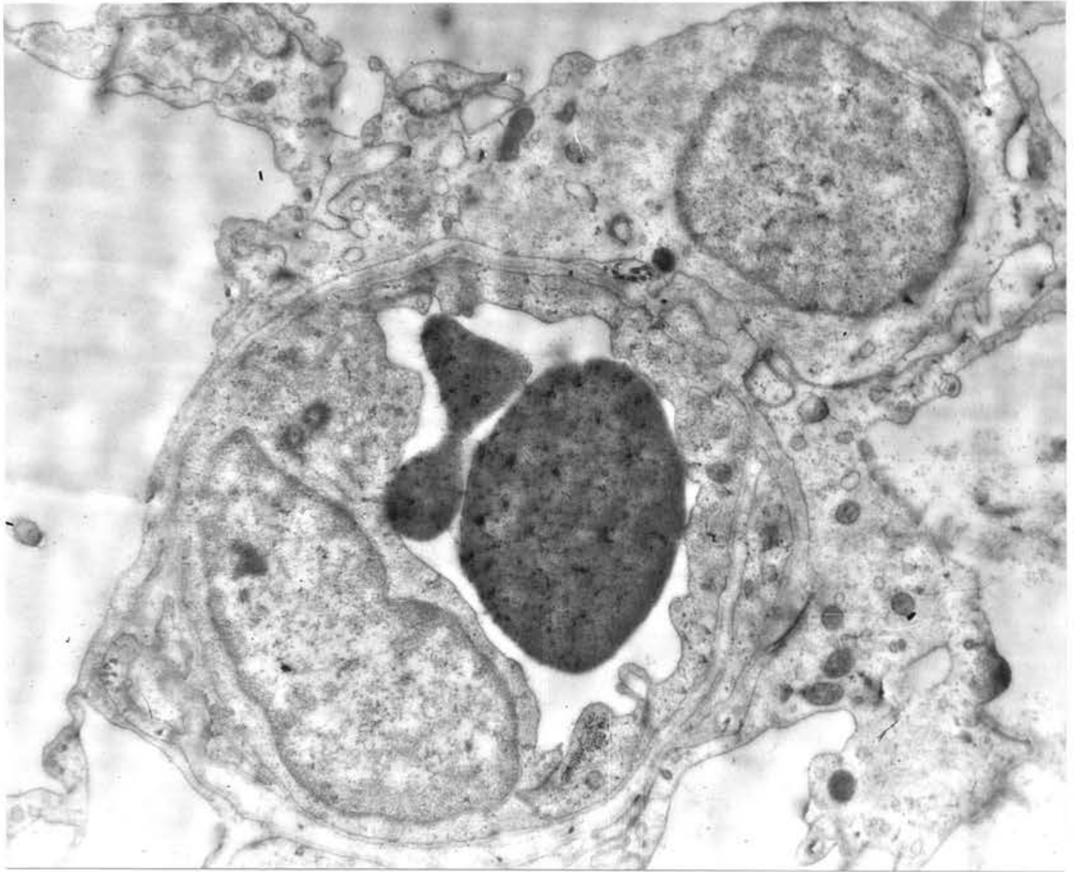

FIG. 28. Electron micrograph of a capillary with two red cells(RBC) in the lumen(arrow); sheep arachnoid granulation.
(x 14,000)

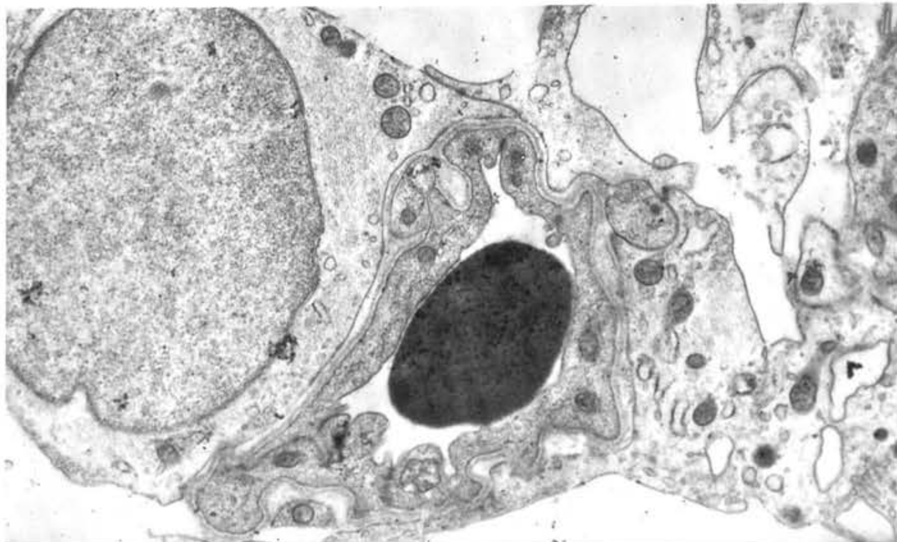

FIG. 29. Electron micrograph of a capillary with a red blood cell(RBC) in the lumen, indicated by arrow.
(x 10,000)

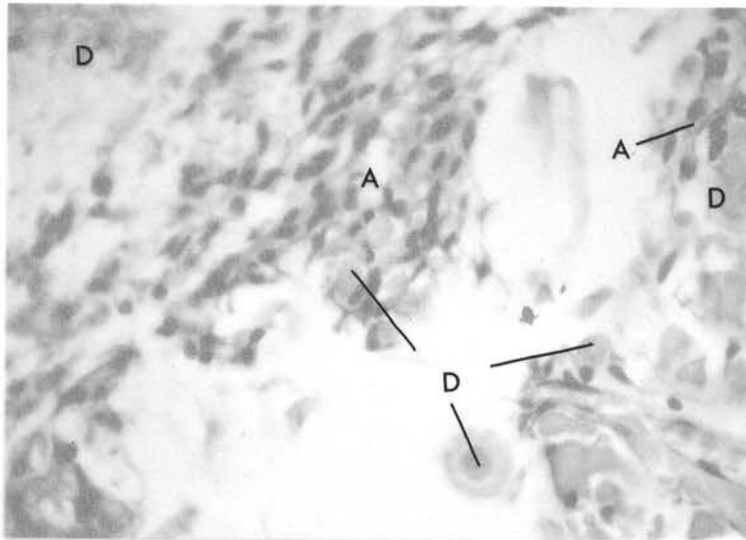

FIG. 30. Photomicrograph of a horizontal(tangential) section of the dura at the point of emergence of arachnoid tissue.
(paraffin embedded, H & E, x 530)

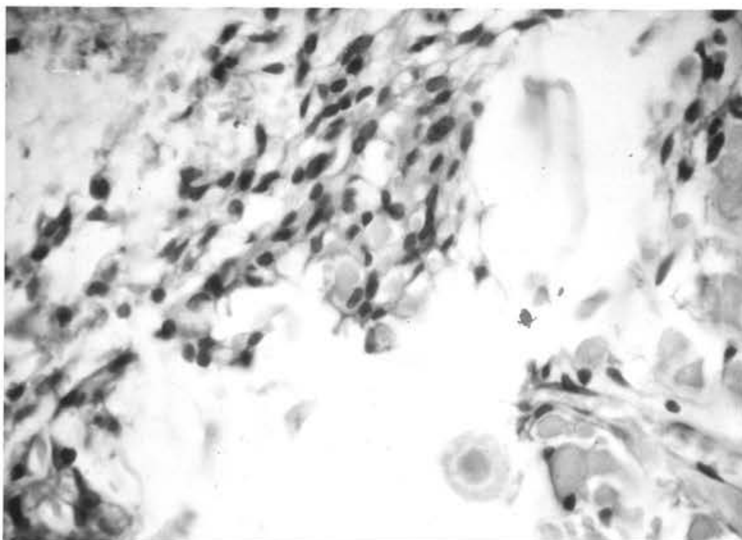

FIG. 30. Photomicrograph of a horizontal(tangential) section of the dura at the point of emergence of arachnoid tissue.
(paraffin embedded, H & E, x 530)

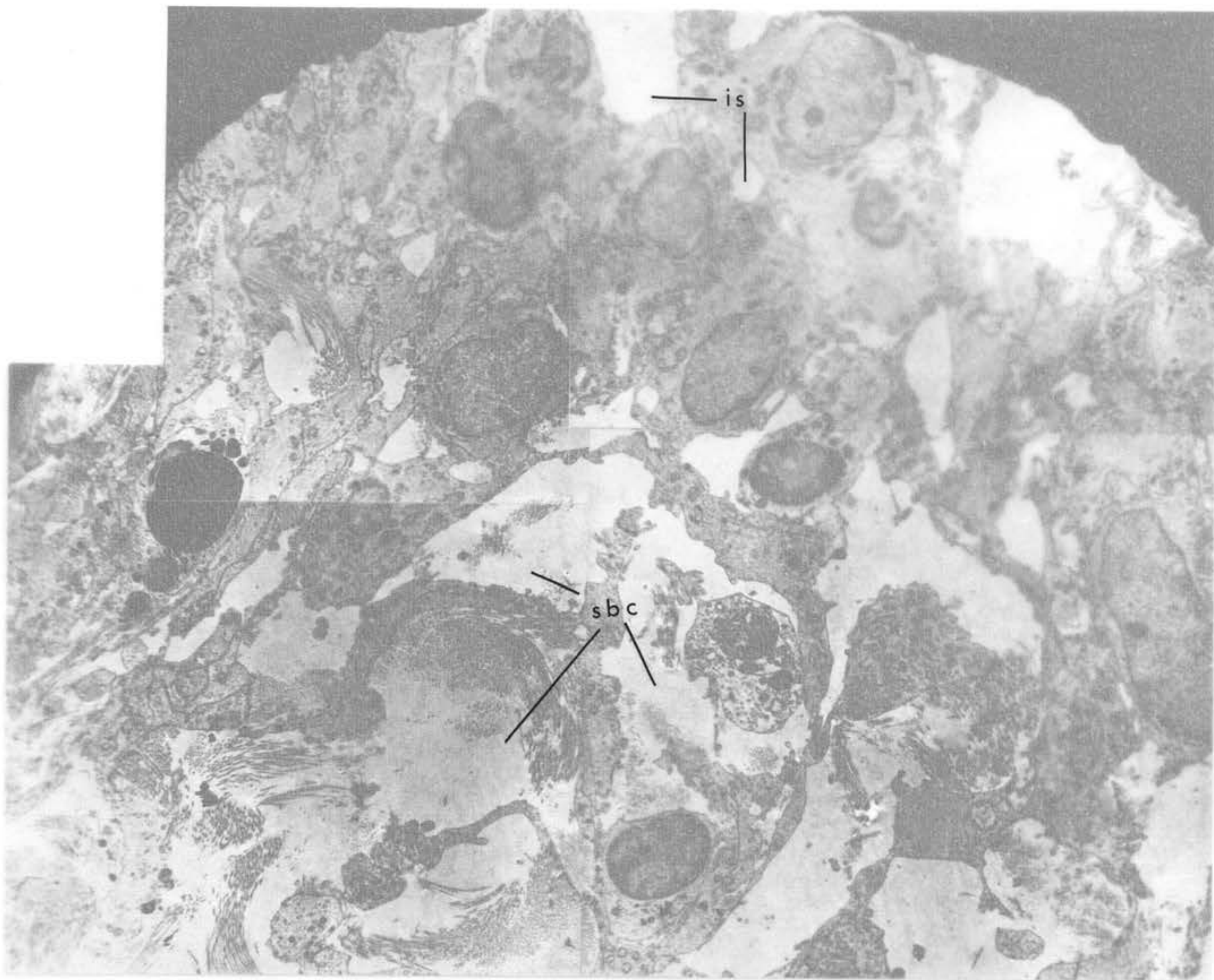

FIG. 31. Electron micrograph montage of core of sheep arachnoid granulation. sbc = spaces between collagen bundles, is = inter-cellular spaces. (x 4,000)

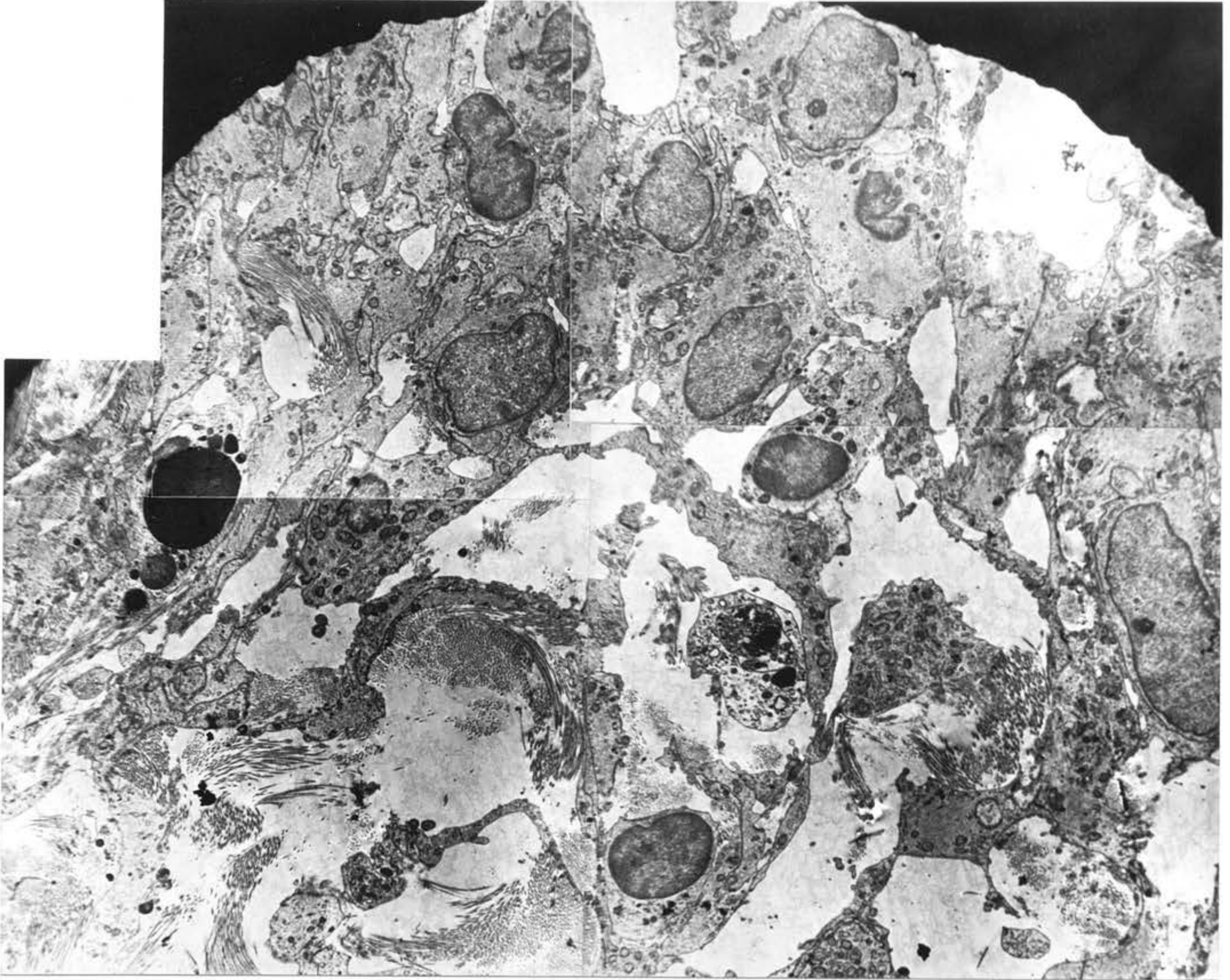

FIG. 31. Electron micrograph montage of core of sheep arachnoid granulation. sbc = spaces between collagen bundles, is = inter-cellular spaces. (x 4,000)

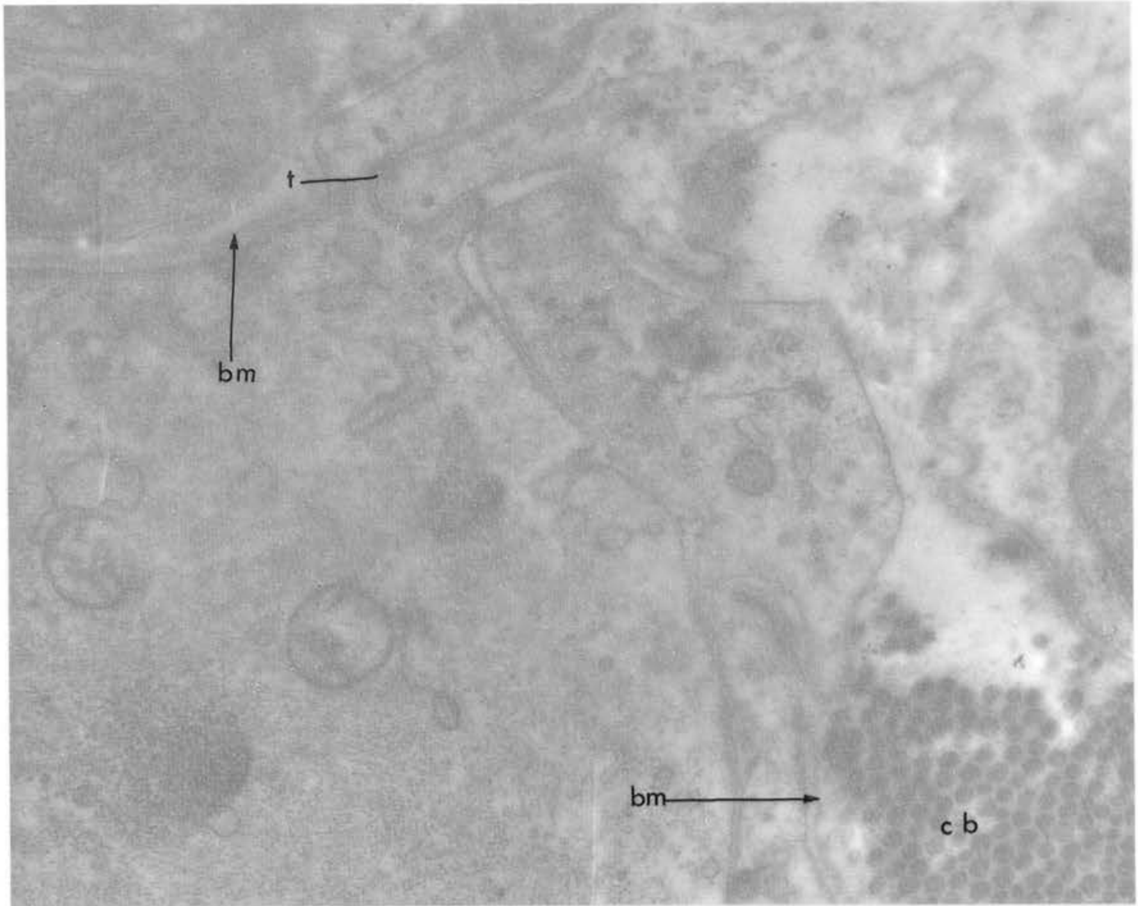

FIG. 32. Electron micrograph showing mesothelial cell lying on basement membrane and separated from an adjacent mesothelial cell by terminal bars. (x 64,000)

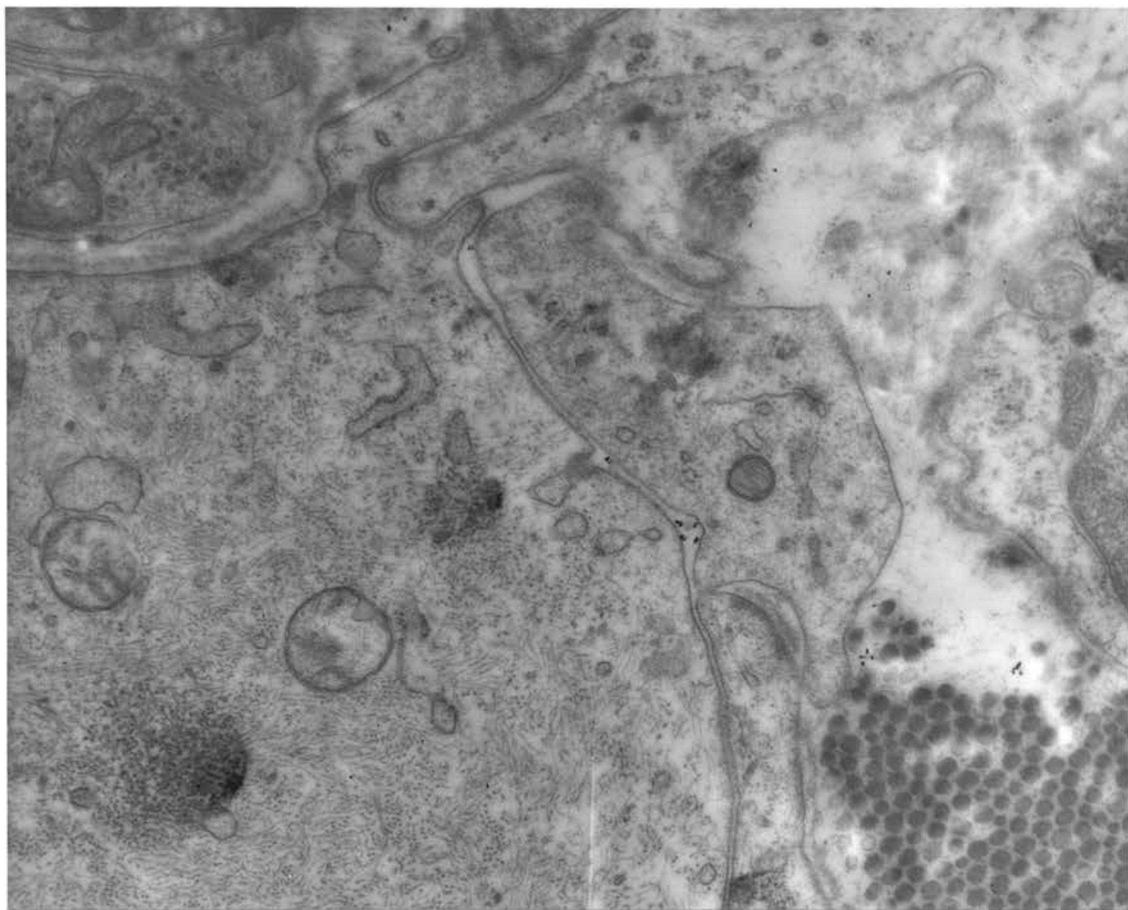

FIG. 32. Electron micrograph showing mesothelial cell lying on basement membrane and separated from an adjacent mesothelial cell by terminal bars. (x 64,000)

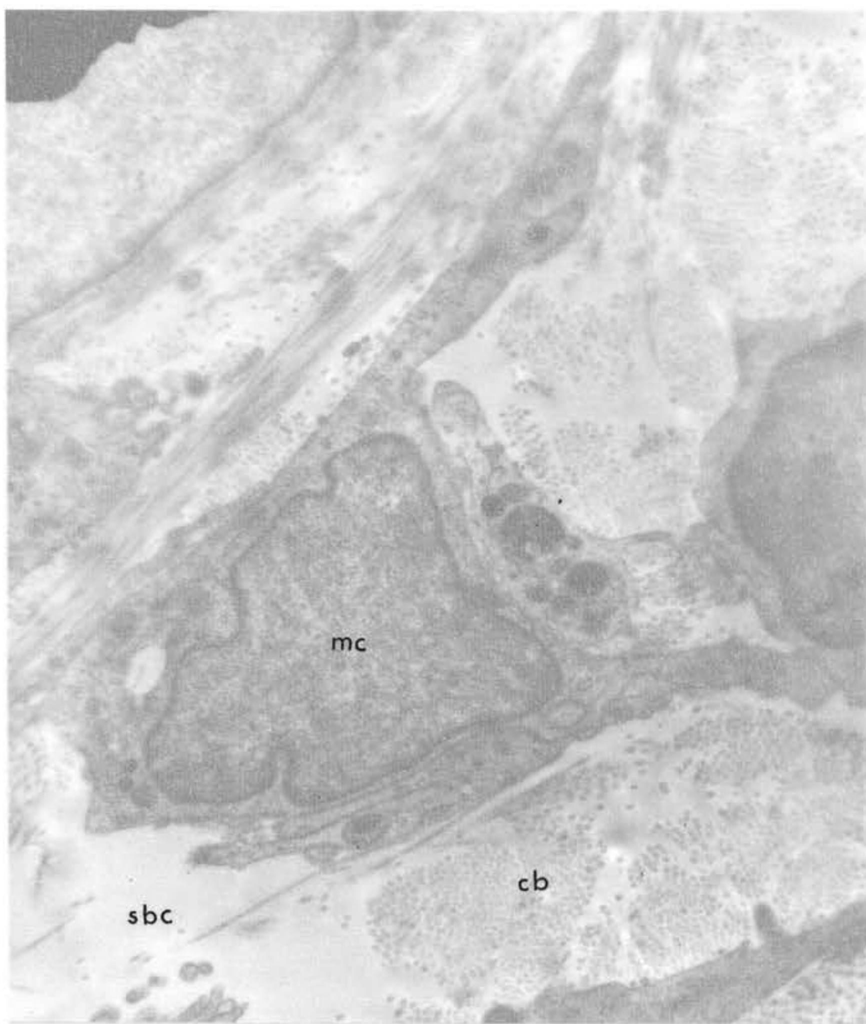

FIG. 33. Electron micrograph of a mesothelial cell(mc) surrounded by collagen bundle (cb). Note absence of basement membrane in this particular cell. (x 14,000)

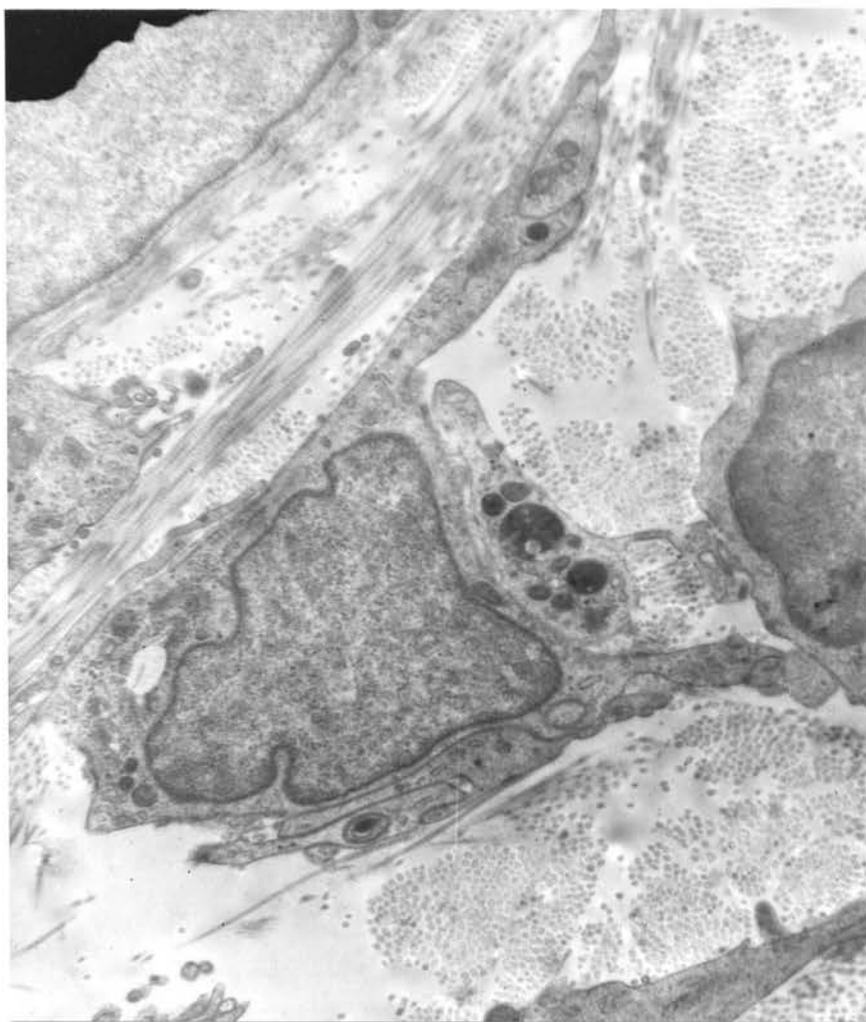

FIG. 33. Electron micrograph of a mesothelial cell(mc) surrounded by collagen bundle (cb). Note absence of basement membrane in this particular cell. (x 14,000)

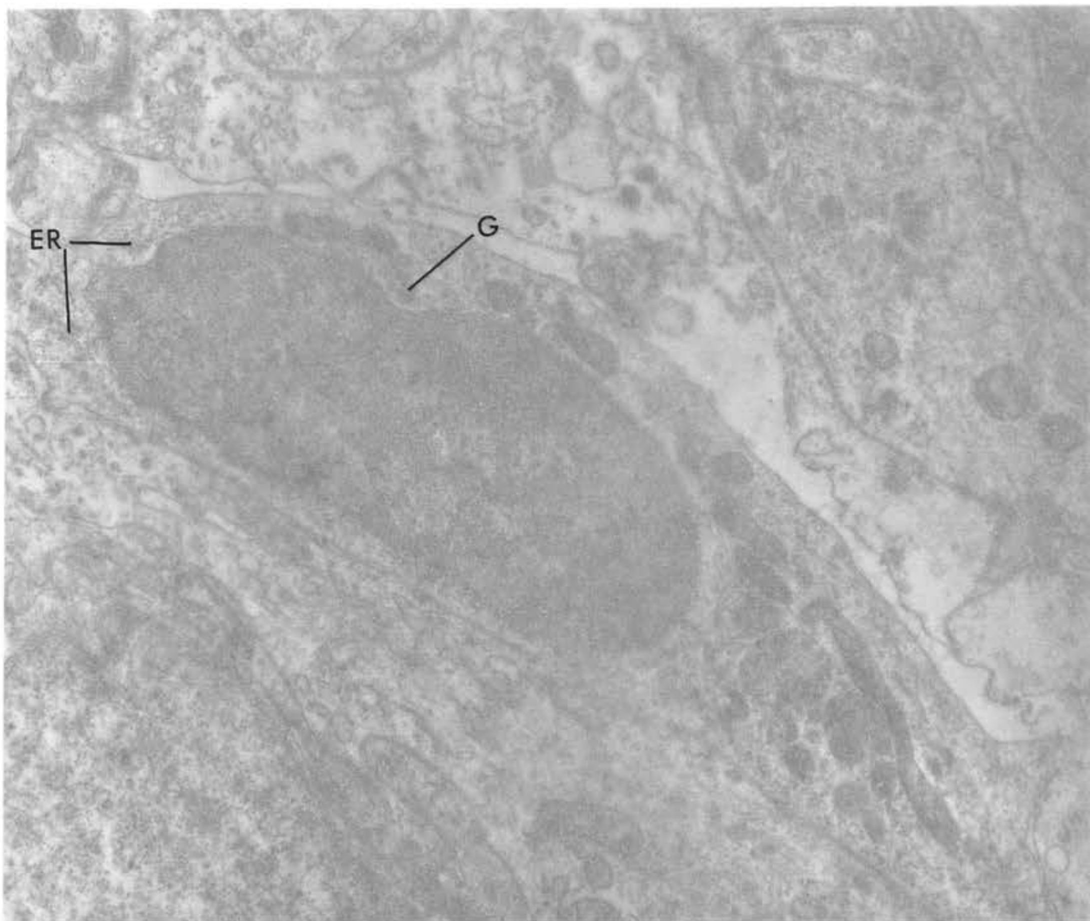

FIG. 34. Electron micrograph of a fibroblast in the core of the granulation. The cell is very similar to endothelial or mesothelial cells. ER = endoplasmic reticulum, G = Golgi apparatus.
(x 28,000)

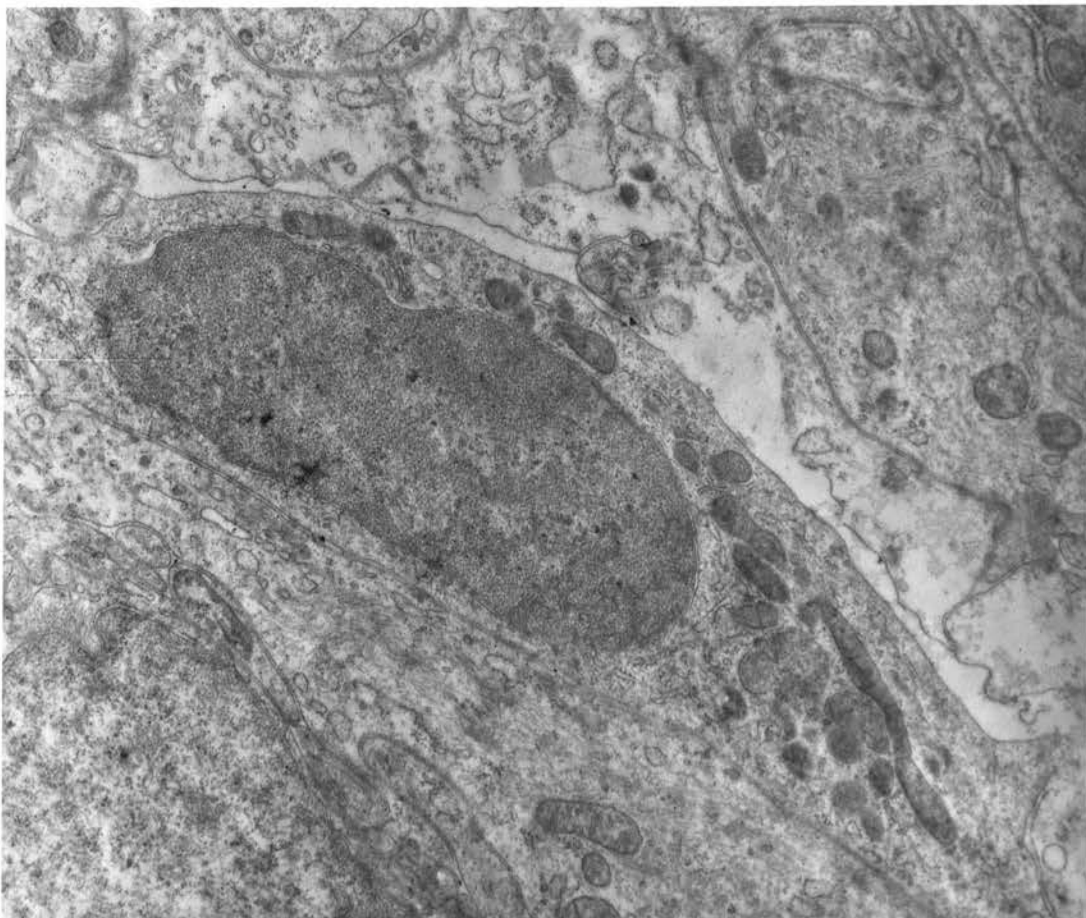

FIG. 34. Electron micrograph of a fibroblast in the core of the granulation. The cell is very similar to endothelial or mesothelial cells. ER = endoplasmic reticulum, G = Golgi apparatus.
(x 28,000)

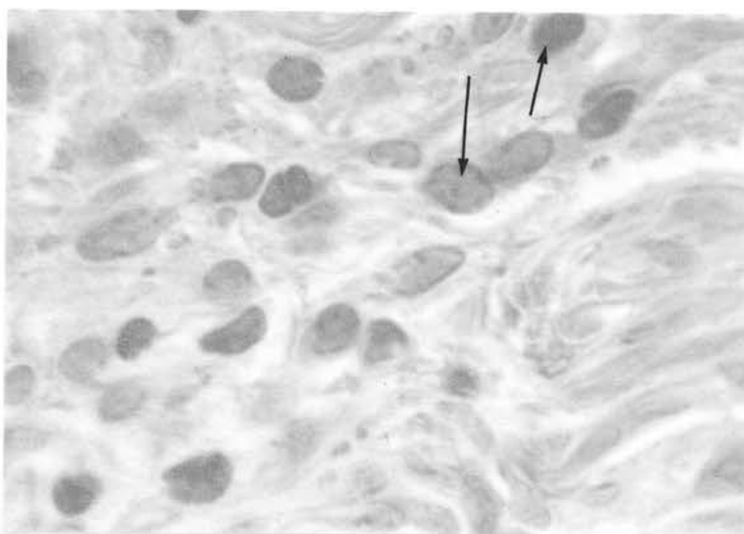

FIG. 35. Photomicrograph of the core of a granulation, showing different cells. Arrows show macrophages. (methacrylate embedded, H & E, x 1,600)

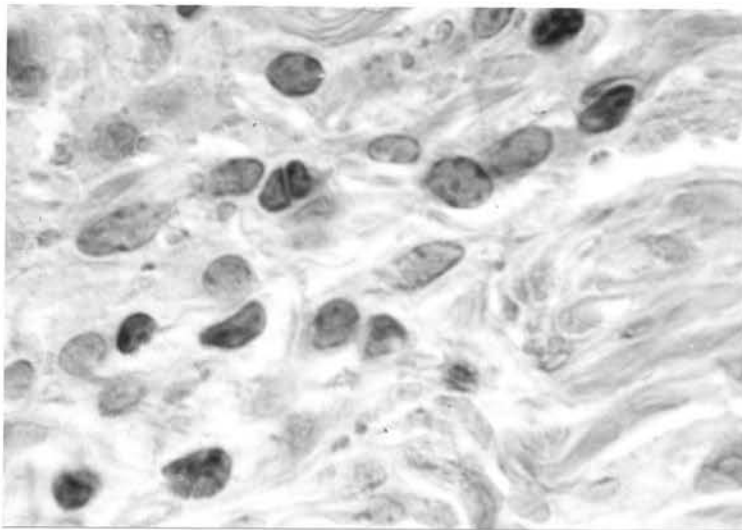

FIG. 35. Photomicrograph of the core of a granulation, showing different cells. Arrows show macrophages. (methacrylate embedded, H & E, x 1,600)

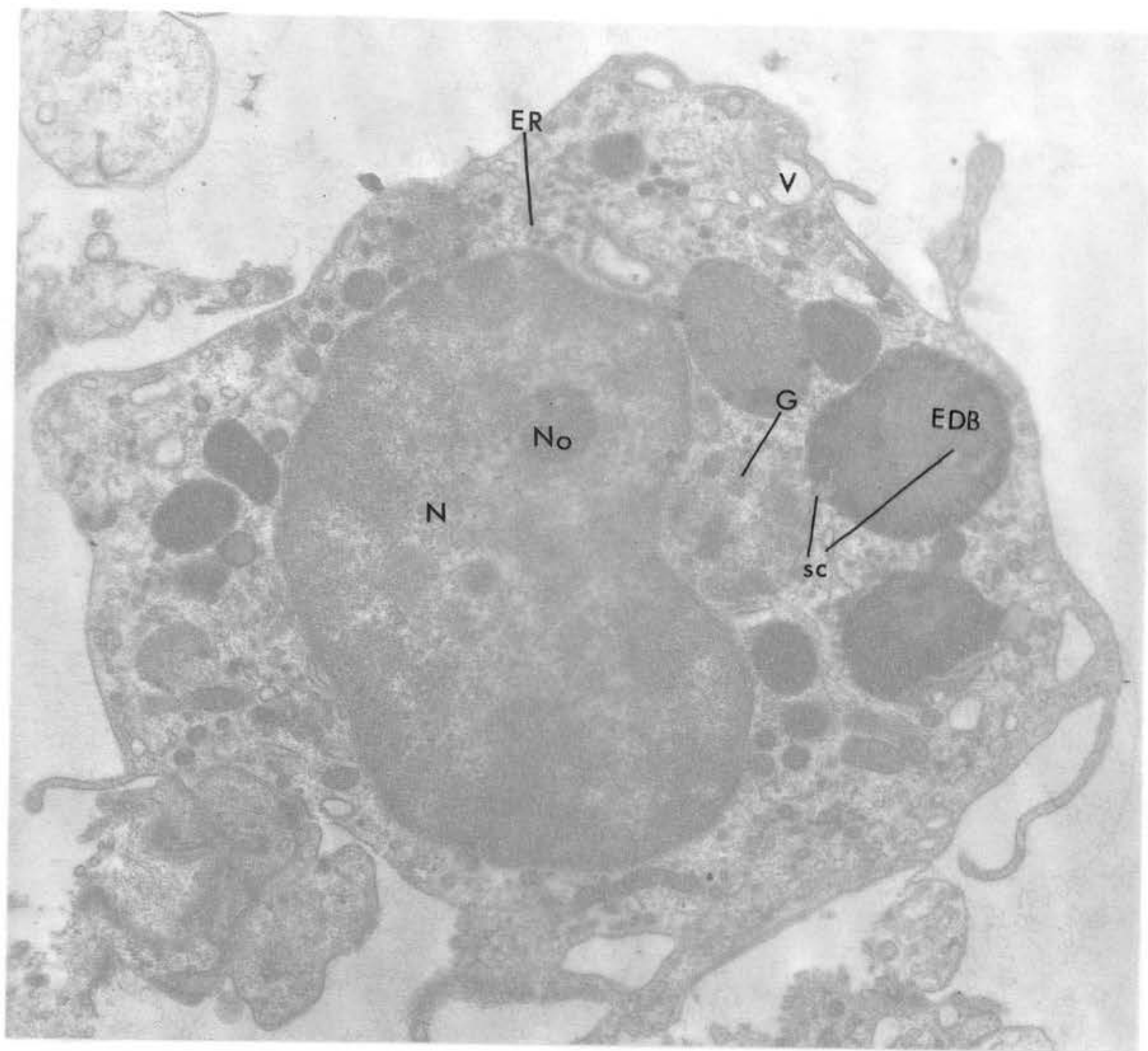

FIG. 36. Electron micrograph of a type 1 macrophage found in the arachnoid granulations of sheep, showing vesicles and electron dense bodies(EDB). ER = endoplasmic reticulum, G = Golgi apparatus, sc = small circular body. (x 14,000)

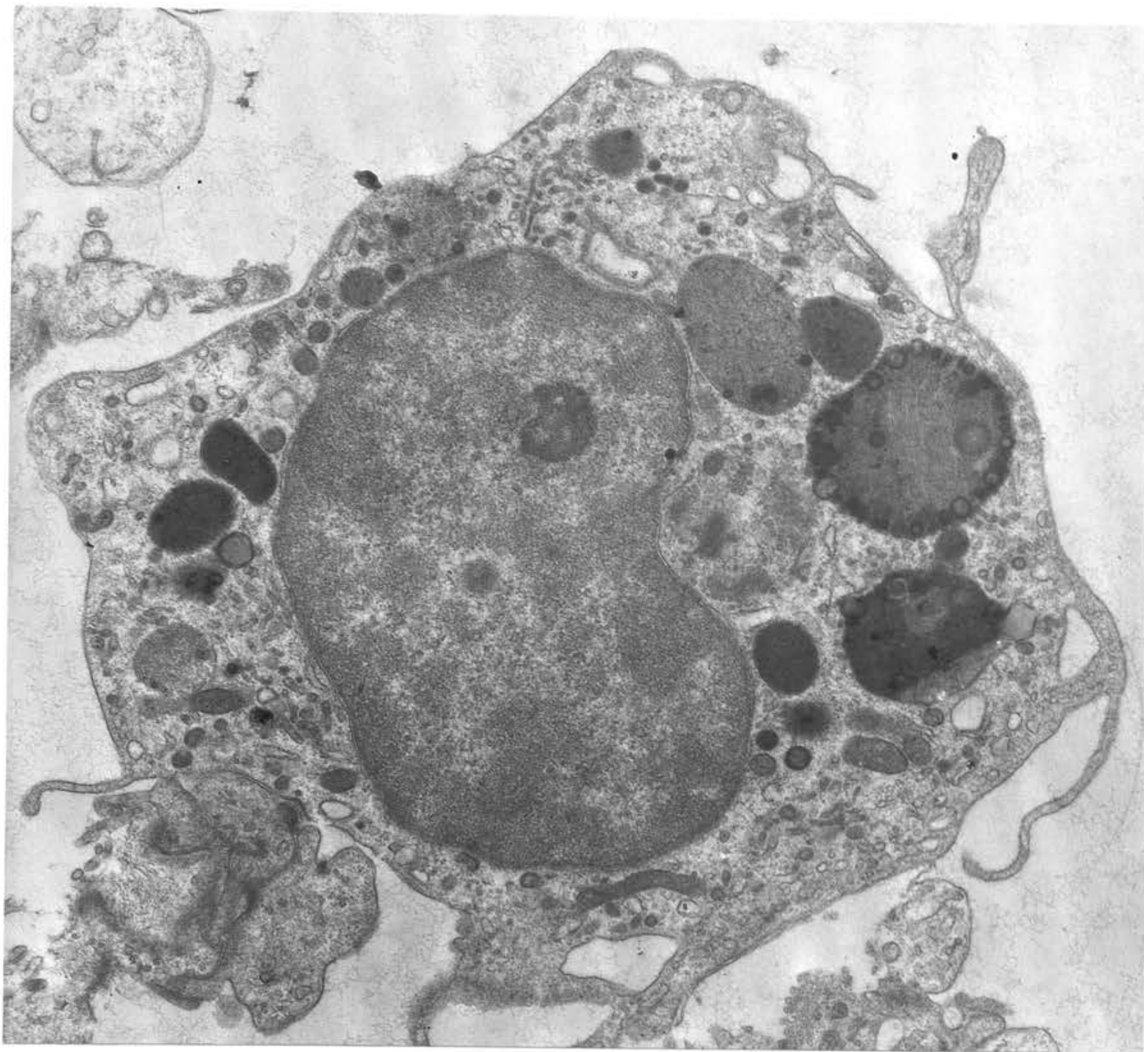

FIG. 36. Electron micrograph of a type 1 macrophage found in the arachnoid granulations of sheep, showing vesicles and electron dense bodies(EDB). ER = endoplasmic reticulum, G = Golgi apparatus, sc = small circular body. (x 14,000)

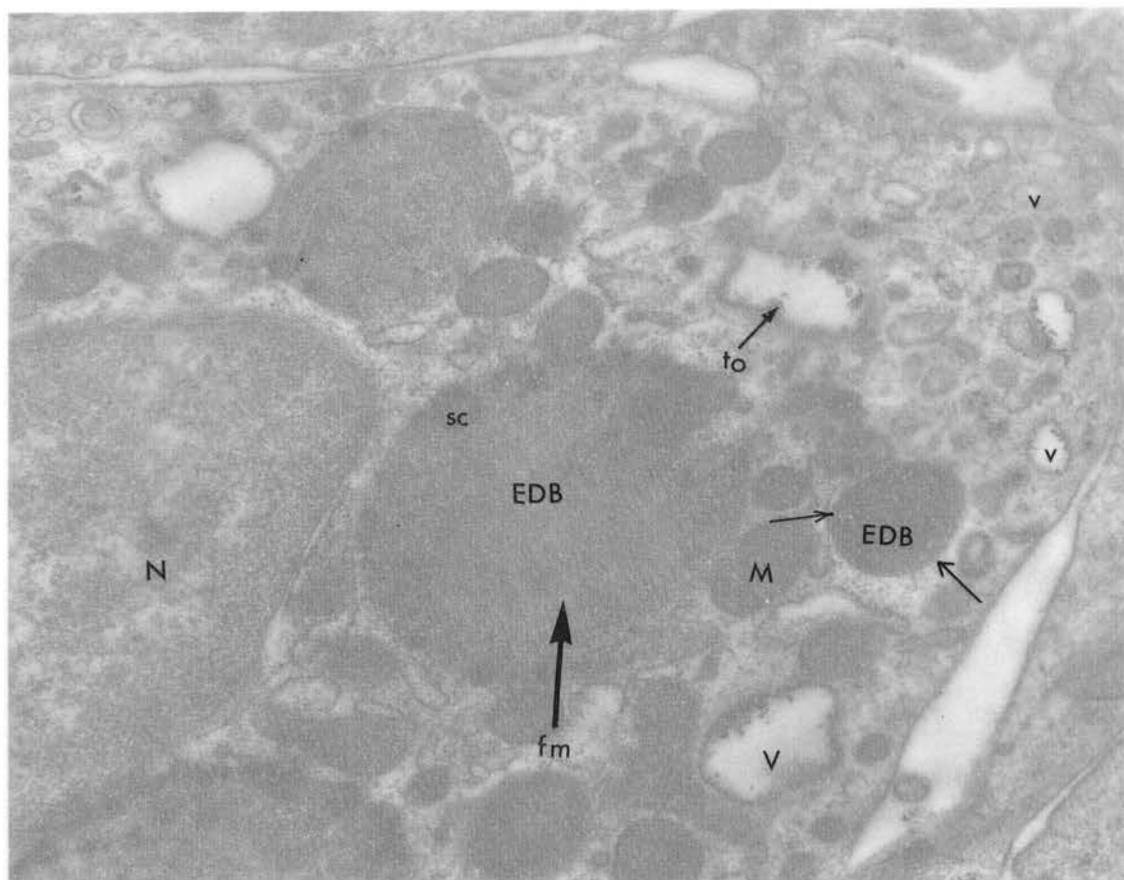

FIG. 37. Electron micrograph of a type 1 macrophage at a higher magnification. Particles of thorium(to) are seen in vesicles. Unlettered arrows show a suggestion of cristae in an electron dense body (EDB) at right of picture. sc = small circular body.
(x 56,000)

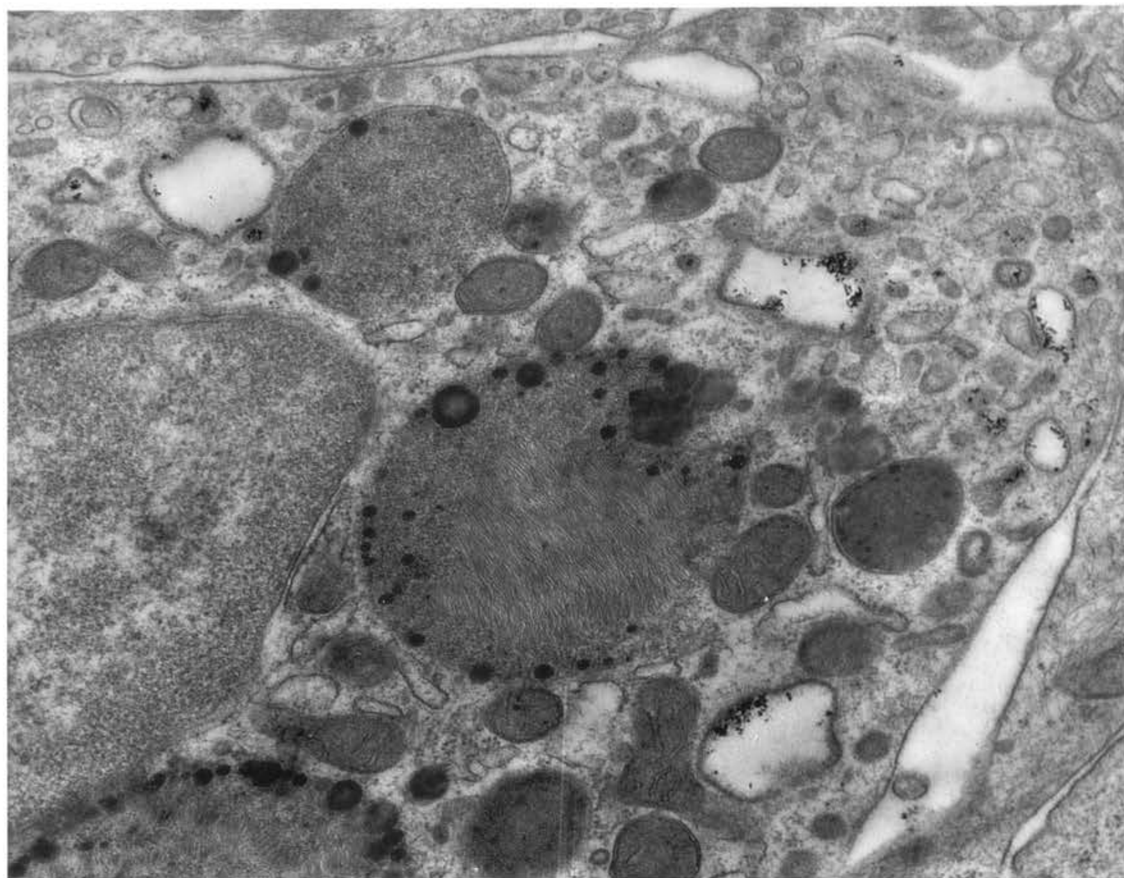

FIG. 37. Electron micrograph of a type 1 macrophage at a higher magnification. Particles of thorium(to) are seen in vesicles. Unlettered arrows show a suggestion of cristae in an electron dense body (EDB) at right of picture. sc = small circular body.
(x 56,000)

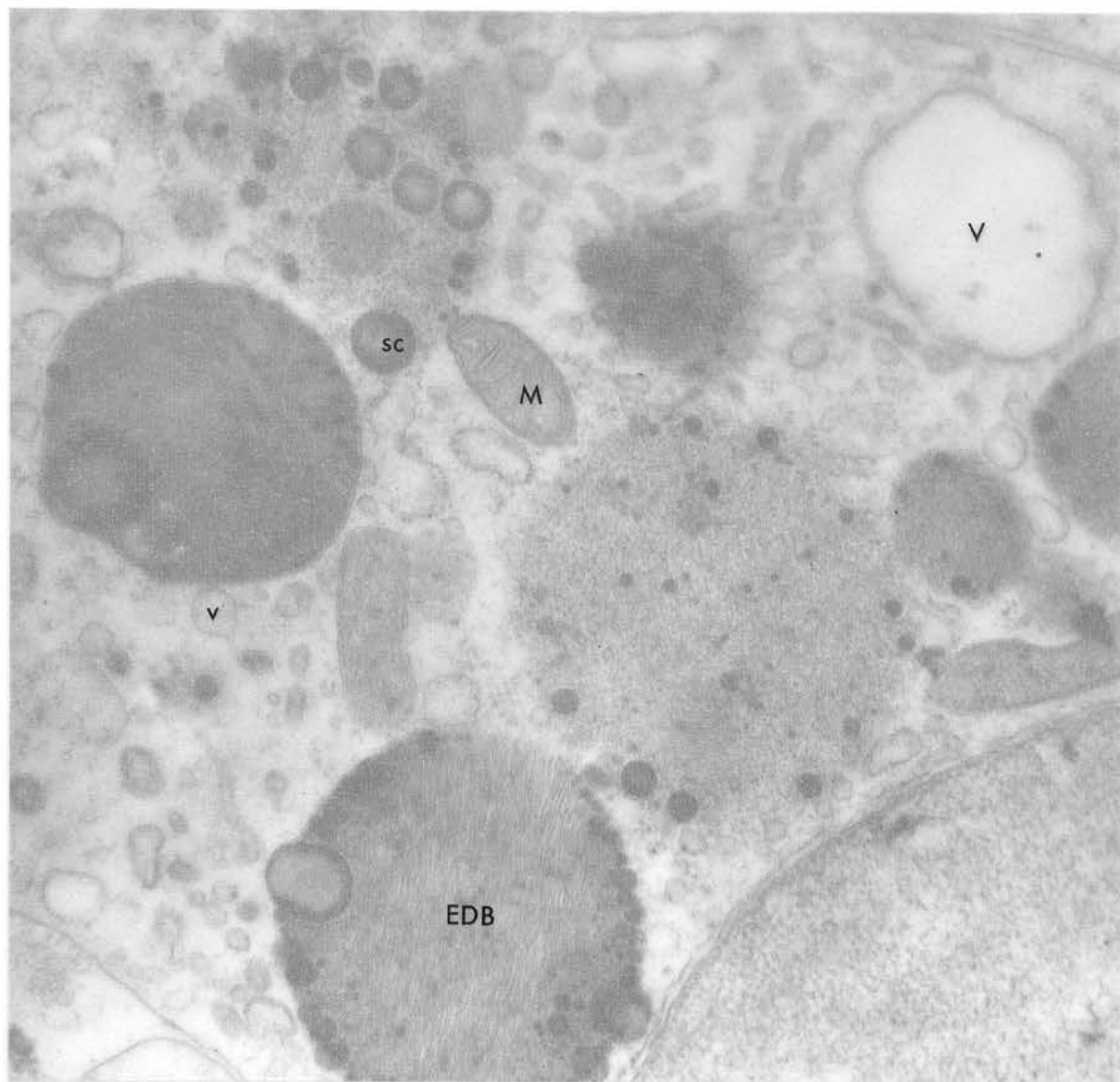

FIG. 38. Electron micrograph of a type 1 macrophage in sheep arachnoid granulation. ER = endoplasmic reticulum, EDB = electron dense body, sc =small circular body.
(130,000)

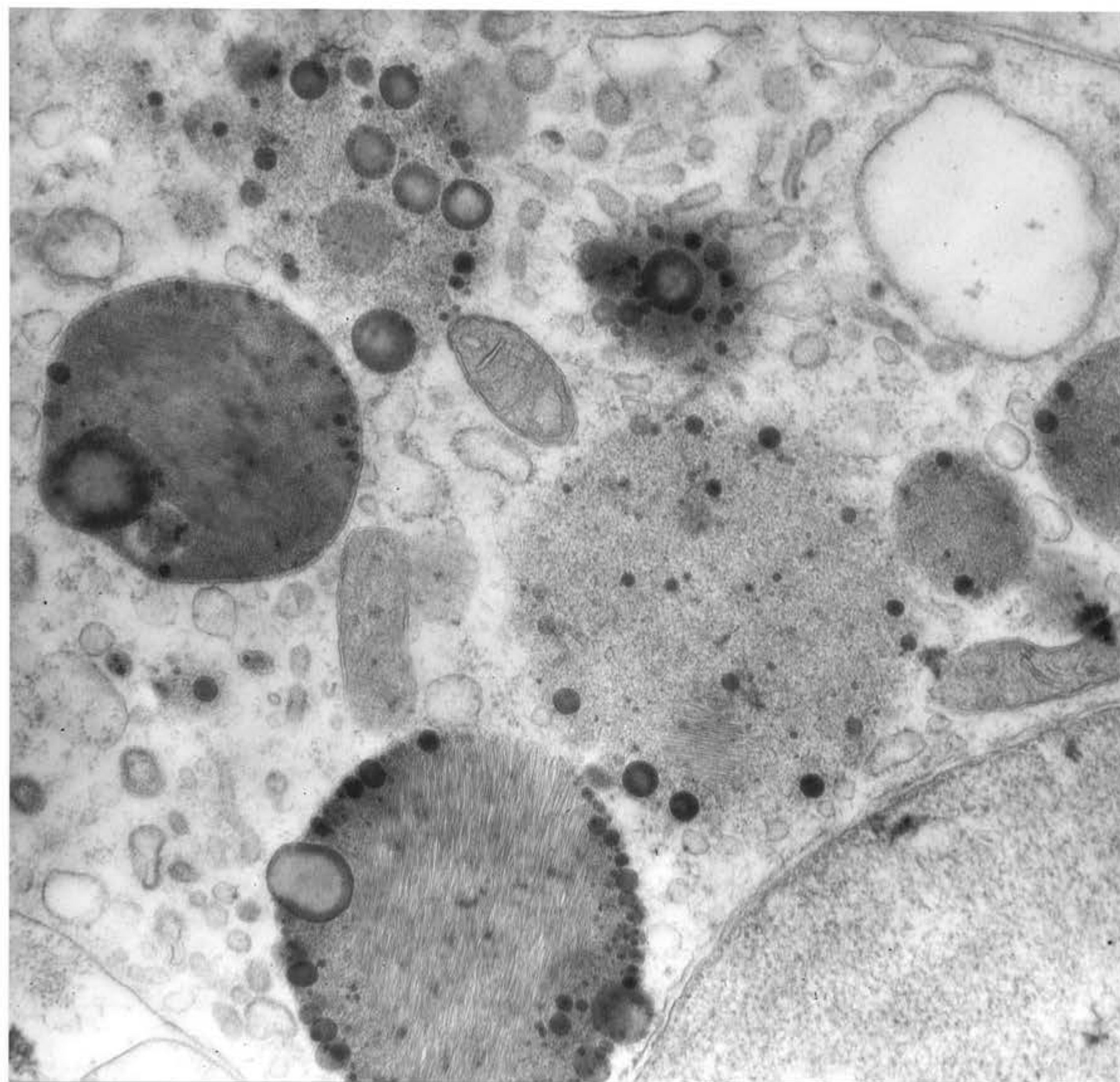

FIG. 38. Electron micrograph of a type 1 macrophage in sheep arachnoid granulation. ER = endoplasmic reticulum, EDB = electron dense body, sc =small circular body.
(130,000)

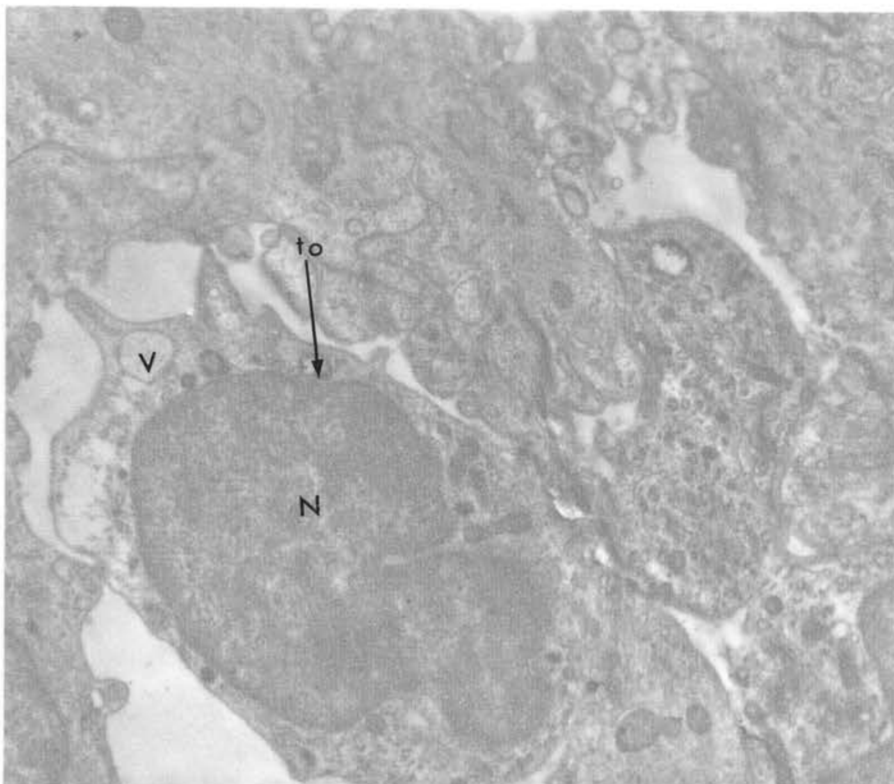

Fig. 39. Electron micrograph of a type 2 macrophage found in sheep granulation showing particles of thorium(to) in vesicles of the cell. (x 14,000)

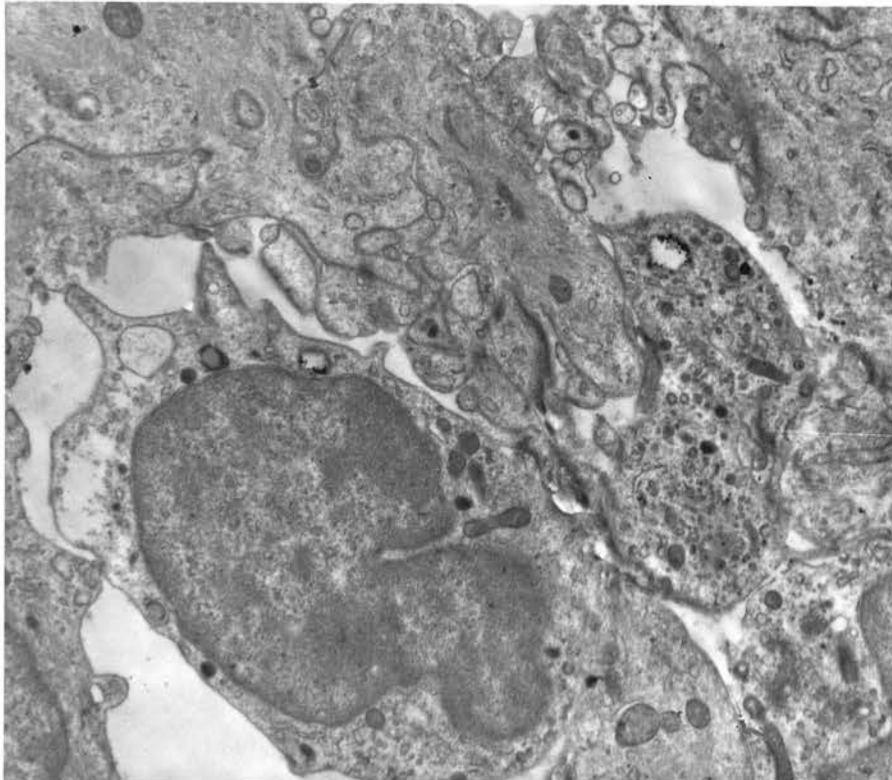

Fig. 39. Electron micrograph of a type 2 macrophage found in sheep granulation showing particles of thorium(to) in vesicles of the cell. (x 14,000)

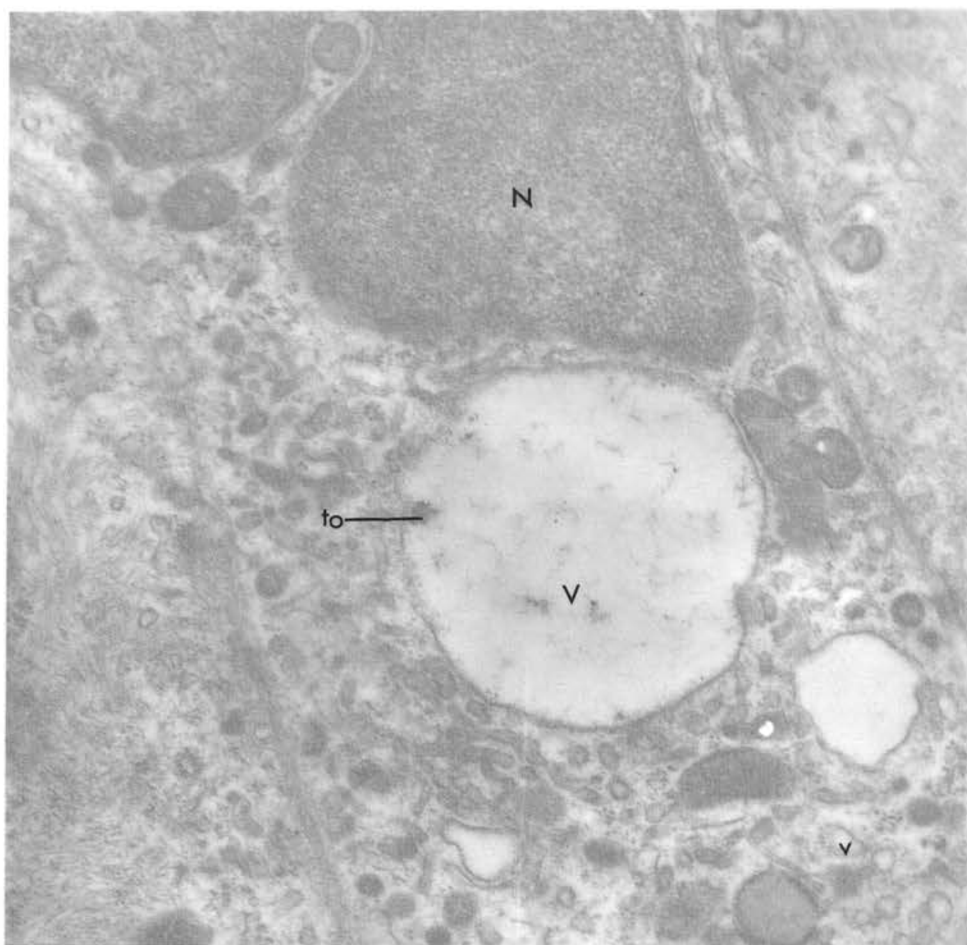

FIG. 40. Electron micrograph of type 2 macrophage showing large vesicle containing thorium(to). (x 56,000)

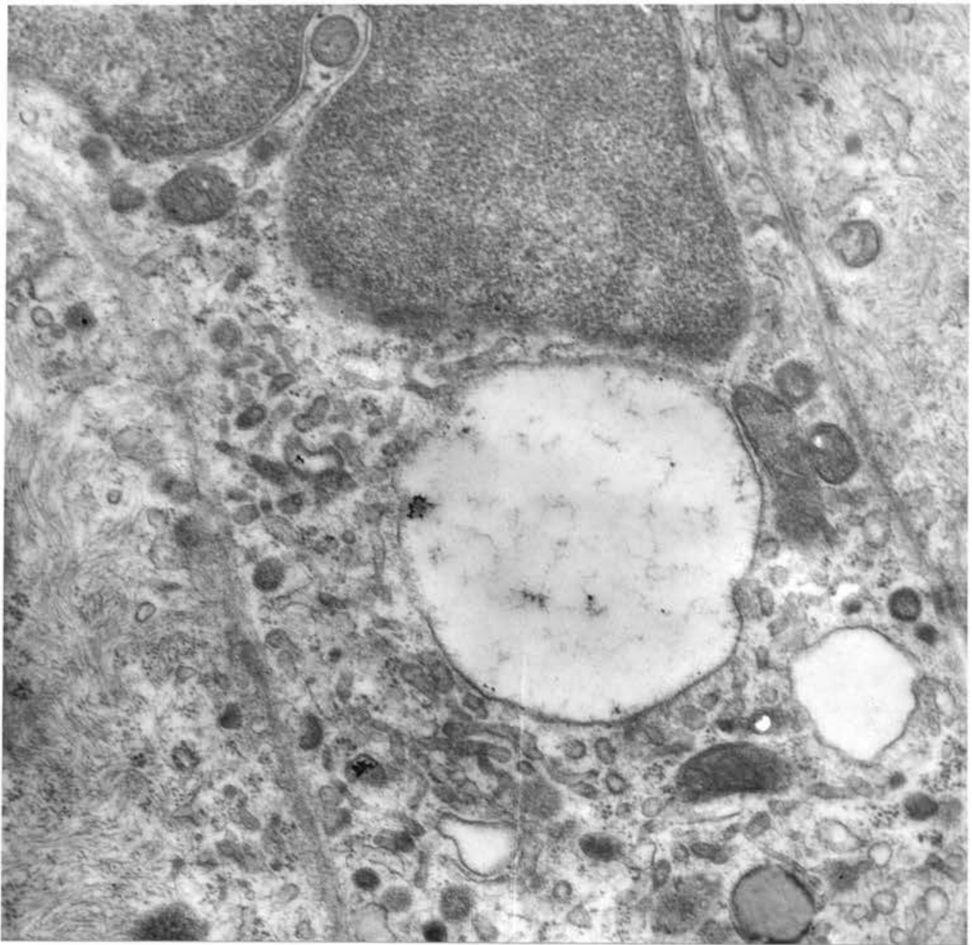

FIG. 40. Electron micrograph of type 2 macrophage showing large vesicle containing thorium(to). (x 56,000)

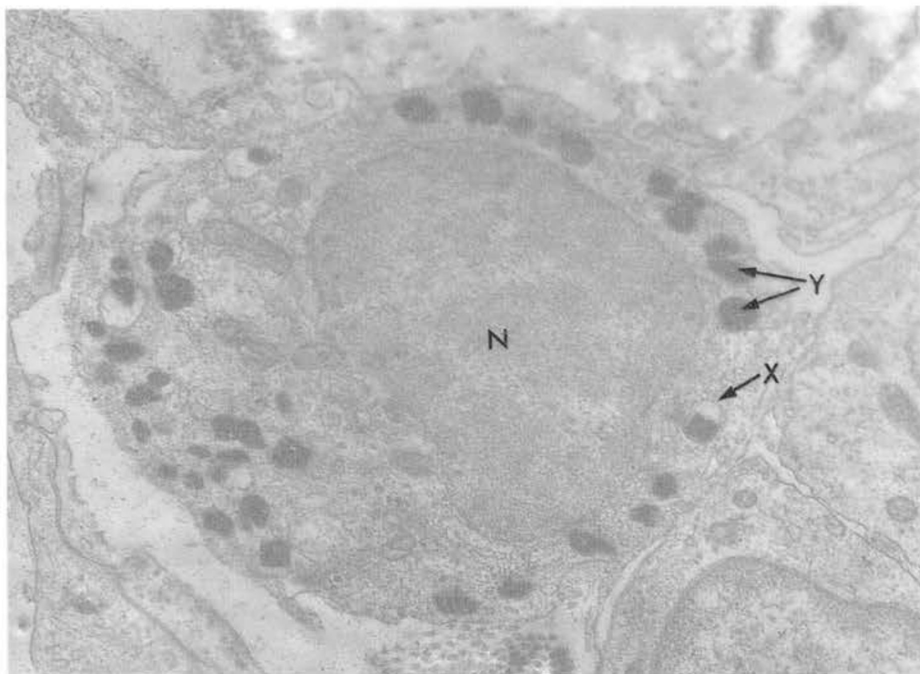

FIG. 41. Electron micrograph of unclassified cell. X is the membranous sac surrounding the polygonalbodies (Y). (x 14,000)

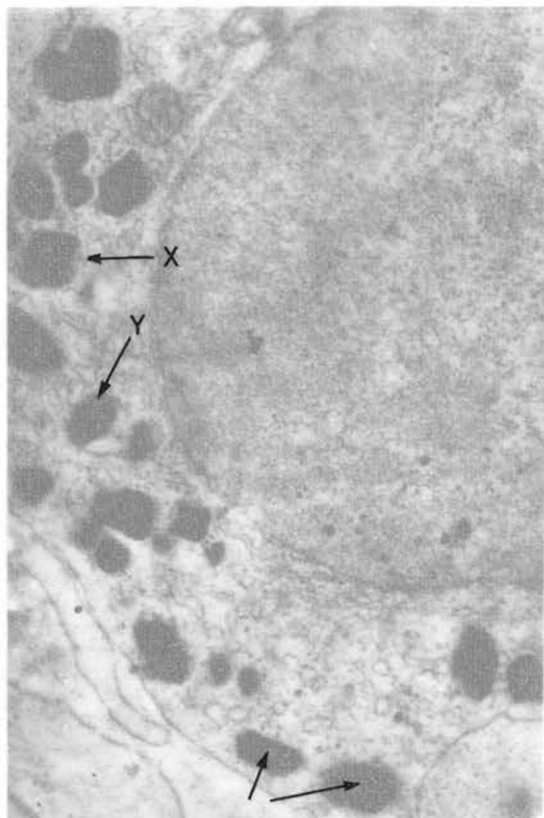

FIG. 42.

FIG. 42 a.

FIGS. 42 and 42 a. Electron micrographs of unclassified cell at higher magnification. X and Y are same as in fig. 41. Arrow indicates the clear spaces within the polygonal bodies.
(x 28,000)

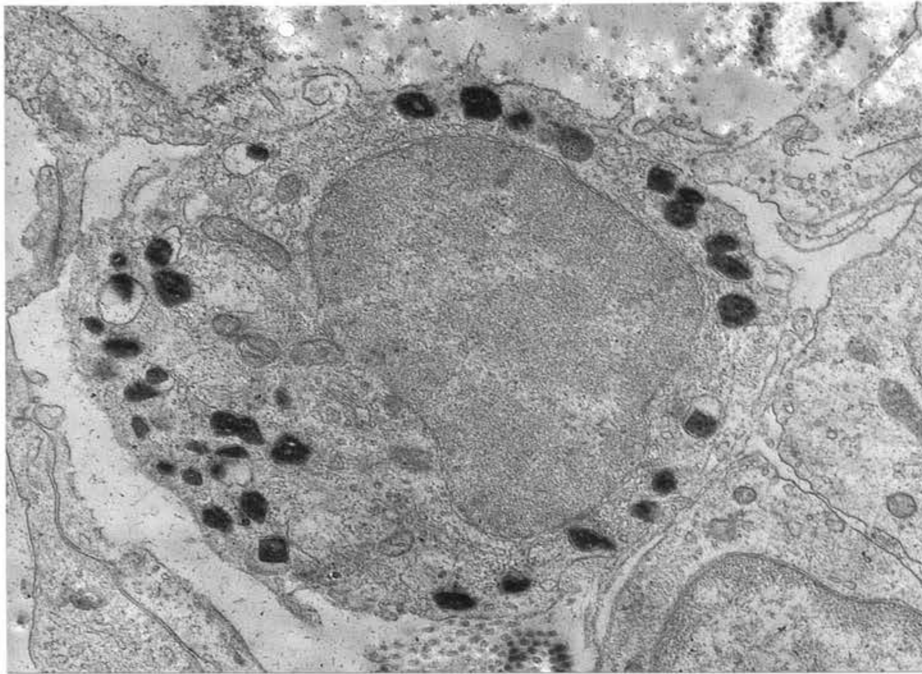

FIG. 41. Electron micrograph of unclassified cell. X is the membranous sac surrounding the polygonalbodies (Y). (x 14,000)

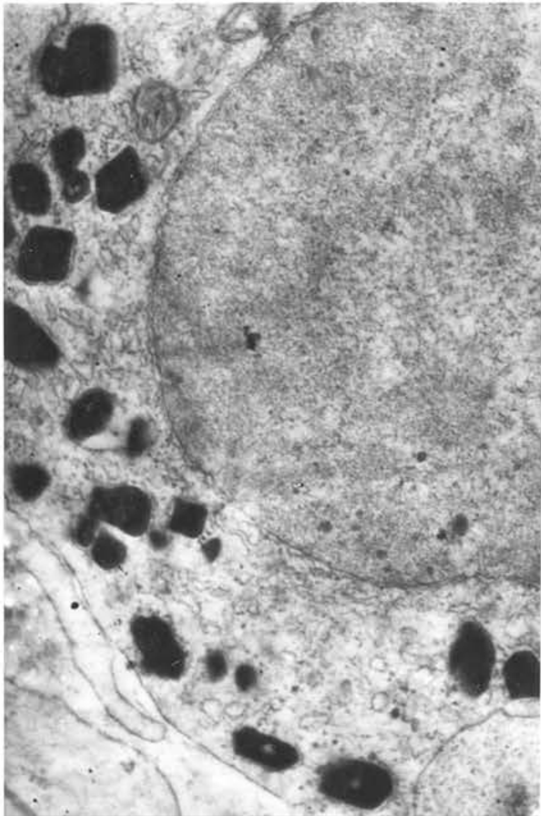

FIG. 42.

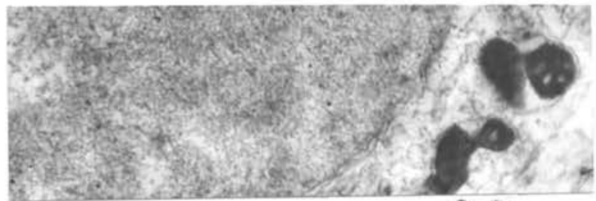

FIG. 42 a.

FIGS. 42 and 42 a. Electron micrographs of unclassified cell at higher magnification. X and Y are same as in fig. 41. Arrow indicates the clear spaces within the polygonal bodies.
(x 28,000)

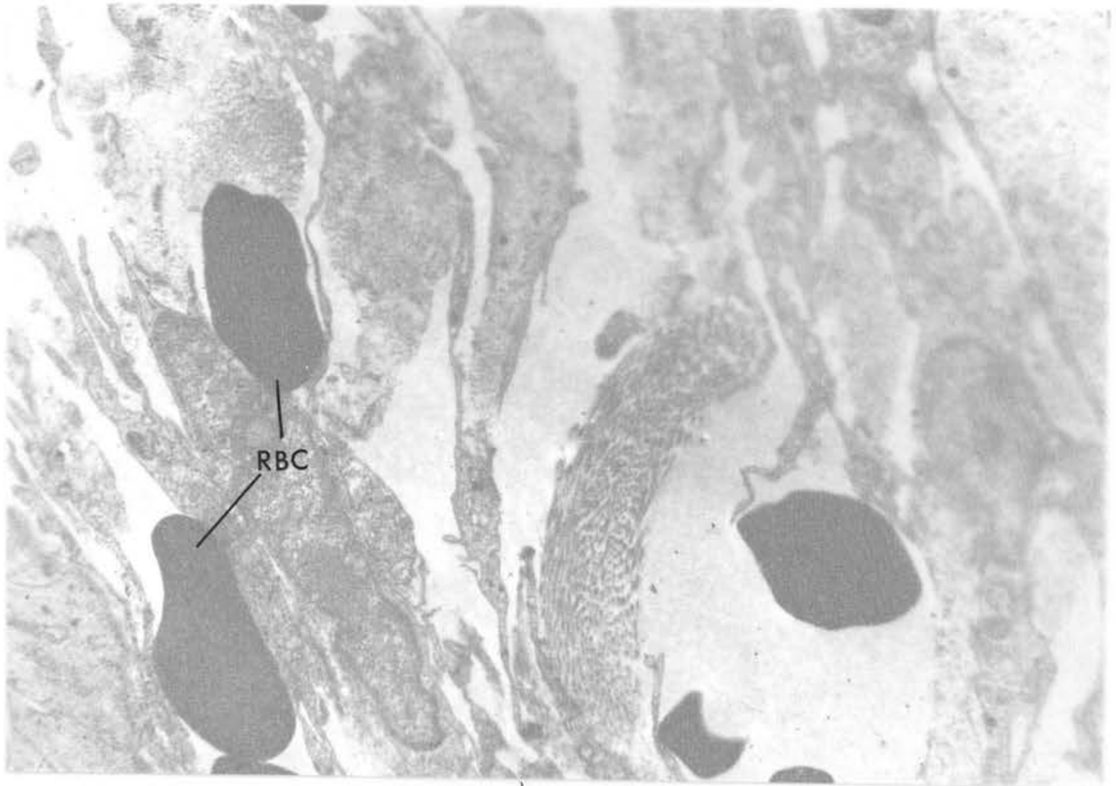

FIG. 43. Electron micrograph showing red blood cells(RBC) towards the centre of the core of a granulation. (x 6,000)

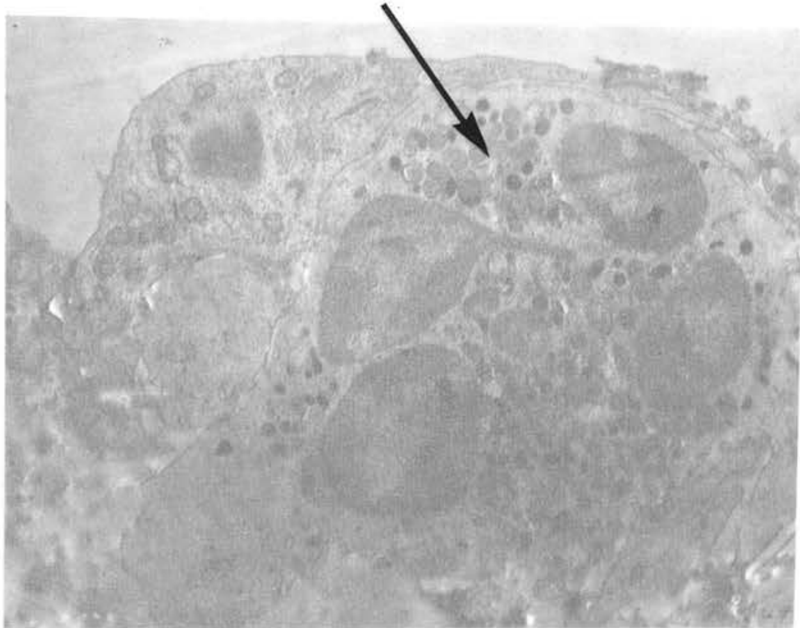

FIG. 44. Electron micrograph of polymorph(thick arrow) adjacent to the surface epithelial cell of a granulation.
(x 14,000)

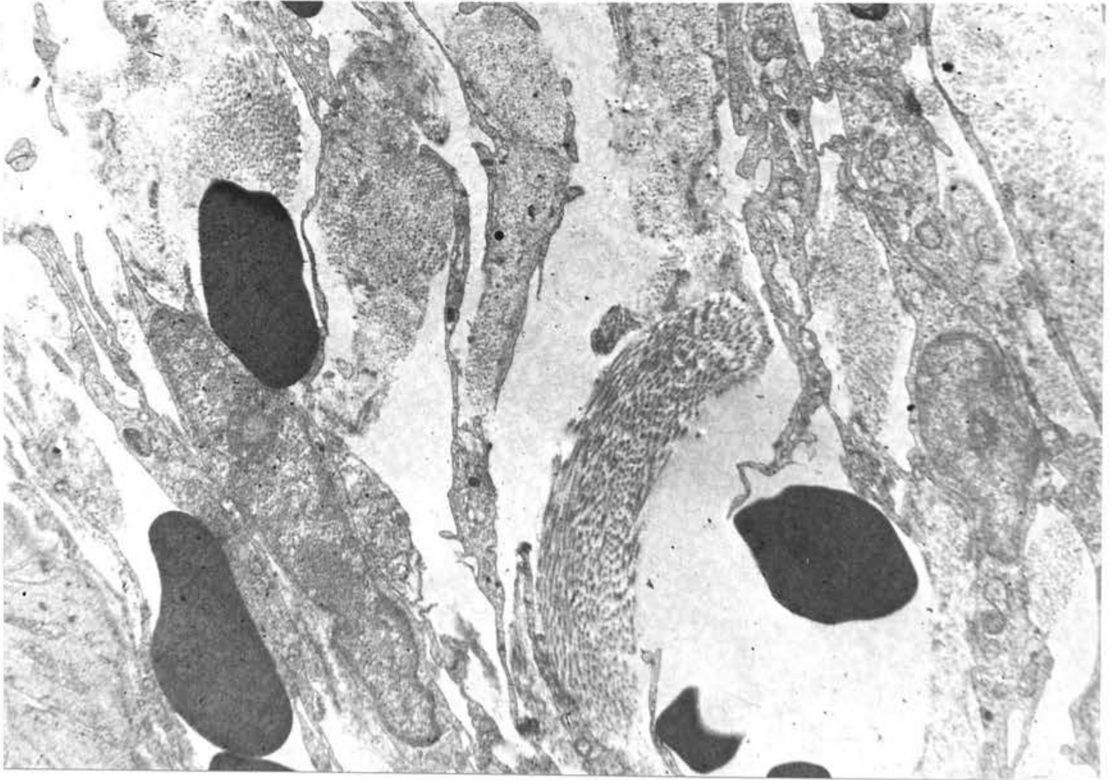

FIG. 43. Electron micrograph showing red blood cells(RBC) towards the centre of the core of a granulation. (x 6,000)

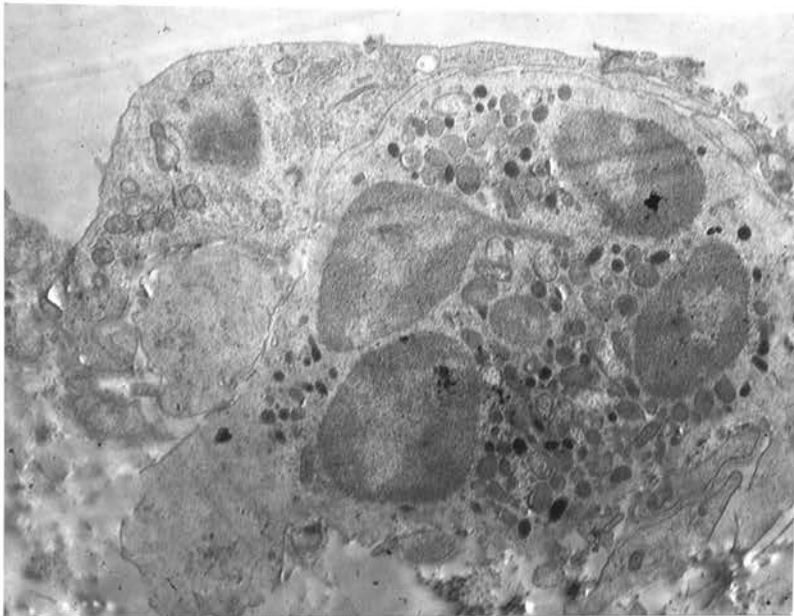

FIG. 44. Electron micrograph of polymorph(thick arrow) adjacent to the surface epithelial cell of a granulation.
(x 14,000)

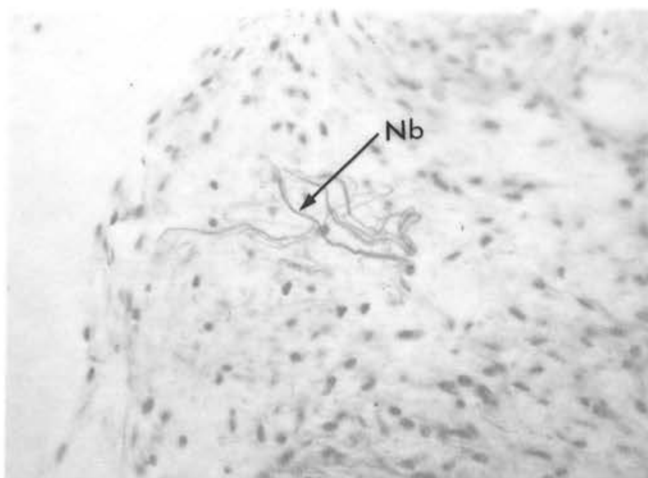

FIG. 45. Photomicrograph of arachnoid granulation of sheep showing nerve fibres.
(Peters' silver-proteinate, x 530)

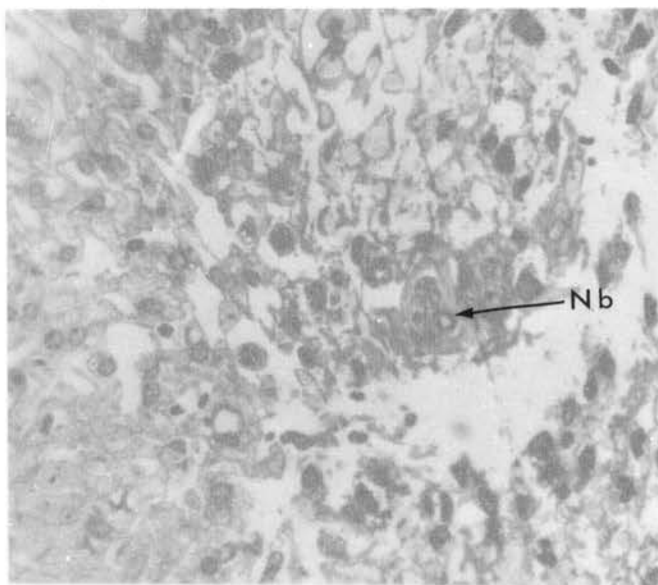

Fig. 46. Photomicrograph of granulation showing a nerve bundle in transverse section.
(araldite embedded, toluidene blue, x 600)

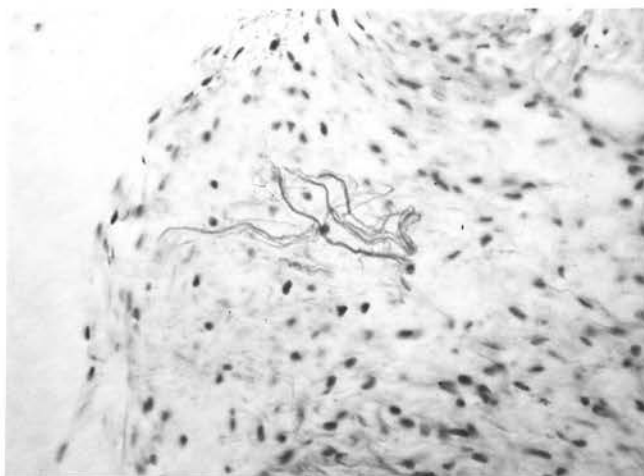

FIG. 45. Photomicrograph of arachnoid granulation of sheep showing nerve fibres.
(Peters' silver-proteinate, x 530)

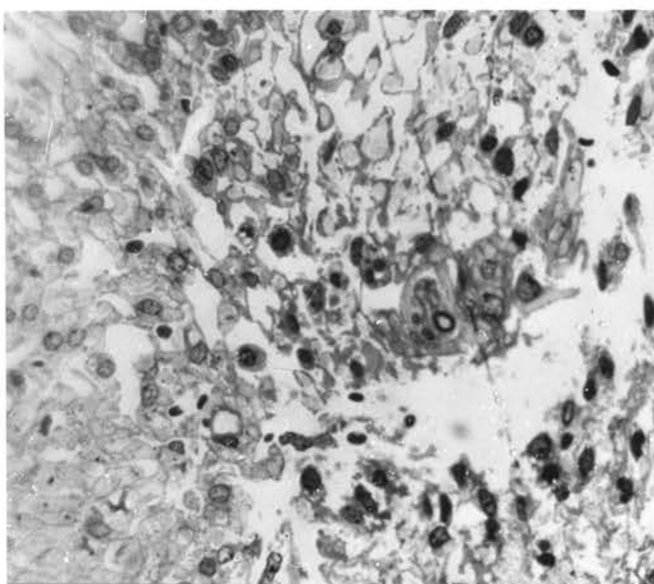

Fig. 46. Photomicrograph of granulation showing a nerve bundle in transverse section.
(araldite embedded, toluidene blue, x 600)

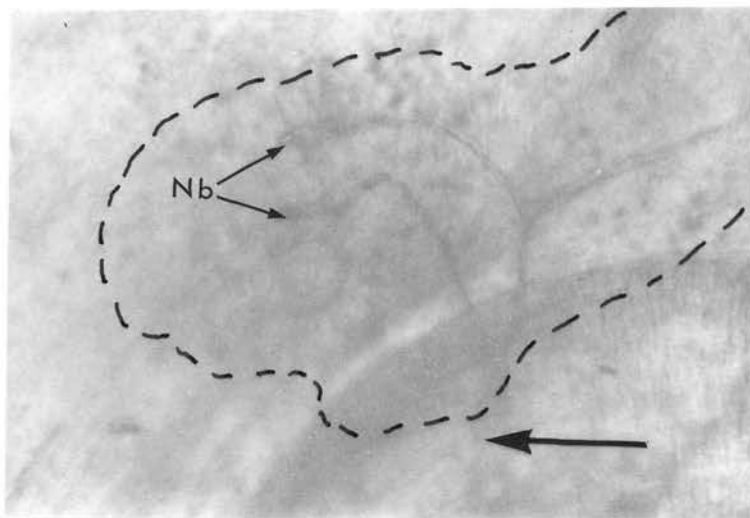

FIG. 47. Photomicrograph of a whole mount of a granulation (methylene blue) to demonstrate nerve fibres. Thick arrow shows nerve fibres in dura and interrupted lines outline granulation. (x 50)

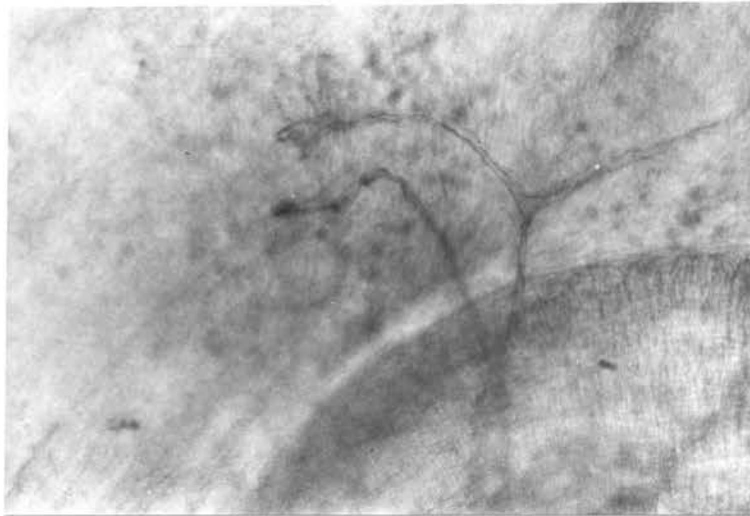

FIG. 47. Photomicrograph of a whole mount of a granulation (methylene blue) to demonstrate nerve fibres. Thick arrow shows nerve fibres in dura and interrupted lines outline granulation. (x 50)

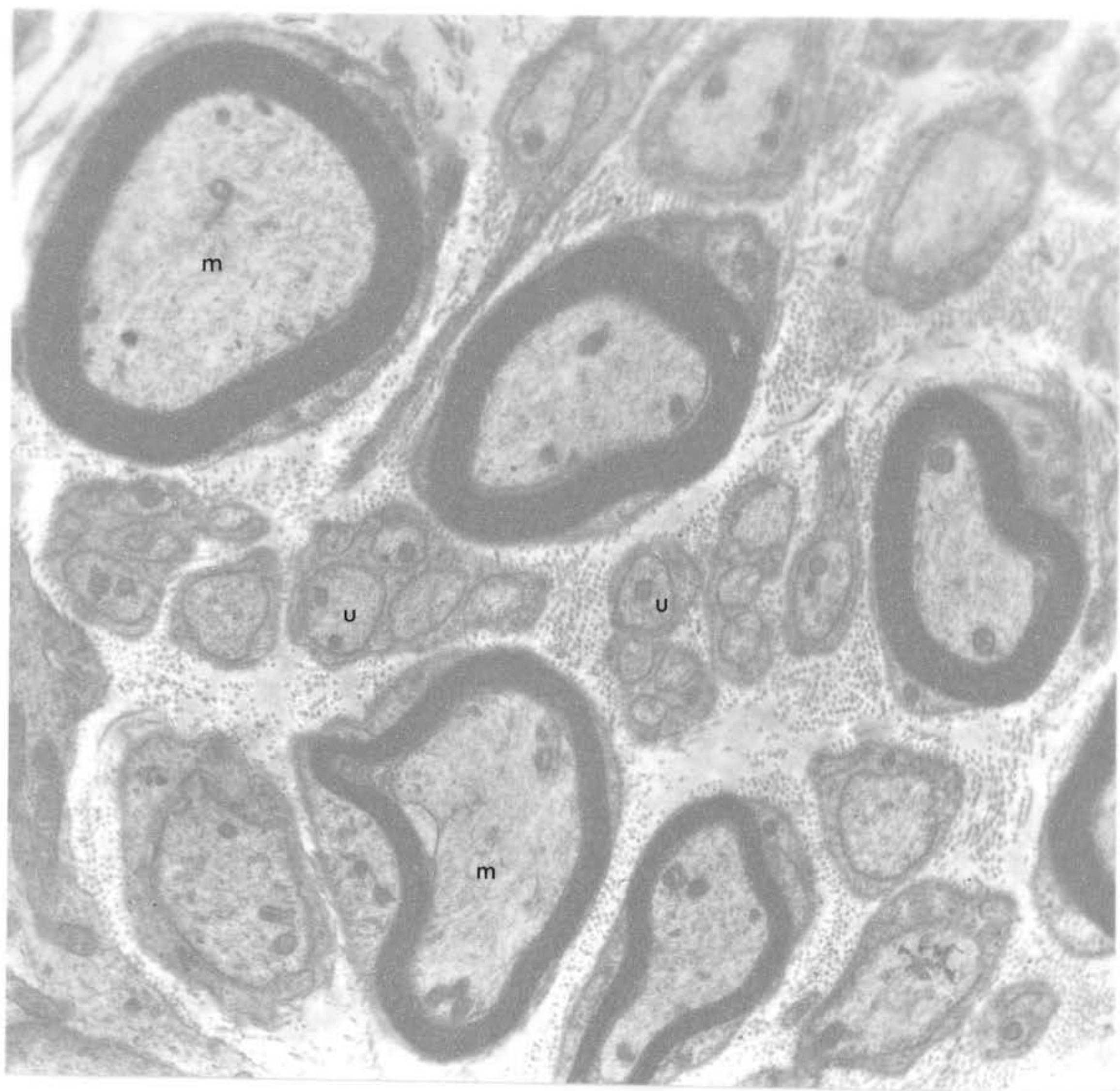

FIG. 48. Electron micrograph showing transverse section of nerve bundle containing both medullated (m) and non-medullated(u) fibres.
(x 21,000)

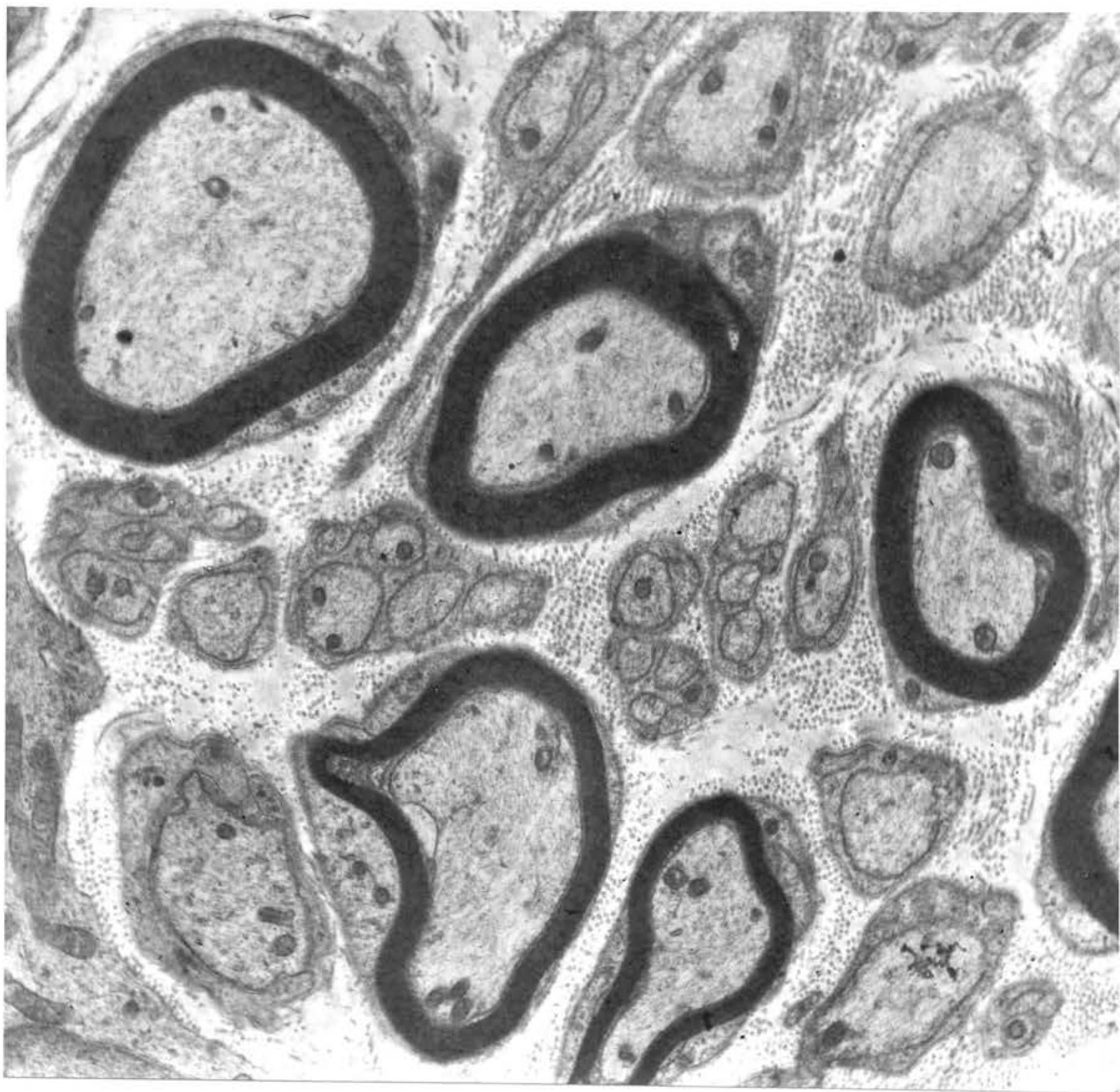

FIG. 48. Electron micrograph showing transverse section of nerve bundle containing both medullated (m) and non-medullated(u) fibres.
(x 21,000)

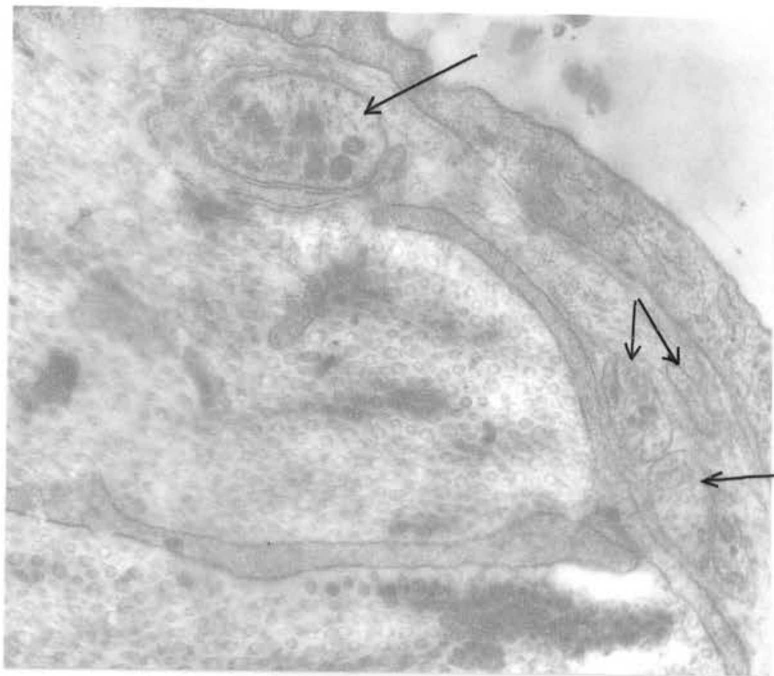

FIG. 49. Electron micrograph showing nerve fibres(arrows) lying adjacent to surface epithelial cells of granulation.
(x 45,000)

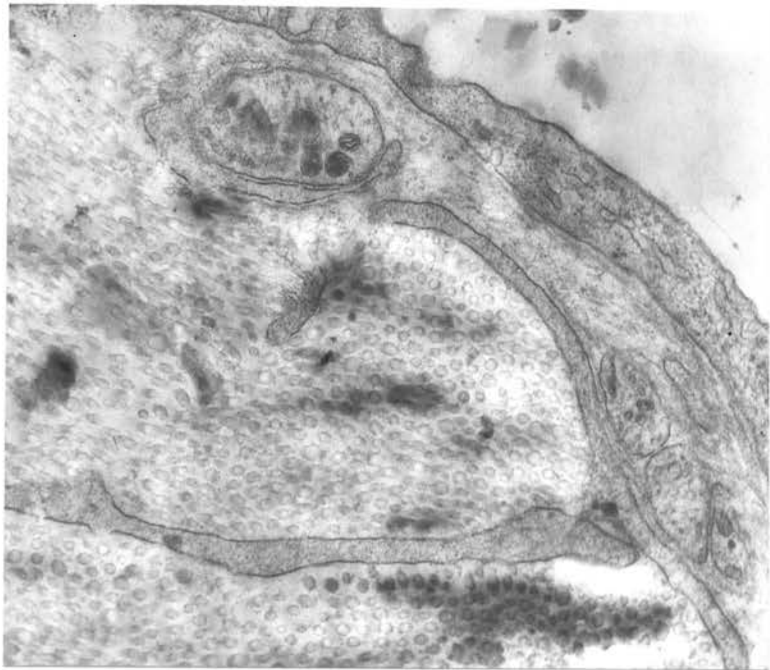

FIG. 49. Electron micrograph showing nerve fibres(arrows) lying adjacent to surface epithelial cells of granulation.
(x 45,000)

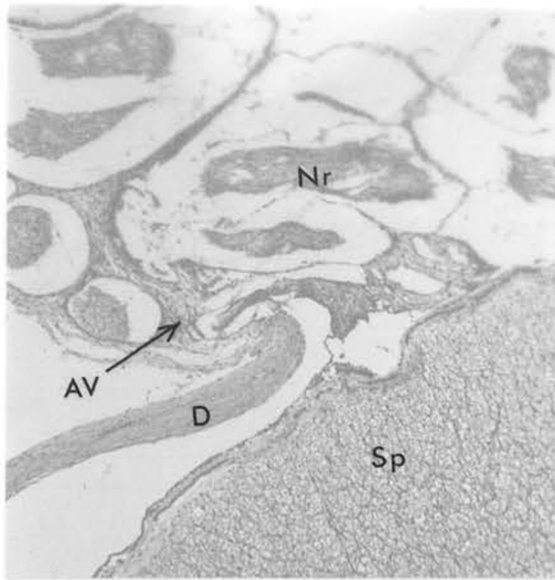

FIG. 50. Photomicrograph of transverse section of sheep spinal cord(Sp) at emergence of ventral nerve root(Nr), showing structures similar to arachnoid villi.
(paraffin embedded, H & E x 54)

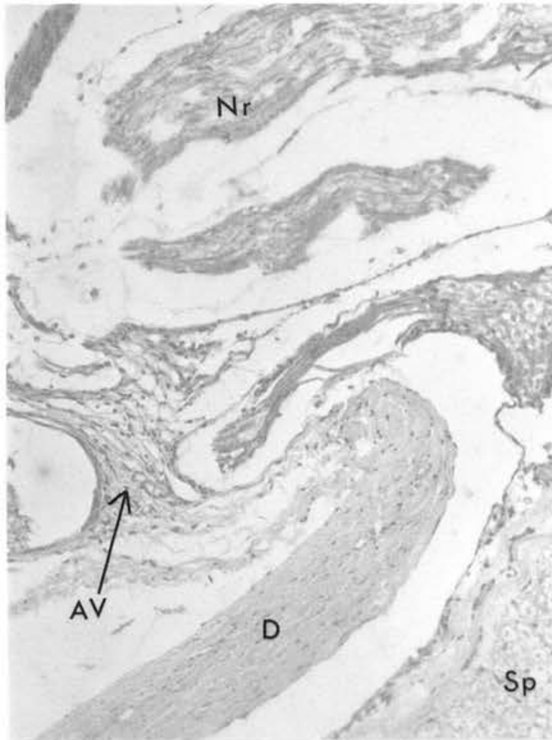

FIG. 51. Same as fig. 50 but at higher magnification.
(paraffin embedded, H & E, x 140)

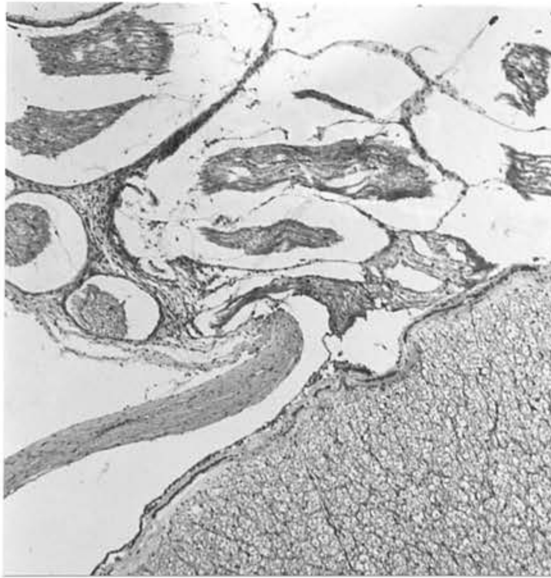

FIG. 50. Photomicrograph of transverse section of sheep spinal cord(Sp) at emergence of ventral nerve root(Nr), showing structures similar to arachnoid villi.
(paraffin embedded, H & E x 54)

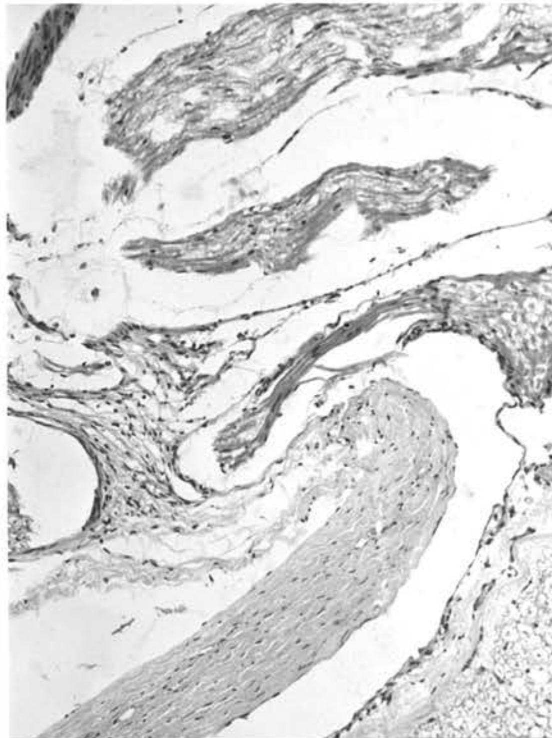

FIG. 51. Same as fig. 50 but at higher magnification.
(paraffin embedded, H & E, x 140)

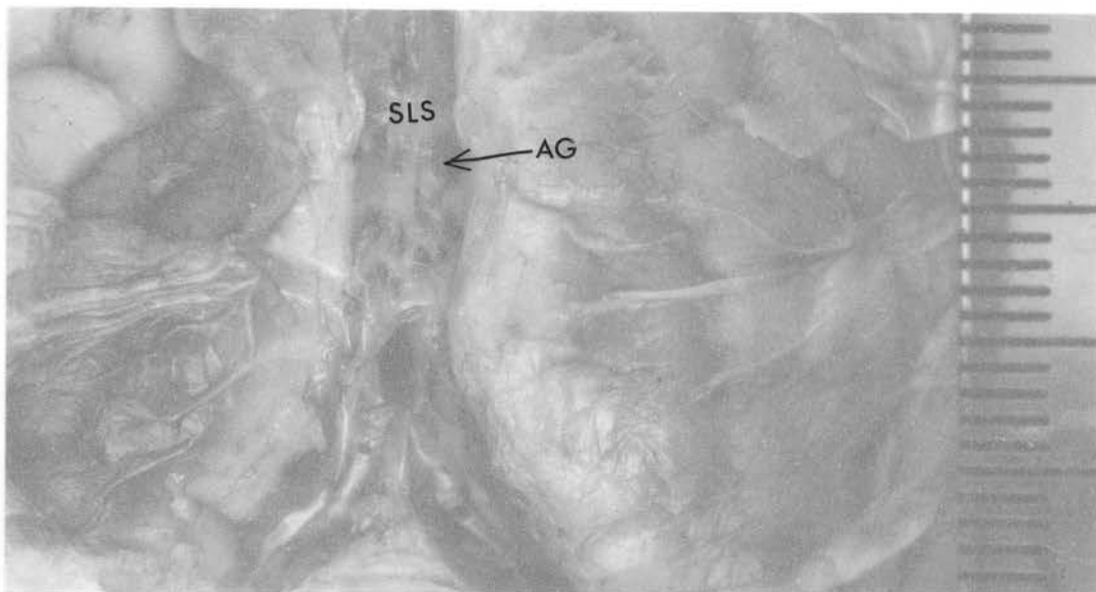

Fig. 52. Photograph of the superior longitudinal sinus of a 140 day sheep foetus, showing arachnoid granulations.
(x 1.5)

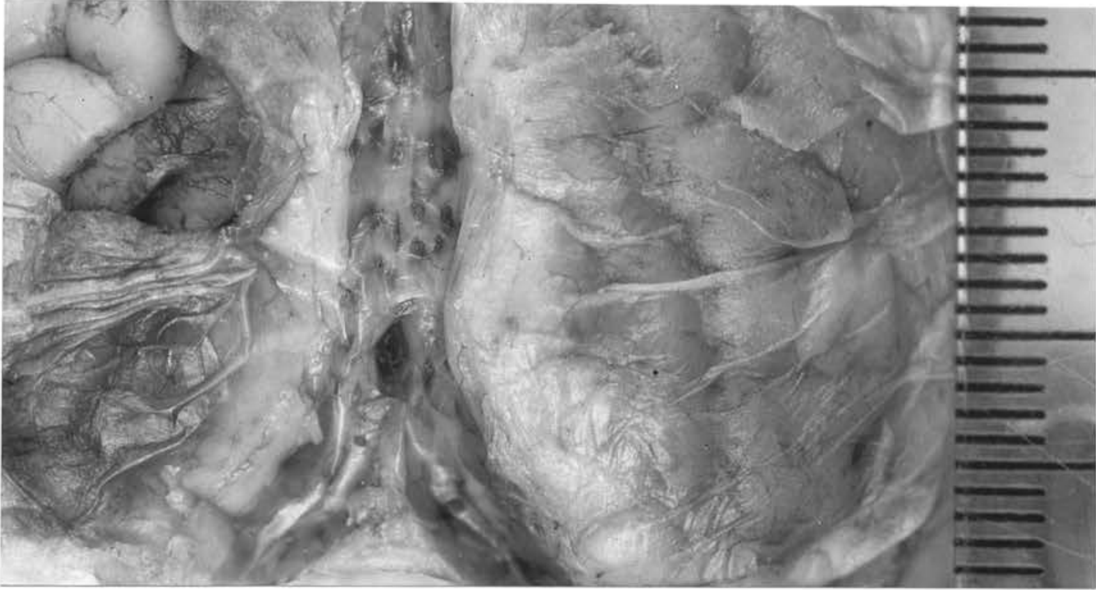

Fig. 52. Photograph of the superior longitudinal sinus of a 140 day sheep foetus, showing arachnoid granulations.
(x 1.5)

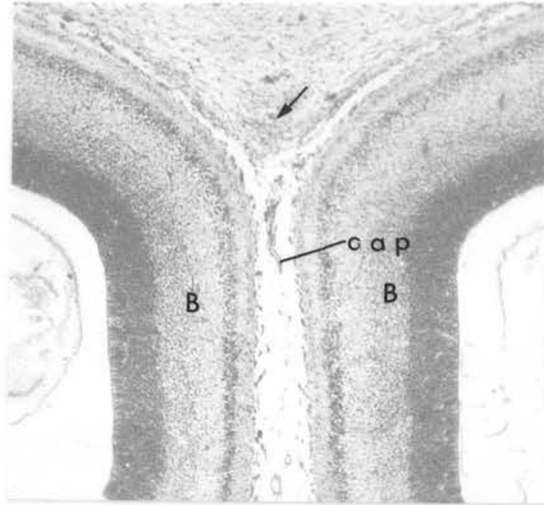

FIG. 53. Photomicrograph of 60 day foetal sheep, showing developing superior longitudinal sinus(arrow) in dura.
(paraffin embedded, H & E, x 70)

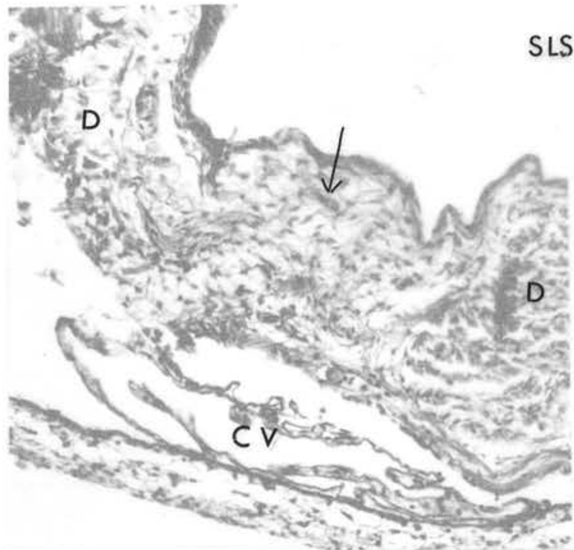

FIG. 54. Photomicrograph of 90 day foetal sheep showing arachnoid cell proliferation(arrow) around cerebral vein.
(paraffin embedded, H & E, x 140)

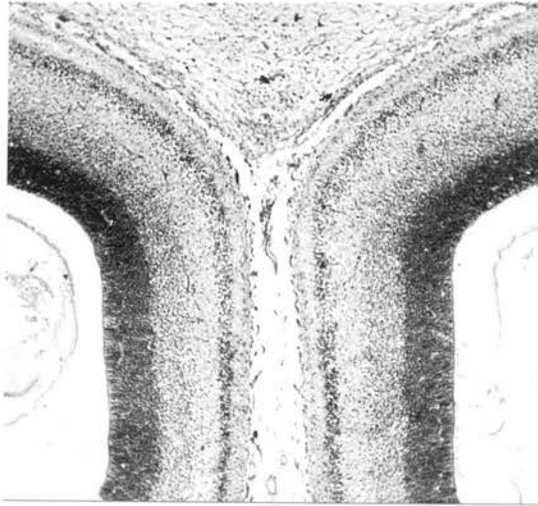

FIG. 53. Photomicrograph of 60 day foetal sheep, showing developing superior longitudinal sinus(arrow) in dura.
(paraffin embedded, H & E, x 70)

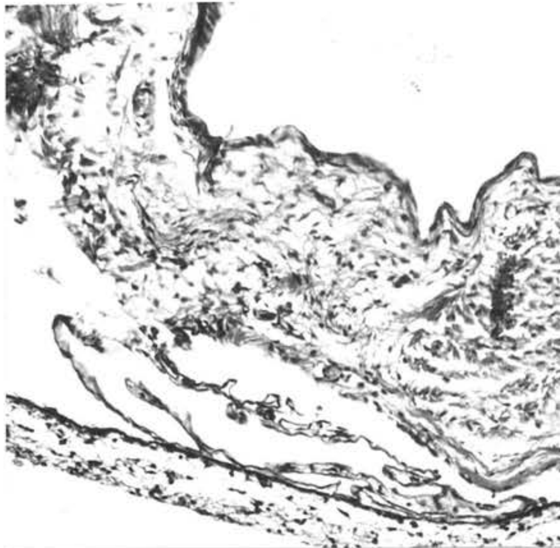

FIG. 54. Photomicrograph of 90 day foetal sheep showing arachnoid cell proliferation(arrow) around cerebral vein.
(paraffin embedded, H & E, x 140)

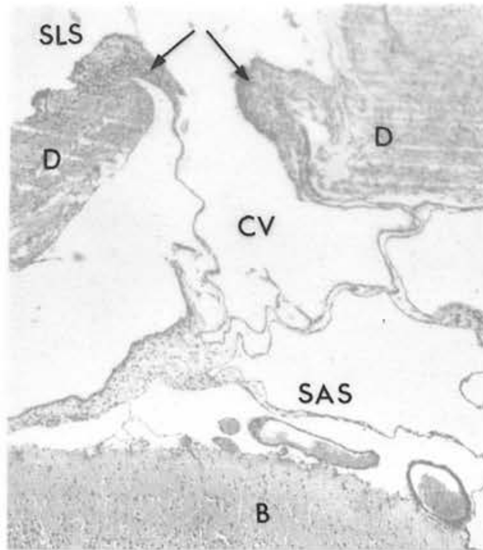

FIG. 55. Photomicrograph of 110 day foetal sheep showing arachnoid cell proliferations(arrow) resembling villi at the opening of cerebral vein into superior longitudinal sinus.
(paraffin embedded, H & E, x 54)

FIG. 56. Same as fig. 55, but showing the cell proliferations meeting to form a granulation.
(paraffin embedded, H & E, x 54)

FIG. 55. Photomicrograph of 110 day foetal sheep showing arachnoid cell proliferations(arrow) resembling villi at the opening of cerebral vein into superior longitudinal sinus.
(paraffin embedded, H & E, x 54)

FIG. 56. Same as fig. 55, but showing the cell proliferations meeting to form a granulation.
(paraffin embedded, H & E, x 54)

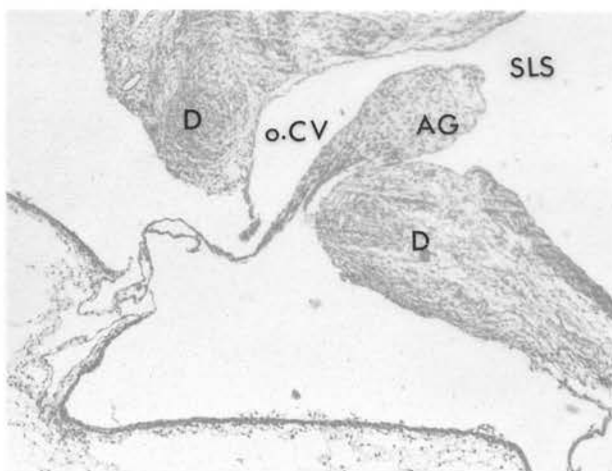

FIG. 57. Photomicrograph of 120 day foetal sheep showing arachnoid granulation at opening of cerebral vein into superior longitudinal sinus.
(paraffin embedded, H & E, x 54)

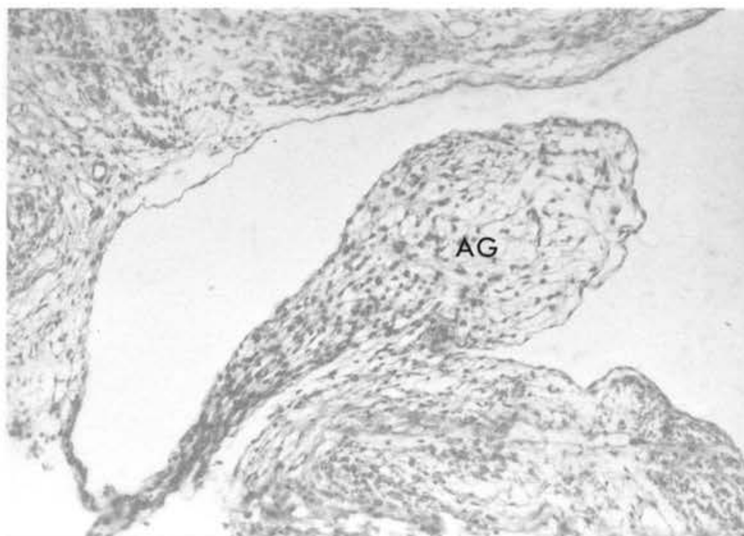

FIG. 58. Arachnoid granulation of 120 day foetus in sheep, showing surface epithelial cells and collagen bundles and the spaces between them in the core of the granulation.
(paraffin embedded, H & E, x 350)

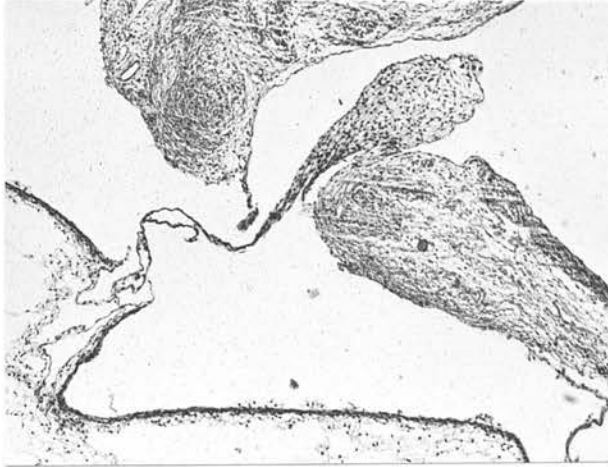

FIG. 57. Photomicrograph of 120 day foetal sheep showing arachnoid granulation at opening of cerebral vein into superior longitudinal sinus.
(paraffin embedded, H & E, x 54)

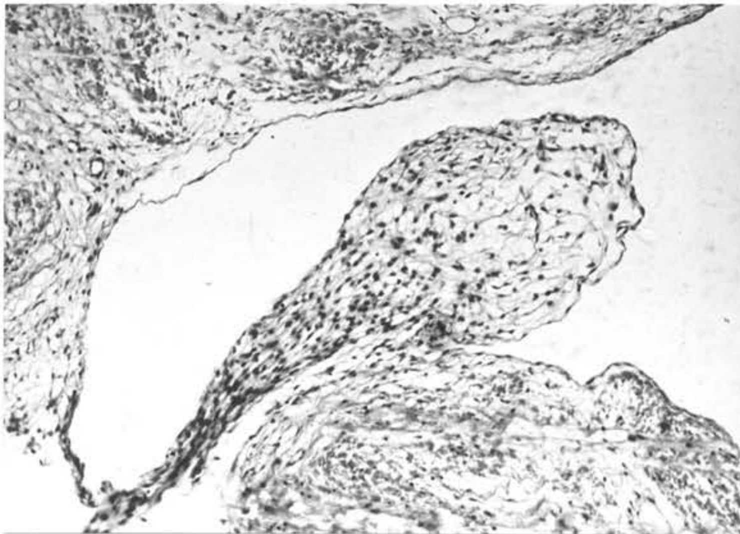

FIG. 58. Arachnoid granulation of 120 day foetus in sheep, showing surface epithelial cells and collagen bundles and the spaces between them in the core of the granulation.
(paraffin embedded, H & E, x 350)

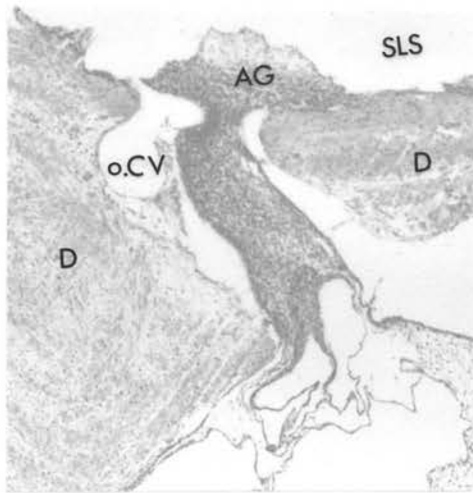

FIG. 59. Photomicrograph of 140 day foetal sheep arachnoid granulation at opening of cerebral vein into sinus.
(paraffin embedded, H & E, x 54)

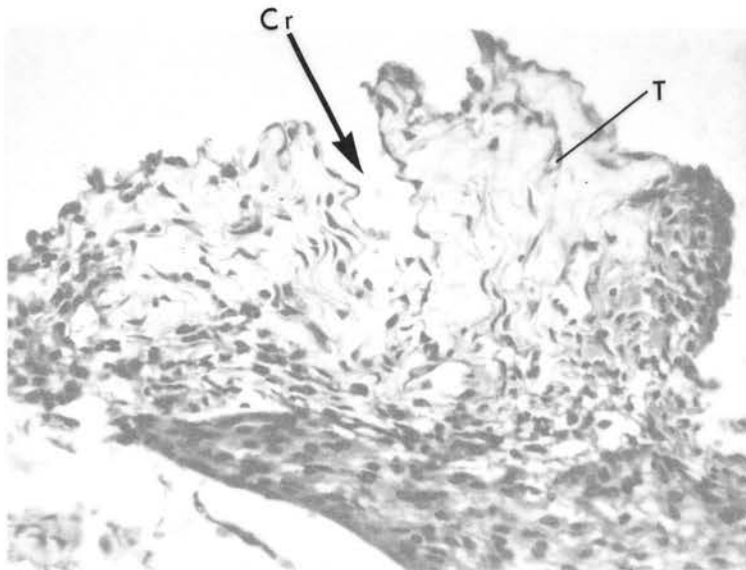

FIG. 60. Photomicrograph of an arachnoid granulation of a 140 day sheep foetus showing crypts and tubules.
(paraffin embedded, H & E, x 350)

FIG. 59. Photomicrograph of 140 day foetal sheep arachnoid granulation at opening of cerebral vein into sinus.
(paraffin embedded, H & E, x 54)

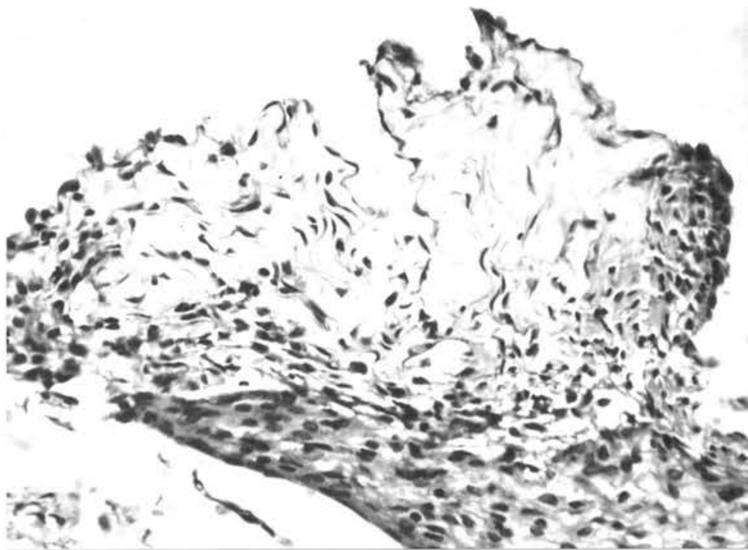

FIG. 60. Photomicrograph of an arachnoid granulation of a 140 day sheep foetus showing crypts and tubules.
(paraffin embedded, H & E, x 350)

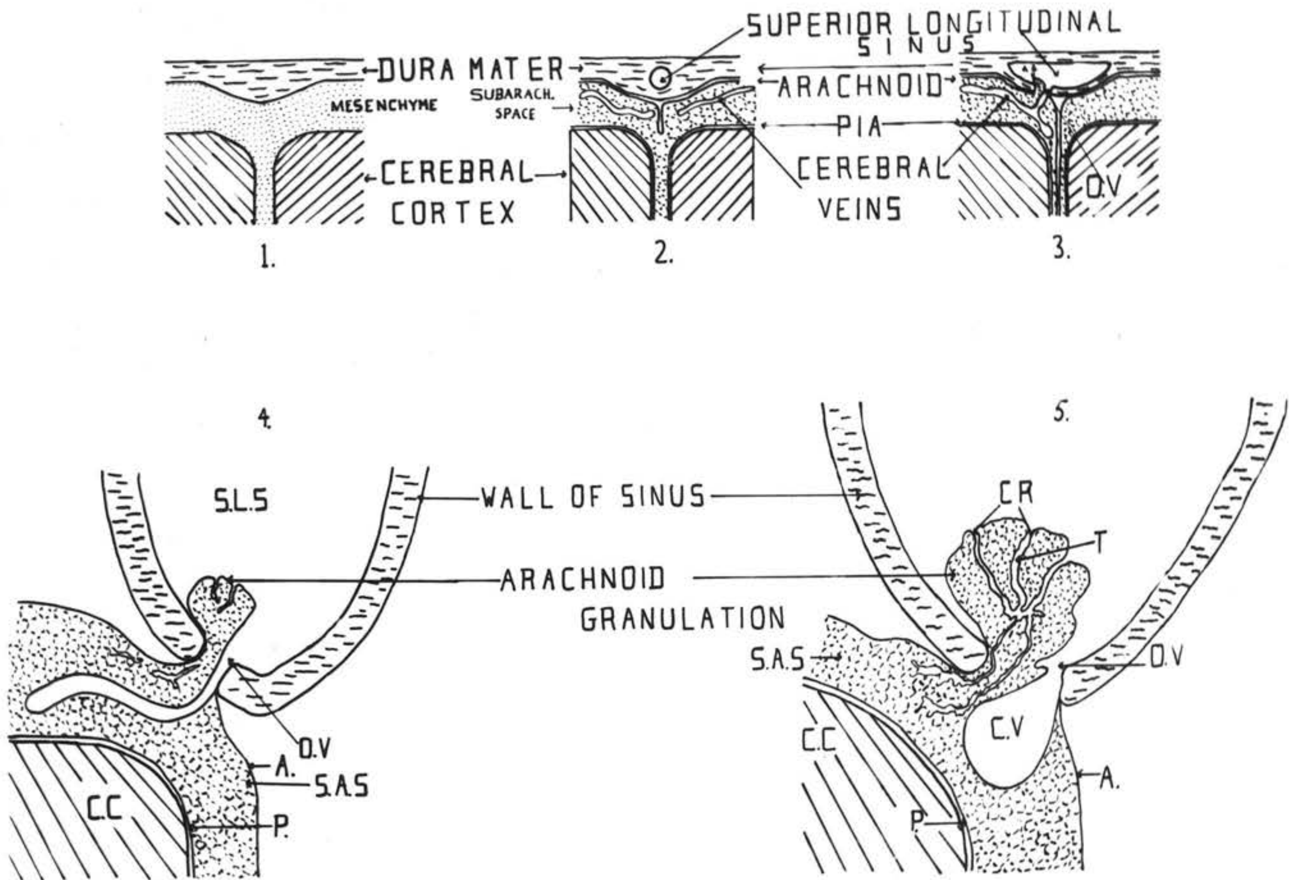

FIG. 61. Diagram summarising stages in development of arachnoid granulations in sheep. CR = crypt, CC = cerebral cortex, OV = opening of cerebral vein.

INJECTION EXPERIMENTS IN SHEEP.

As described in a previous section, two sets of channels lined by flattened endothelial cells have been observed in the arachnoid granulations of sheep by both light and electron microscopy. One set appears to arise from the blood vessels of the dura, while the other, which forms continuous channels, appears to connect the subarachnoid space to the venous sinus. In order to confirm this observation and to assess whether the two systems are separate or are linked, the following experiments were carried out:

If the arterial system is injected with India ink, only those channels arising from arteries should contain the dye, and not the other; conversely, if the subarachnoid space is injected the channels joining the subarachnoid space to the venous sinus should show the ink, but not those arising from the blood vessels. If there is communication between the two systems, then injection into either the arteries or into the subarachnoid space should blacken both sets of channels. Such experiments were performed on the decapitated heads of sheep, and whole mounts and microscopic sections of the injected granulations have been examined by light microscopy.

In order to study the communicating channels between the subarachnoid space and the venous sinus (tubules) at ultra structural level, another set of experiments was designed. Electron dense particulate matter (thorium dioxide) was injected into the subarachnoid space in live sheep, and the arachnoid granulations examined by electron microscopy.

MATERIAL AND METHOD.

Light microscopy.

(a) Intra-arterial injection.

3 adult sheep were used.

The/

The animals were killed by decapitation and the arteries washed out by perfusing with 3.8% sodium citrate (to clear away clotted blood and prevent further clot formation) through both common carotid arteries. When clear perfusing fluid leaked out of the veins, the skull was opened and the superior longitudinal sinus incised. The superior cerebral veins opening into the sinus were ligated. This was done in order to prevent India ink from flowing into the sinus, from which the ink could enter the tubules of the granulation. As a further precaution that part of the superior longitudinal sinus containing the granulations was isolated by ligatures and severed from the rest of the sinus, in order to prevent any possible leak of ink from the anterior or posterior end of the sinus. The sinus was then thoroughly washed with 1.N saline after which 125 mls of a solution of India ink (containing 1 part of 'Higgins' India ink in 5 parts of 1.N saline) were injected into each common carotid artery by gravitational flow. The column of injection fluid measured approximately 1 meter. As it was impossible to ligate all the venules and the leakage of India ink could invalidate the results, the granulations were continuously washed with 1.N saline during the period of injection, so that India ink in any channel could only have reached it via the arteries, and not from the venous sinus. When the injection of the India ink was complete, the sinus was examined by naked eye, and the brain fixed in situ in 10% formol saline for double-embedding in paraffin. (vide pages 32 and 33). Granulations were also removed for embedding in methacrylate as described on page 34.

(b) <u>Subarachnoid injection.</u>

3 animals were used.

After decapitation the vascular system was again/

again washed out with sodium citrate. A small bore tube was then passed along the dorsal aspect of the spinal medulla between the pia and arachnoid membranes, towards the brain. The cut edges of the spinal medulla and the polythene tube were tightly tied together to prevent any back flow of fluid between the pia and arachnoid membranes. 50 mls of India ink were injected through the polythene tube by gravitational flow (similar to the intra-arterial injection) until drops of India ink were seen to leak from the nostrils. The skull was opened, the sinus examined and blocks of tissue removed for examination by light microscopy using paraffin and methacrylate as embedding media. Whole mounts were also prepared.

Electron microscopy.

Two sets of experiments were carried out. In one set the animal was sacrificed half an hour after subarachnoid injection of thorium dioxide, and in the other, the animal was sacrificed 4 hours after injection.

1st set

2 adult sheep were used.

Each animal was anaesthetized by injecting 15 mls of nembutal (containing 60 mgms of nembutal per 1 ml) into the internal jugular vein. When the animal was fully anaesthetized, cisternal puncture was performed with a needle and 2 mls of cerebro-spinal fluid collected. Next, 2 mls of thorium dioxide were injected slowly (in 5 minutes) through the same needle into the cisterna magna and the stillette of the cisternal puncture needle replaced to prevent leakage. Twenty minutes after the injection with thorium, surgical anaesthesia began to wear off. Thirty minutes after injection of thorium, a few drops of cerebro-spinal fluid was collected in order to check the/

the presence of thorium particles. After this, the cisternal puncture needle was withdrawn and the animal sacrificed. The skull was opened with a saw, the superior longitudinal sinus incised, and two separate blocks of tissue, one containing arachnoid granulations and sinus wall, and the other, cerebral cortex and meninges were removed. The blocks were processed for embedding in araldite as described on pages 35 and 36.

2nd set.

2 animals were used.

The procedure adopted was similar to the 1st set, except that the animals were sacrificed 4 hours after subarachnoid injection of thorium dioxide. Before sacrifice, the cerebro-spinal fluid was once again examined. After 4 hours following the thorium injection, the anaesthesia had completely worn off, the animal stood upright again and moved its head freely.

RESULTS.

Intra-arterial injection.

Macroscopic.

The dural blood vessels contain particles of the injected India ink, and so do the cerebral veins up to the point of their ligation (fig. 62).

Microscopic.

On low power microscopic examination of the whole mount preparations, channels filled with India ink are observed in the granulations. On viewing the granulations at different level of focus, a large number of channels showing India ink are observed, which are continuous with the dural blood vessels (fig. 63).

In both paraffin and methacrylate sections, India ink is observed in channels arising from dural blood vessels but not in the channels (tubules) connecting the subarachnoid space to the venous sinus (figs. 64 and 65). These India ink filled channels must be capillaries as they connect with the dural blood vessels. Two graphic reconstructions were made from serial sections of the injected granulations embedded in methacrylate, by plotting the width of granulations, diameters of tubules and capillaries as abscissae and the thickness of the sections as ordinates (the thickness of each section was 1 μ and every tenth section taken). These reconstructions are shown in figs. 66 and 67. It was possible to demonstrate the relative positions of blood vessels and the arachnoid tubules.

The tubules, which are in continuity with the crypts at the surface of the granulation, appear to communicate with similar tubules of the subarachnoid space. Each tubule does not pass through the granulation independently, but joins up with/

with others to form a network of tubules in the centre of the granulation. From this network, separate tubules arise inferiorly and pass through the neck of the granulation to join the subarachnoid space (figs. 66 and 67).

Subarachnoid injection.

Macroscopic.

On opening the sinus, no blood is observed in it, though there are particles of India ink. The granulations stand out as black beads (fig. 68). Examination of the surface of the granulation, shows darker pitted areas, probably the openings of the crypts filled with India ink (fig. 69). The entire subarachnoid space is blackened but not the dura.

Microscopic.

On viewing the whole mounts with a microscope, tubules filled with India ink are outlined as shown in fig. 70. In subarachnoid injections, the India ink fills both tubules and the spaces between the collagen bundles in the core of the granulation, thereby masking many tubules.Fig. 70 a to d are photomicrographs of a granulation taken at different levels of focus, with the granulation slightly tilted. Photographs a, b and c show the tubules and d shows India ink in the spaces between the collagen bundles. Photographs a, b and c also show masses of India ink particles quite separate from the tubules, and these appear to be particles of the ink lying in other tubules or on the surface of the granulation at the opening of crypts. (a is at the highest point of focus).

In paraffin and methacrylate sections, the arachnoid tubules are observed to be filled with India ink but not capillaries (fig. 71). It is possible in most sections to identify separately the tubules and the/

the spaces between the collagen bundles (fig. 72). In a few isolated areas, however, where the granulation is packed with India ink, separation between tubules and the spaces between the collagen bundles is impossible (fig. 73). India ink is also present on the surface of the granulation as well as in the sinus, thus suggesting the existence of a direct communication between subarachnoid space and venous sinus through the tubules. There was no evidence of rupture of a membrane, i.e. lining of tubule or surface epithelium.

On examining the junction between granulations and subarachnoid space, India ink was found in tubule-like spaces of the subarachnoid tissue (fig. 74). Whether these spaces in the subarachnoid tissue represent a tubular system similar to that described in granulations or to the spaces between collagen bundles has not been determined.

Electron microscopy.

Particles of thorium dioxide are observed in the cerebro-spinal fluid, as well as in sections of granulations from sheep killed half an hour after subarachnoid injection. Particles have been noted on the sinus surface of the granulations, in the crypts, in the tubules and in the spaces between collagen bundles. Some particles are also seen in macrophage cells.

Clumps of thorium dioxide particles are observed lying close to the plasma membrane of the surface epithelial cells, on the venous side (fig. 75). In the crypts and tubules, clumps of thorium dioxide particles are also present in the lumina close to the plasma membrane (figs. 76 and 77), and in pockets between adjacent cells (fig. 78). Thorium is also found in the spaces between the collagen bundles and around individual collagen fibrils (fig. 79). Not only/

only are they found in these spaces but they are also found in the inter-cellular spaces of the core of the granulation (fig. 80). With the exception of one vesicle in a section of a single surface cell (fig.75), particles of thorium have not been observed in cytoplasmic vesicles of the endothelial cells of the surface epithelium of the granulation and lining the tubules.

Large numbers of particles are present in the cytoplasmic vesicles of both type 1 and type 2 macrophages of sheep arachnoid granulations (figs. 37 and 40). In some of the type 1 macrophages, vesicles (containing particles) are arranged in a linear fashion (fig. 81). This appearance could be due to pinocytosis by the 'membrane flow and membrane vesiculation' mechanism as suggested by Bennett (1956). Half an hour after the injection of thorium dioxide into the subarachnoid space, large numbers of type 1 and type 2 macrophages are observed in sheep granulations in about equal proportions.

In the arachnoid membrane and in the subarachnoid tissue away from the granulations, the distribution of both types of macrophages is similar to that in the granulations, and such macrophages also have vesicles containing particles of thorium. The cells of the arachnoid epithelium lining the subdural space also show vesicles containing particles of thorium dioxide (fig. 83). The uptake of particulate matter by vesicles of the arachnoid epithelial cells, shown in this investigation, is similar to the findings of Odor (1956) who demonstrated the presence of thorium dioxide particles in vesicles of the cells lining the peritoneal cavity, after thorium was injected into the peritoneal cavity. The arachnoid epithelial cells lining the subdural space are morphologically similar to the cells lining the peritoneum or/

or the pleura. In this respect, the subdural space could be compared to either the pleural or peritoneal cavities.

In the cerebro-spinal fluid withdrawn from sheep 4 hours after injection of thorium, fewer particles were observed as compared to the half hour specimen. In the granulations examined 4 hours after injection the following features are apparent:

1. Thorium particles are not present in the tubules, in the crypts or on the surface epithelial cells of the granulation.
2. Few particles are present in the spaces between collagen bundles and in the inter-cellular spaces, in comparison with the half hour specimen.
3. The number of type 1 macrophages is greatly reduced and hardly any of the type 2 variety are observed, the approximate ratio of type 1 to type 2 being in the order of 10:1.
4. The concentration of thorium particles in the type 1 macrophage seems to be greater in the 4 hour specimen (fig 82) than in the ½ hour one (vide fig.81).
5. A large collection of thorium dioxide particles is found interspersed with the basement membrane of the surface epithelial cells of the granulation (fig. 85). The reduced number of particles in the spaces between the collagen bundles, with the presence of thorium in large numbers, at the basement membrane of the surface epithelial cells, indicates a slow movement of fluid from the core to the periphery of the granulation. This probably suggests a slow filtration of cerebro-spinal fluid through the surface epithelium.
6. No thorium particles are observed in the cytoplasm of any of the surface epithelial cells or in the cells lining the crypts and the tubules.

In the arachnoid membrane away from the granulations/

granulations, and lining the subdural space, the following features are observed in the 4 hour specimen.

1. The vesicles in the arachnoid epithelial cells, show an increase in the number of particles (fig. 84), as compared to the $\frac{1}{2}$ hour one (vide fig. 83).
2. Few particles are present in spaces between collagen bundles of the subarachnoid tissue, with a
3. Similar reduction in the number of both types of macrophages to that noted in the granulations.

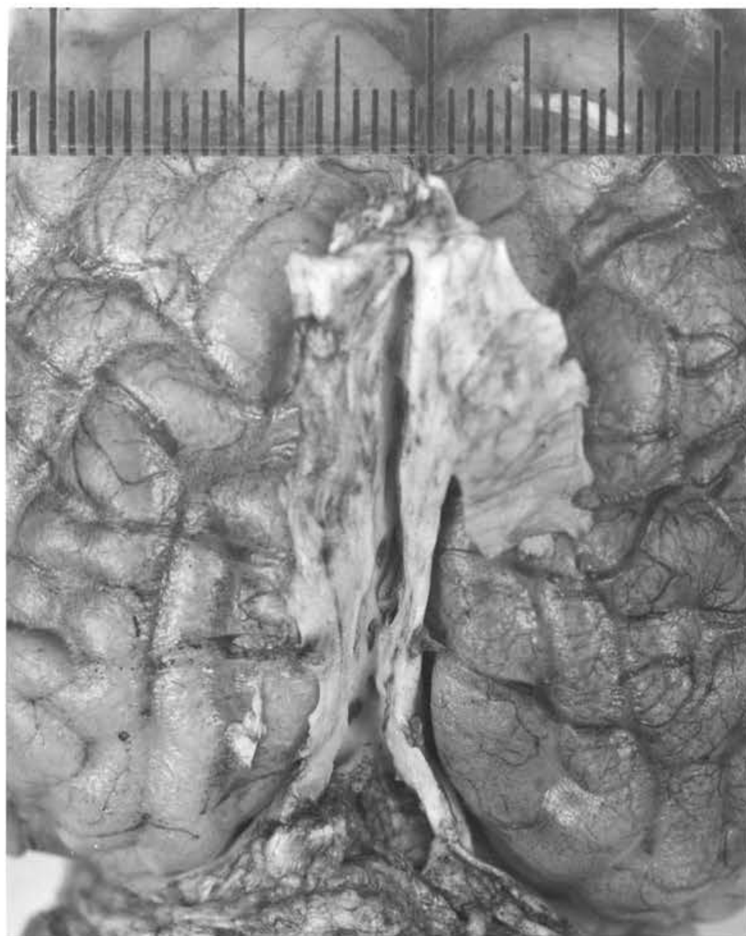

FIG. 62. Photograph of sheep brain showing cerebral and dural blood vessels filled with India ink. The cerebral vessels look incompletely injected because the photograph was taken, after the brain had been in fixative for 24 hours. (x 1)

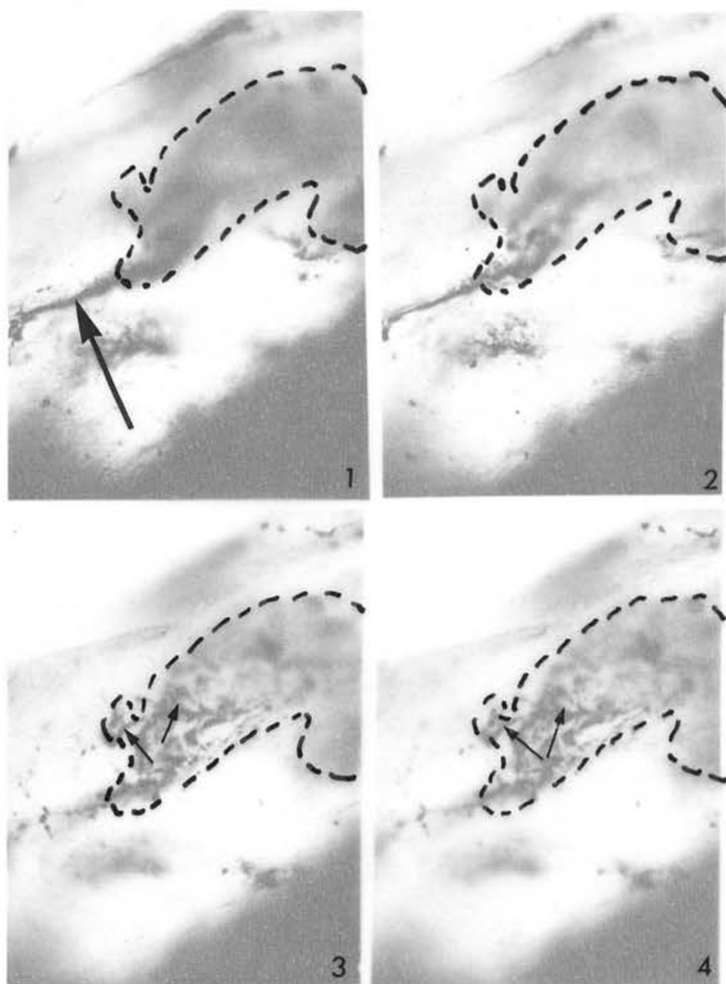

FIG. 63. Photomicrograph of a whole mount of a sheep arachnoid granulation after intra-arterial injection with India ink. Thick arrow shows dural blood vessels and thin arrows show capillaries. Interrupted lines outline granulation.
(x 50)

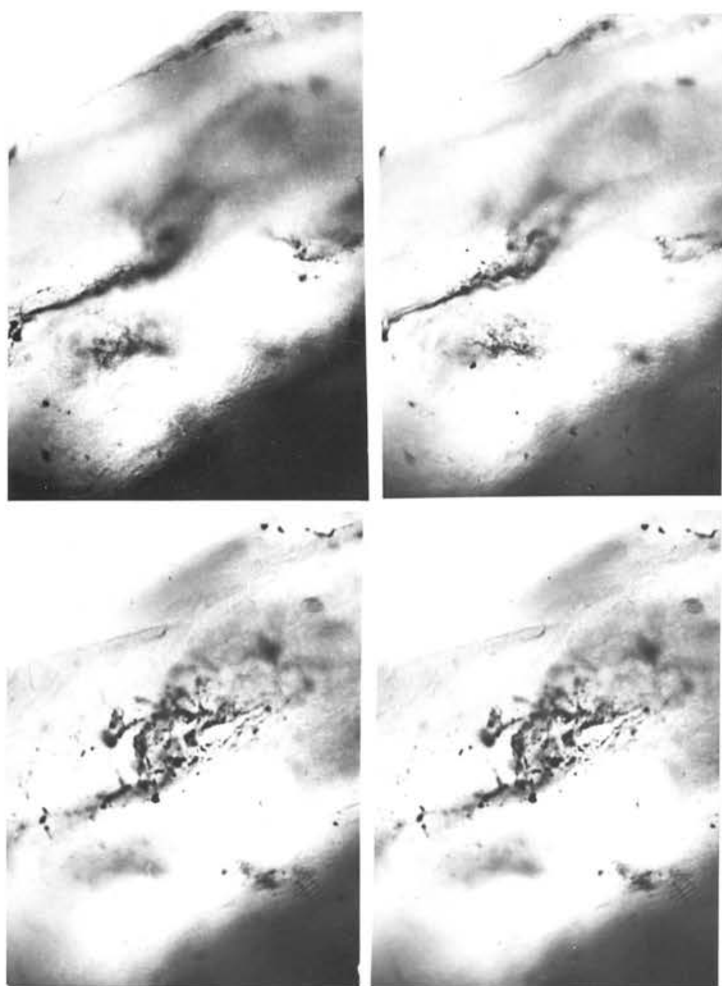

FIG. 63. Photomicrograph of a whole mount of a sheep arachnoid granulation after intra-arterial injection with India ink. Thick arrow shows dural blood vessels and thin arrows show capillaries. Interrupted lines outline granulation.
(x 50)

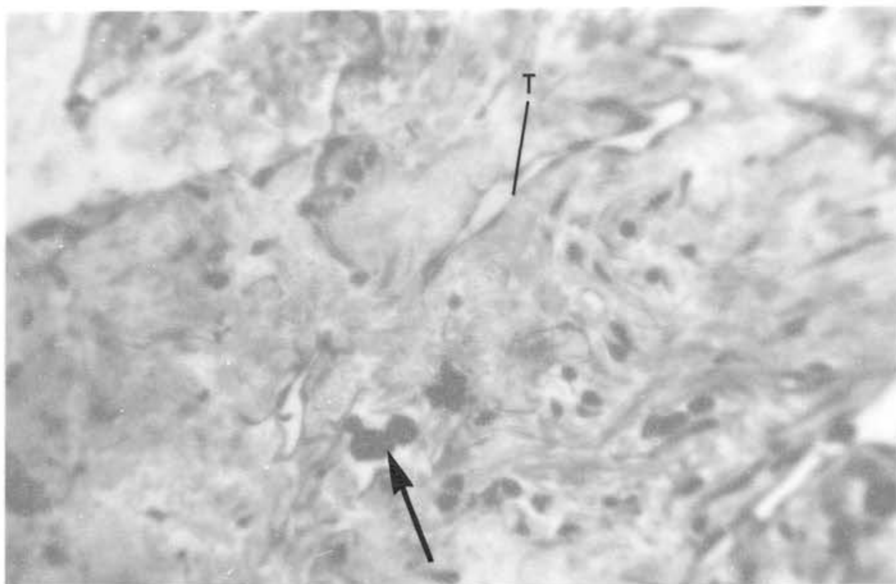

FIG. 64. Photomicrograph of a sheep arachnoid granulation after intra-arterial injection with India ink. Thick arrow shows capillaries filled with India ink while tubule is empty.
(araldite embedded, toluidene blue, x 530)

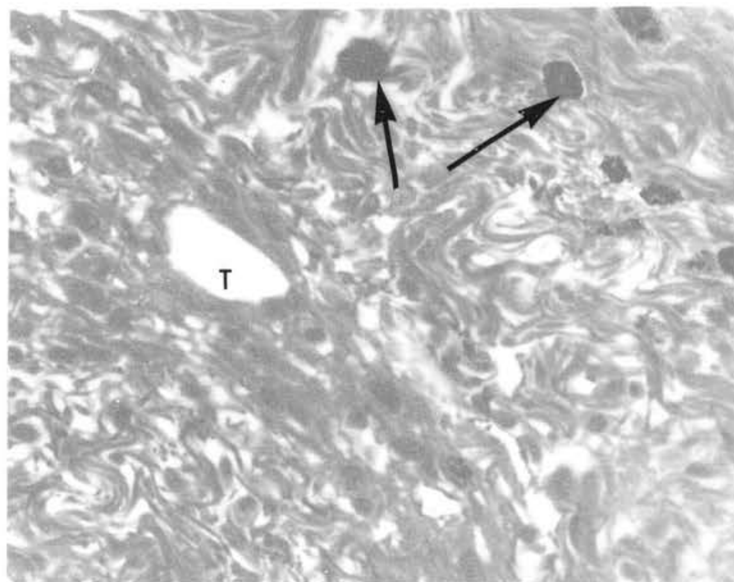

FIG. 65. Same as fig. 64, with some cells showing particles of ink in cytoplasm.
(methacrylate embedded, H & E, x 530)

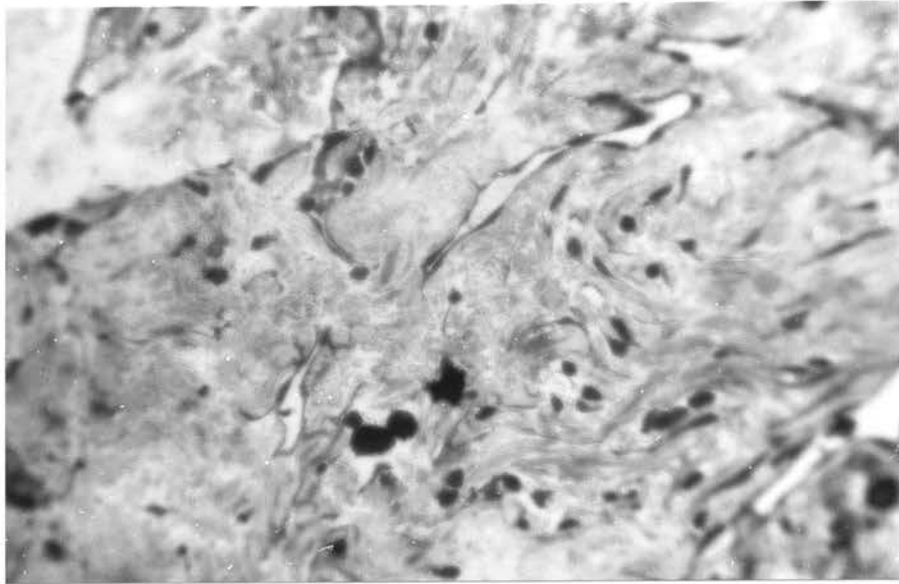

FIG. 64. Photomicrograph of a sheep arachnoid granulation after intra-arterial injection with India ink. Thick arrow shows capillaries filled with India ink while tubule is empty.
(araldite embedded, toluidene blue, x 530)

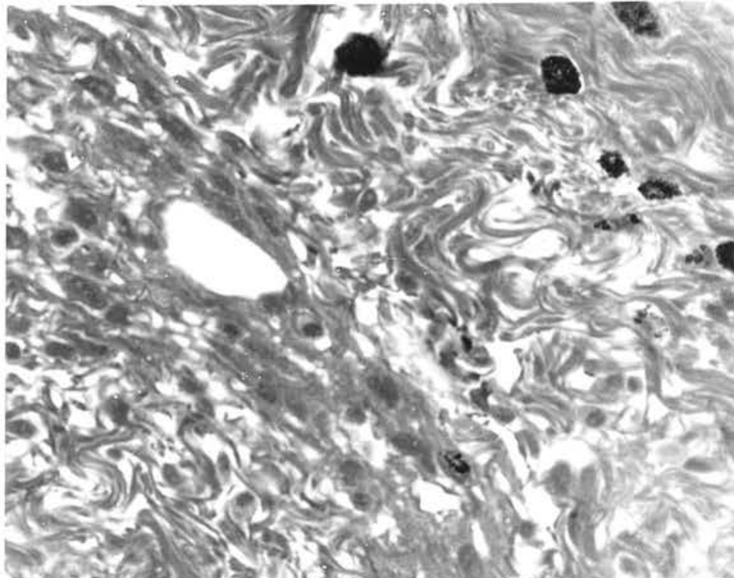

FIG. 65. Same as fig. 64, with some cells showing particles of ink in cytoplasm.
(methacrylate embedded, H & E, x 530)

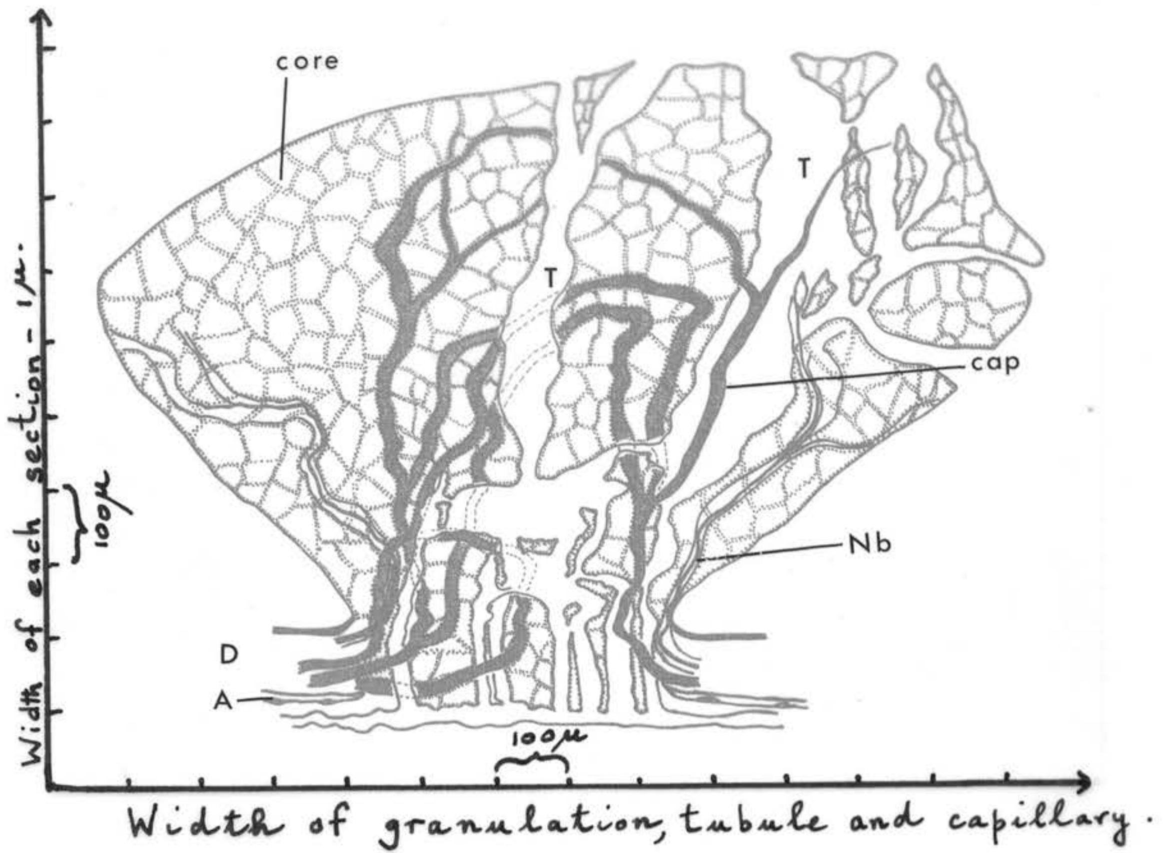

FIG. 66. Graphic reconstruction of sheep arachnoid granulation in which blood vessels were injected with India ink.

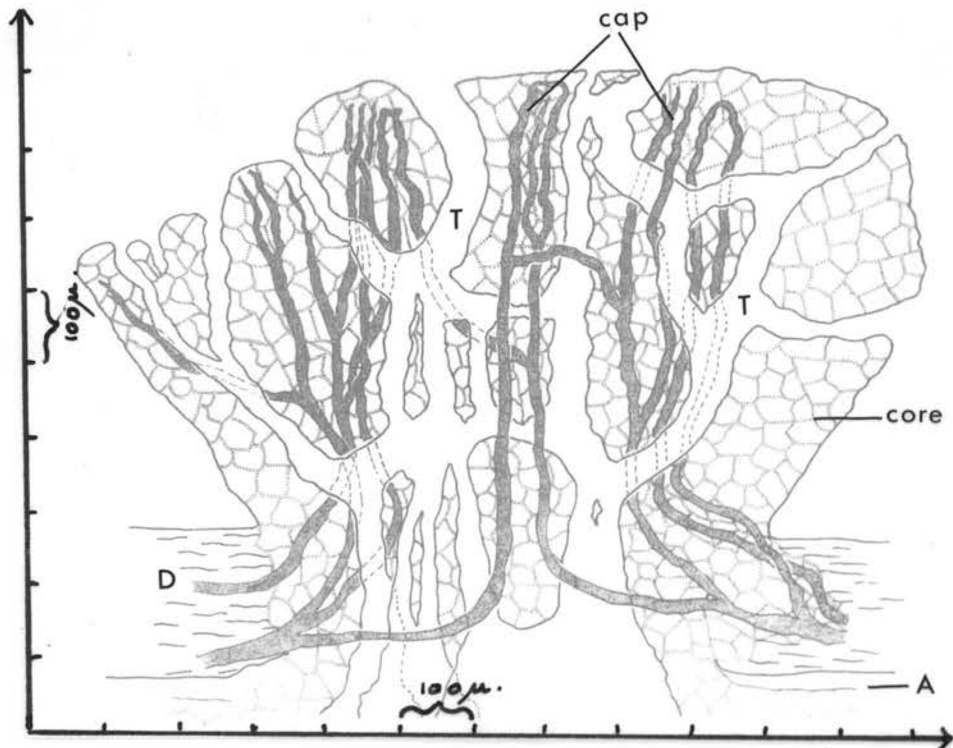

FIG. 67. Graphic reconstruction of sheep arachnoid granulation.

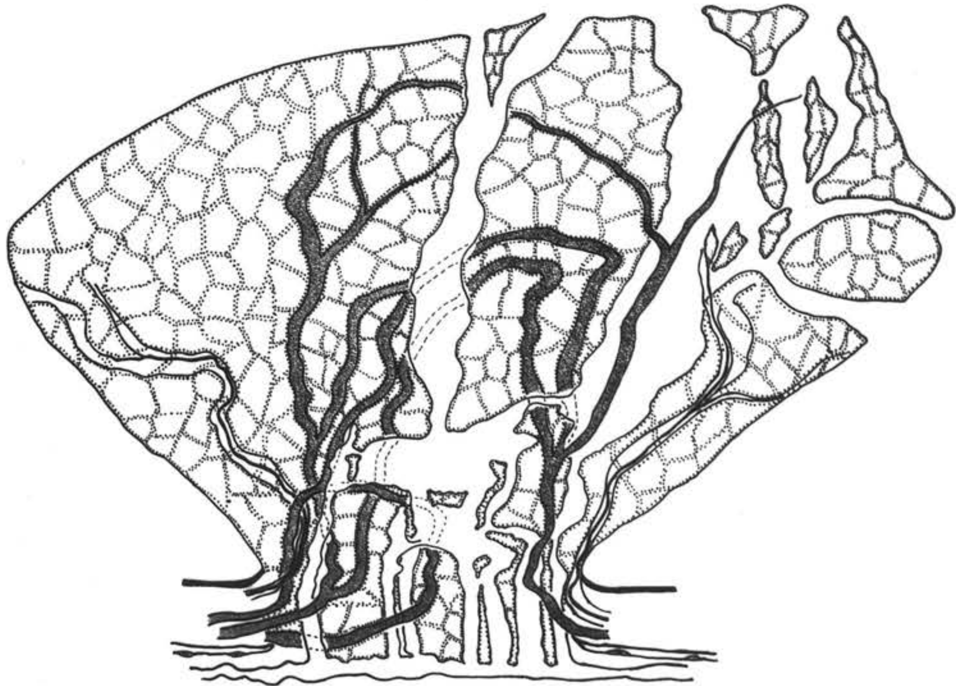

FIG. 66. Graphic reconstruction of sheep arachnoid granulation in which blood vessels were injected with India ink.

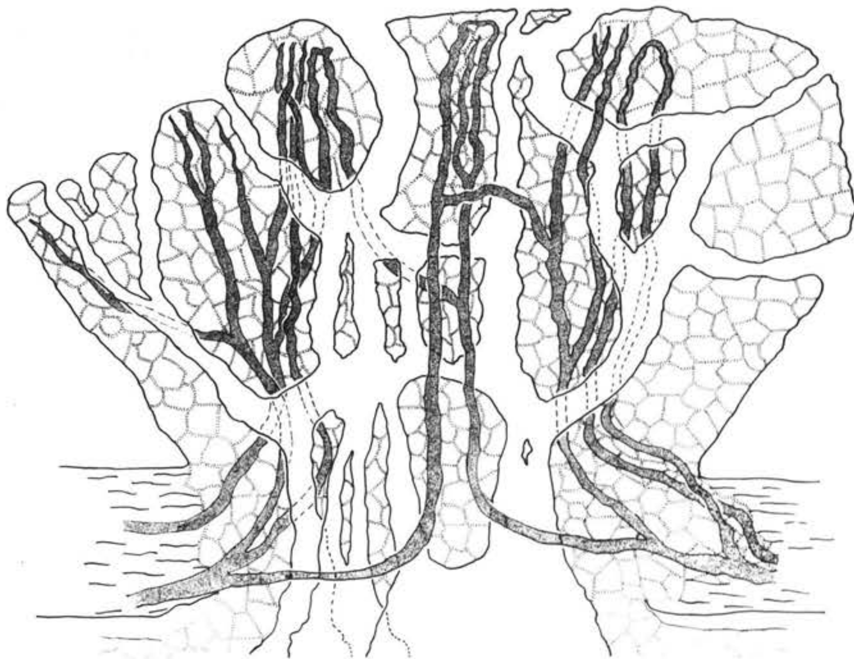

FIG. 67. Graphic reconstruction of sheep arachnoid granulation.

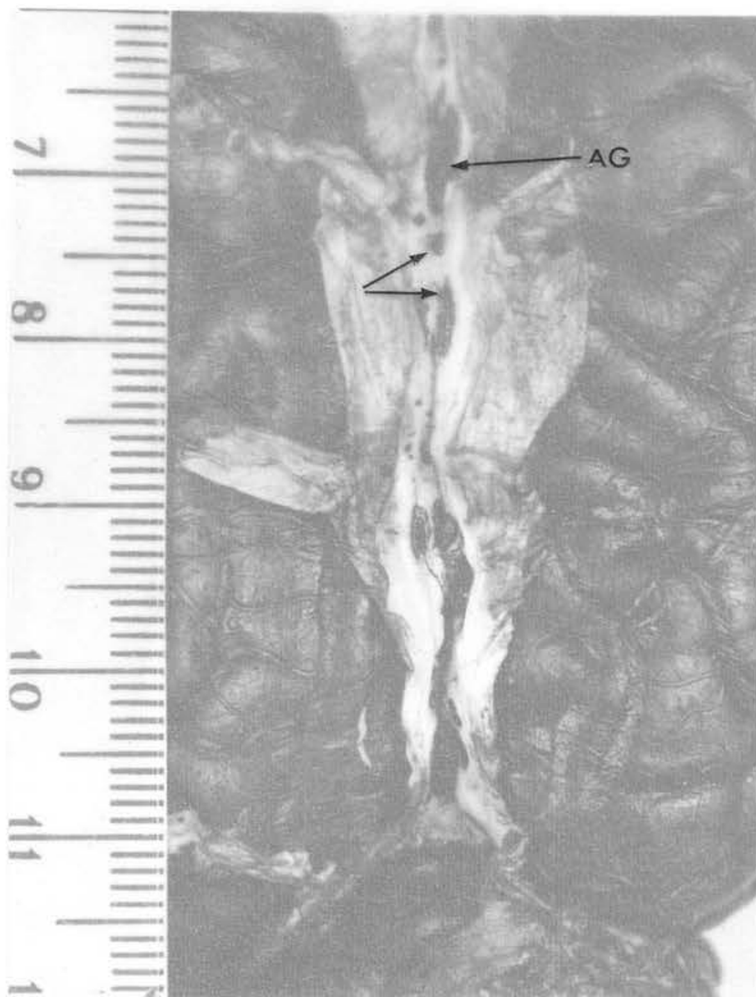

FIG. 68. Photograph of sheep brain after subarachnoid injection with India ink, showing blackening of arachnoid membrane. Granulations stand out as black beads in the superior longitudinal sinus. Thin arrow shows India ink particles in sinus.
(x 1)

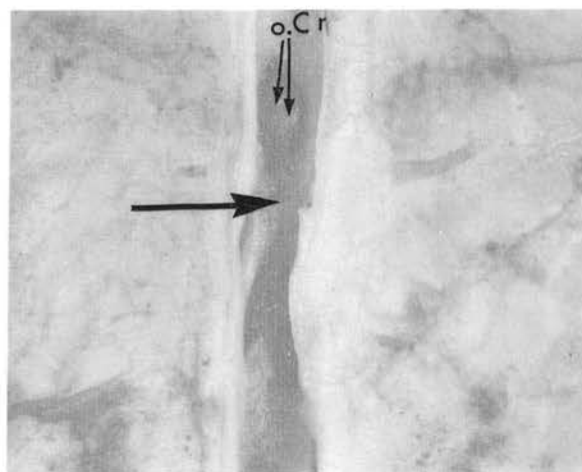

FIG. 69. Photograph of granulation of sheep showing openings of crypts. Thick arrow shows India ink in sinus.
(x 3)

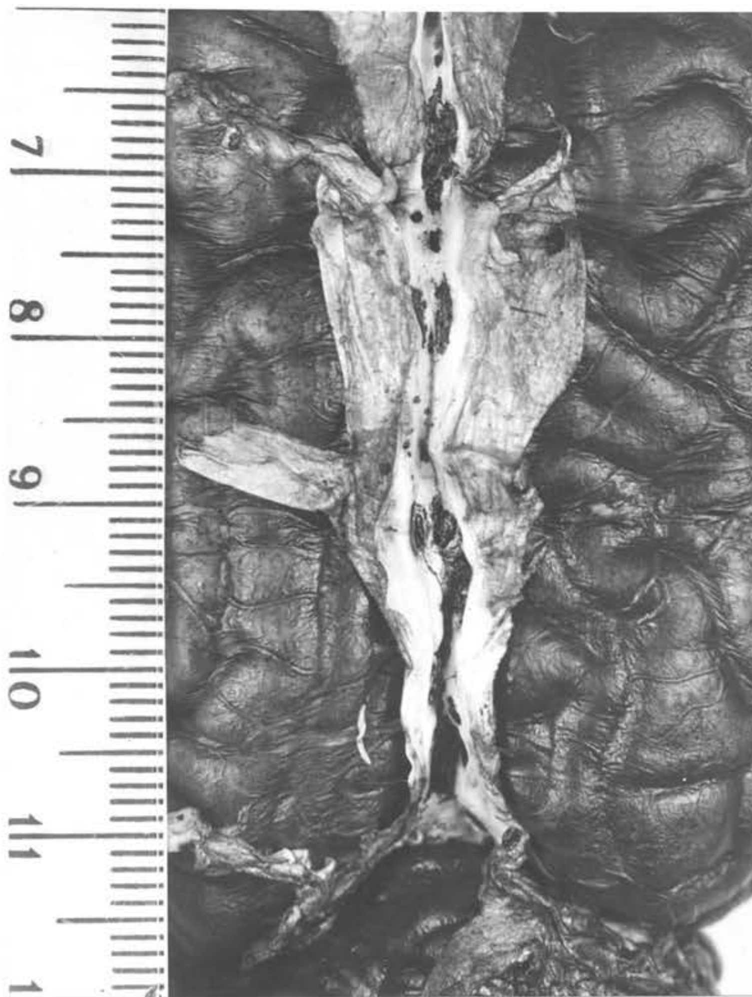

FIG. 68. Photograph of sheep brain after subarachnoid injection with India ink, showing blackening of arachnoid membrane. Granulations stand out as black beads in the superior longitudinal sinus. Thin arrow shows India ink particles in sinus.
(x 1)

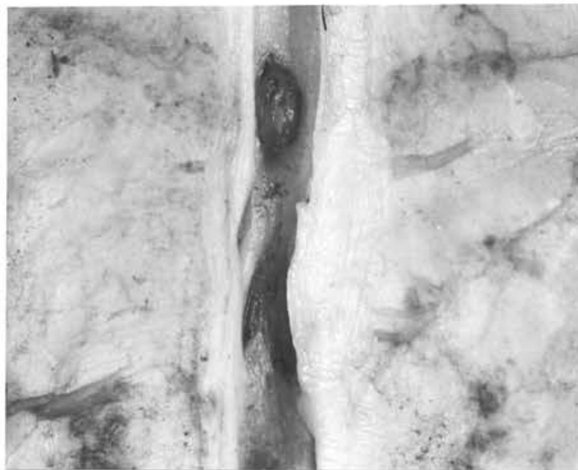

FIG. 69. Photograph of granulation of sheep showing openings of crypts. Thick arrow shows India ink in sinus.
(x 3)

Towards S.A.S. ←

→ Towards S.L.S.

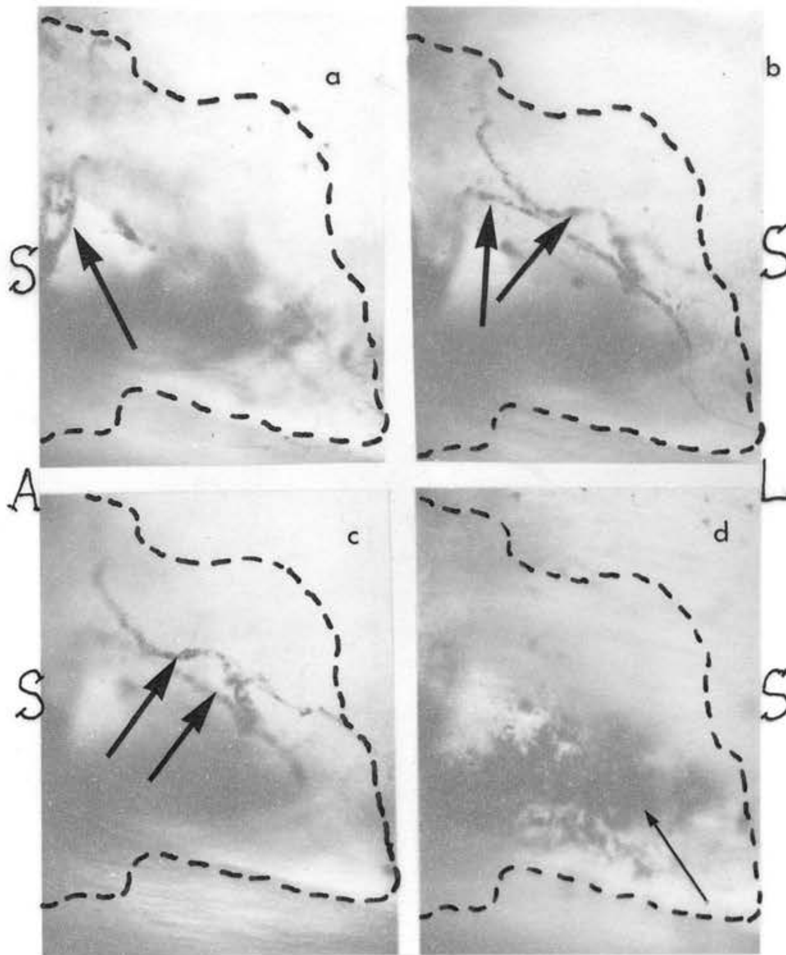

FIG. 70. Photomicrographs of whole mount of a sheep arachnoid granulation, following subarachnoid injection with India ink. Interrupted lines outline granulation. (x 75)

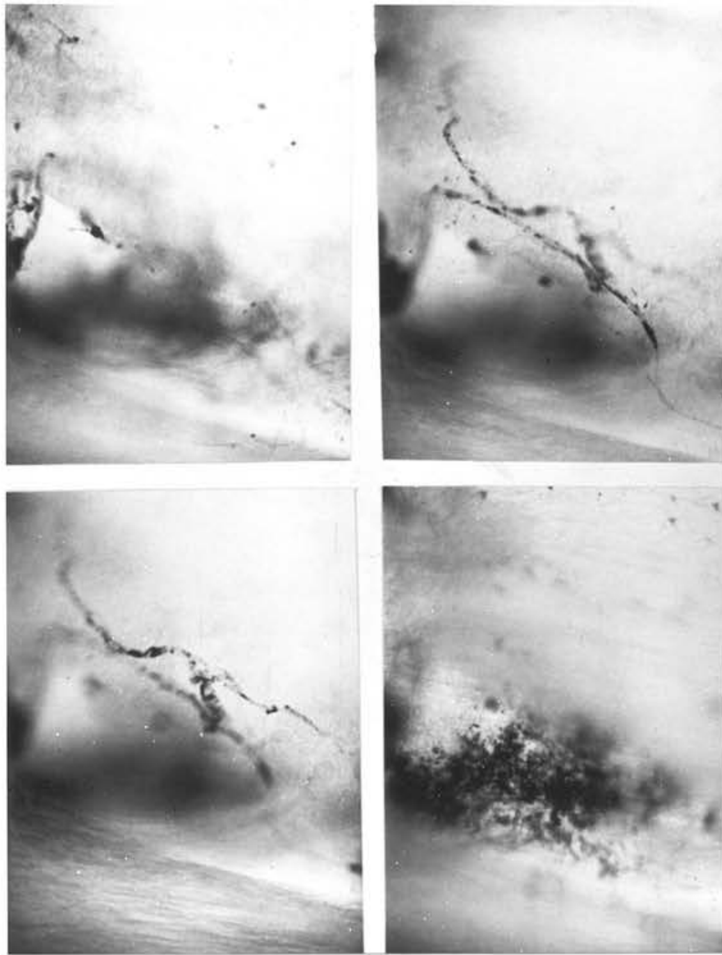

FIG. 70. Photomicrographs of whole mount of a sheep arachnoid granulation, following subarachnoid injection with India ink. Interrupted lines outline granulation. (x 75)

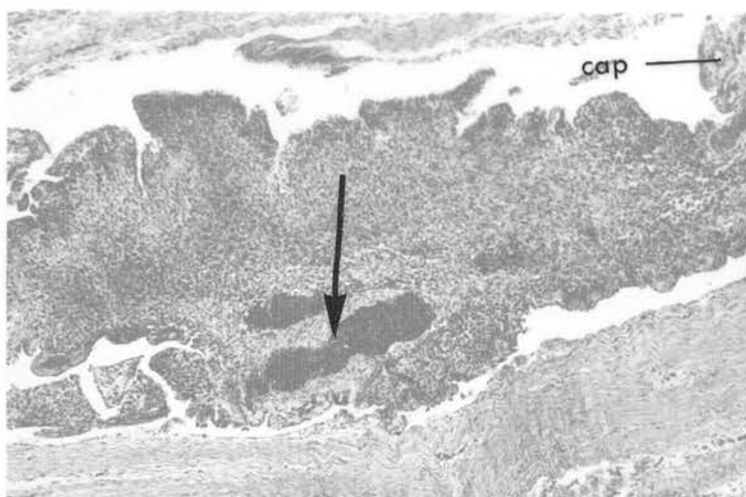

FIG. 71. Photomicrograph of horizontal(tangential) section of sheep granulation after subarachnoid injection with India ink. Arrow shows India ink in tubules and in spaces between collagen bundles but not in capillaries. (paraffin embedded, H&E, x 54)

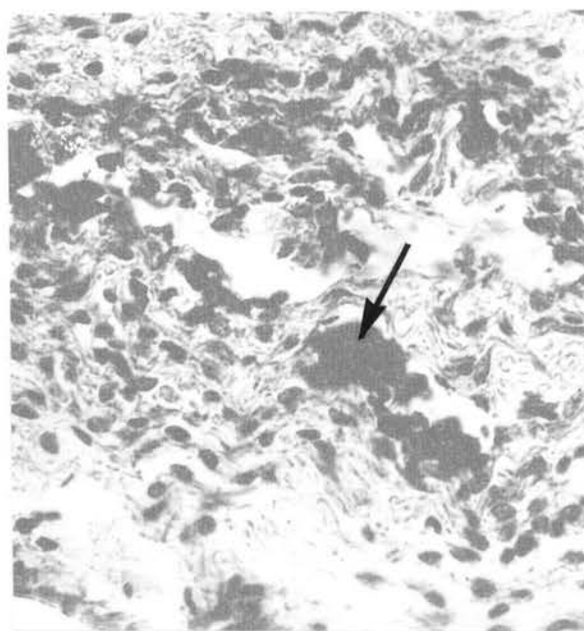

FIG. 72. Photomicrograph of a horizontal (tangential) section of sheep arachnoid granulation following injection of India ink into subarachnoid space. Arrow shows India ink in tubules. India ink is also seen in spaces between collagen bundles.
(methacrylate embedded, H & E, x 530)

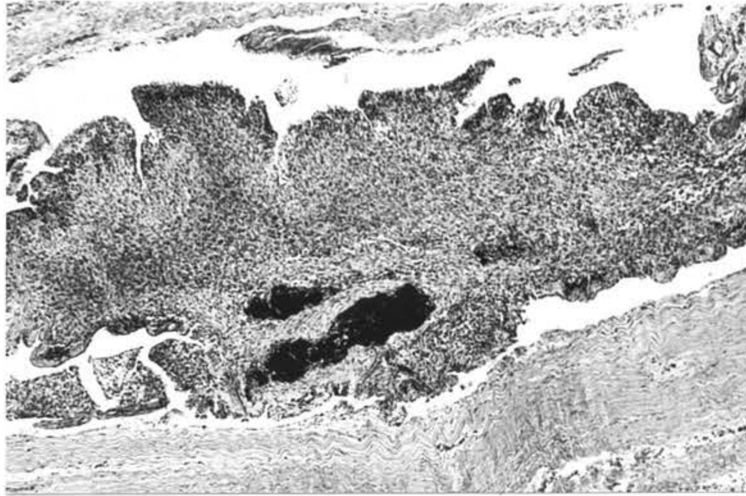

FIG. 71. Photomicrograph of horizontal(tangential) section of sheep granulation after subarachnoid injection with India ink. Arrow shows India ink in tubules and in spaces between collagen bundles but not in capillaries. (paraffin embedded, H&E, x 54)

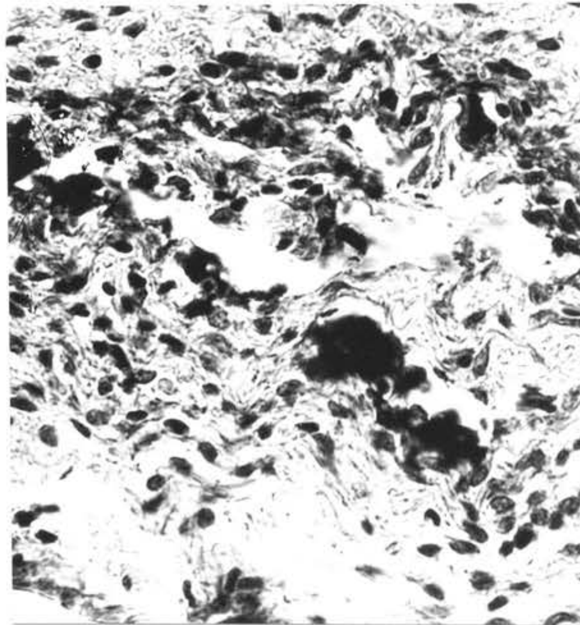

FIG. 72. Photomicrograph of a horizontal (tangential) section of sheep arachnoid granulation following injection of India ink into subarachnoid space. Arrow shows India ink in tubules. India ink is also seen in spaces between collagen bundles. (methacrylate embedded, H & E, x 530)

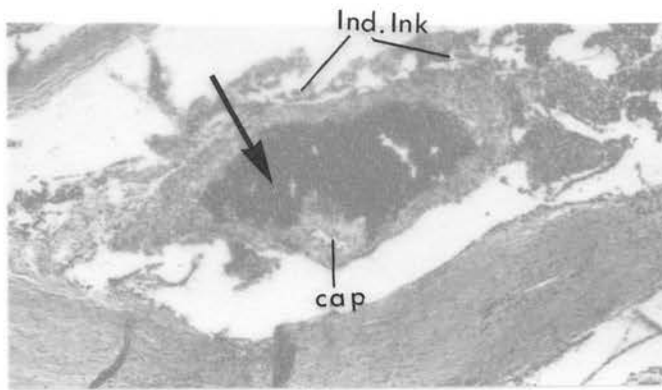

FIG. 73. Photomicrograph of a horizontal(tangential) section of sheep granulation after subarachnoid injection of India ink. Thick arrow shows India ink in core of granulation, which masks the tubules. The capillaries are not filled. India ink is seen in sinus.
(paraffin embedded, H & E, x 54)

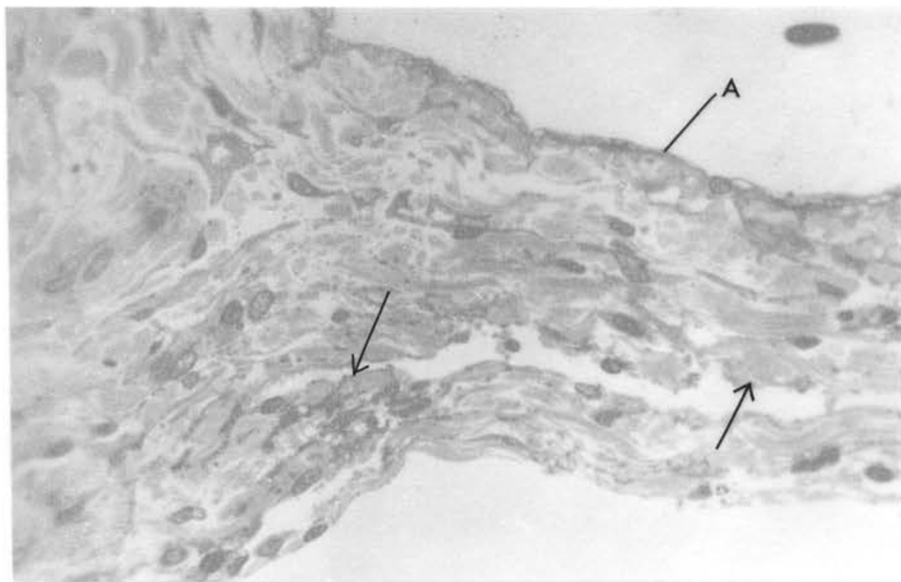

FIG. 74. Photomicrograph of section of junction of granulation with arachnoid tissue after subarachnoid injection with India ink, in sheep. Arrows show India ink particles adherent to the wall of tubule-like structure in subarachnoid space.
(araldite embedded, toluidene blue, x 580)

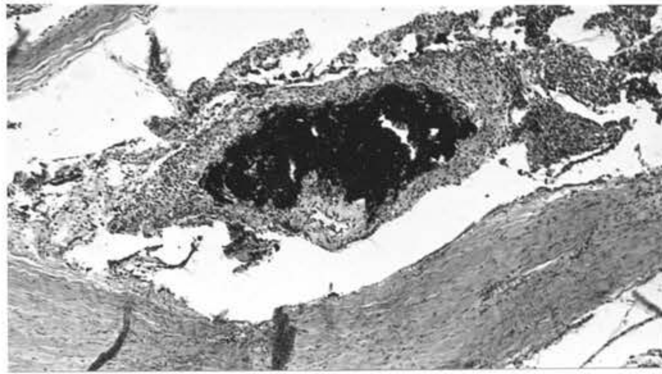

FIG. 73. Photomicrograph of a horizontal(tangential) section of sheep granulation after subarachnoid injection of India ink. Thick arrow shows India ink in core of granulation, which masks the tubules. The capillaries are not filled. India ink is seen in sinus.
(paraffin embedded, H & E, x 54)

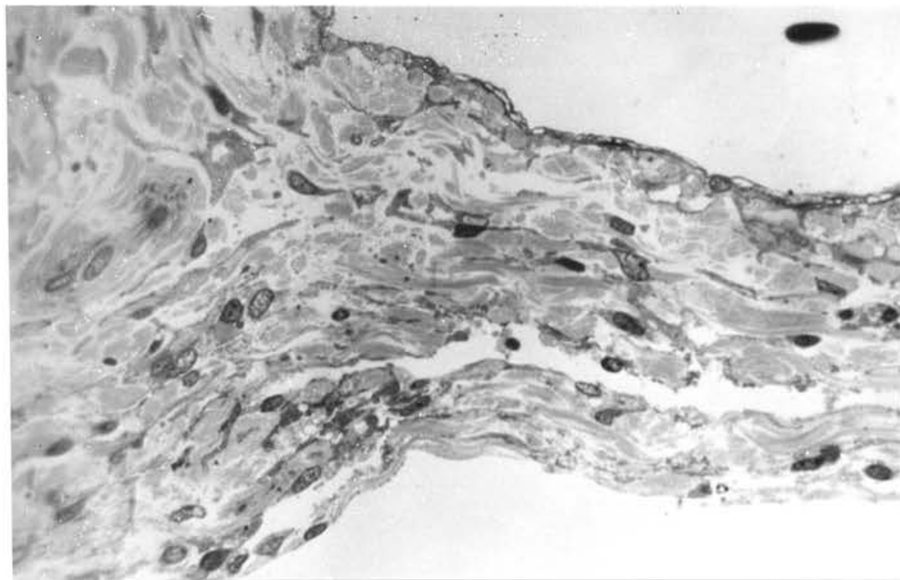

FIG. 74. Photomicrograph of section of junction of granulation with arachnoid tissue after subarachnoid injection with India ink, in sheep. Arrows show India ink particles adherent to the wall of tubule-like structure in subarachnoid space.
(araldite embedded, toluidene blue, x 580)

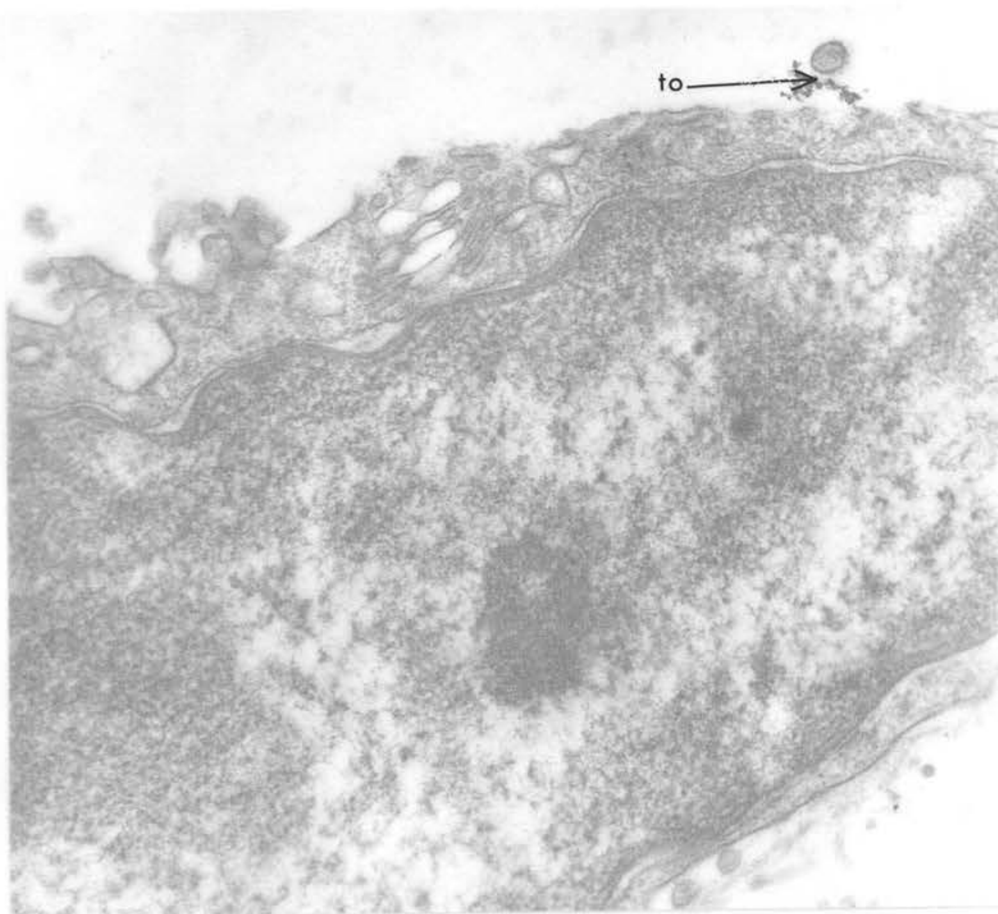

FIG. 75. Electron micrograph of surface epithelial cell showing particles of thorium(to) adherent to the plasma membrane towards the venous sinus, $\frac{1}{2}$ hour after injection into the subarachnoid space.
(x 105,000)

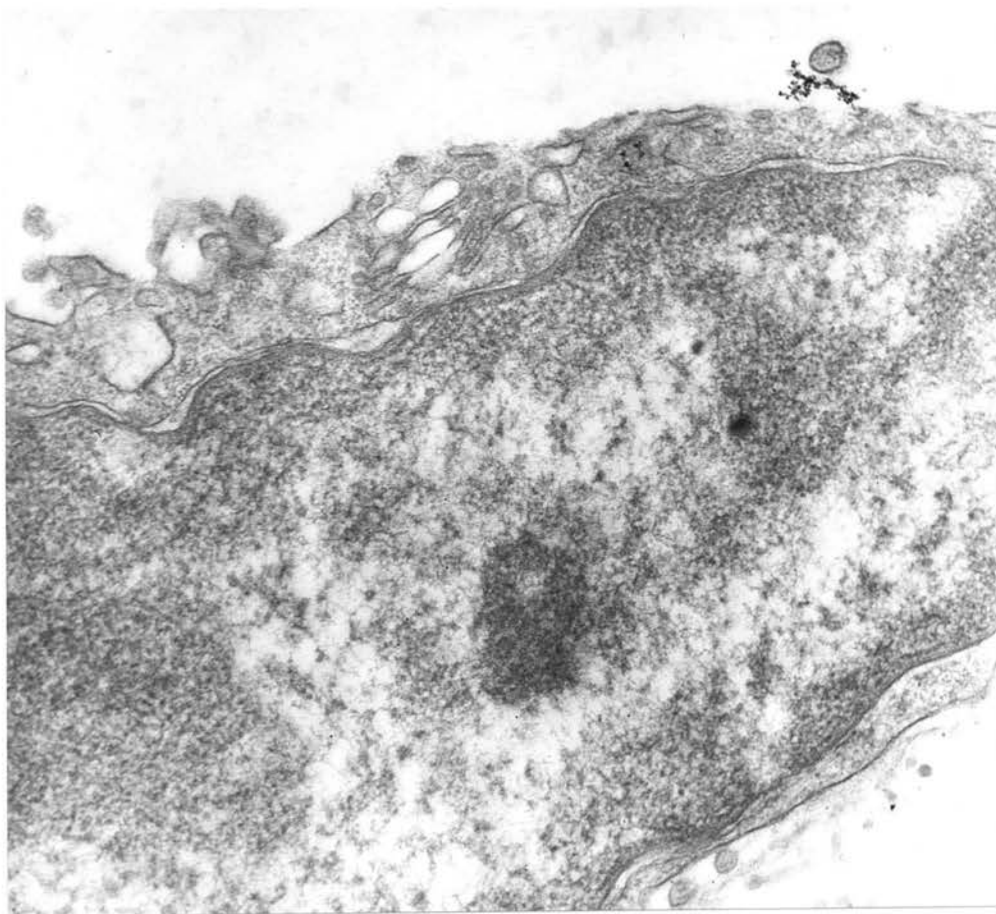

FIG. 75. Electron micrograph of surface epithelial cell showing particles of thorium(to) adherent to the plasma membrane towards the venous sinus, ½ hour after injection into the subarachnoid space.
(x 105,000)

FIG. 76. Montage of electron micrographs showing crypt at the surface of sheep granulation. Square shows particles of thorium, $\frac{1}{2}$ hour after injection into the subarachnoid space.
(x 4,000)

FIG. 76. Montage of electron micrographs showing crypt at the surface of sheep granulation. Square shows particles of thorium, $\frac{1}{2}$ hour after injection into the subarachnoid space.
(x 4,000)

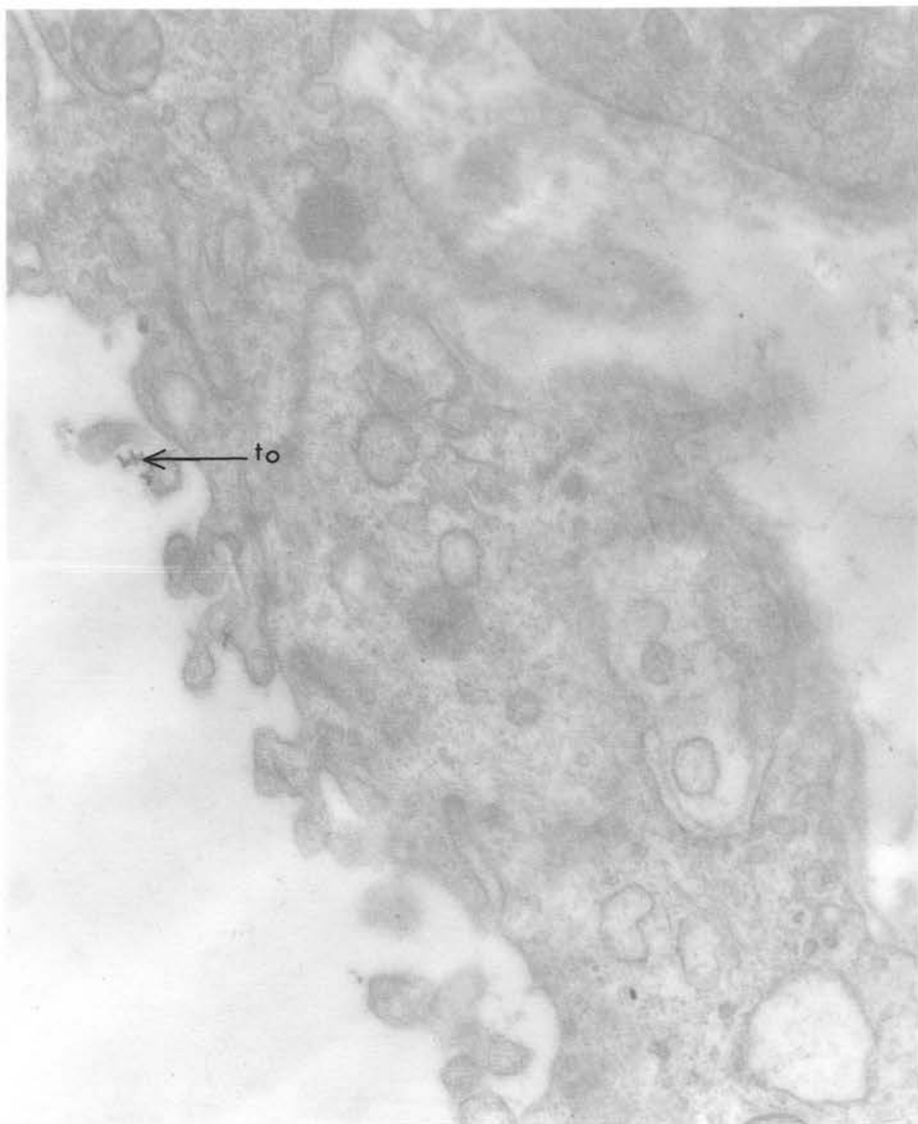

FIG. 77. High power electron micrograph of square seen in fig. 76. Particles of thorium (to) seen adhering to the plasma membrane of a lining cell of the crypt.
(x 70,000)

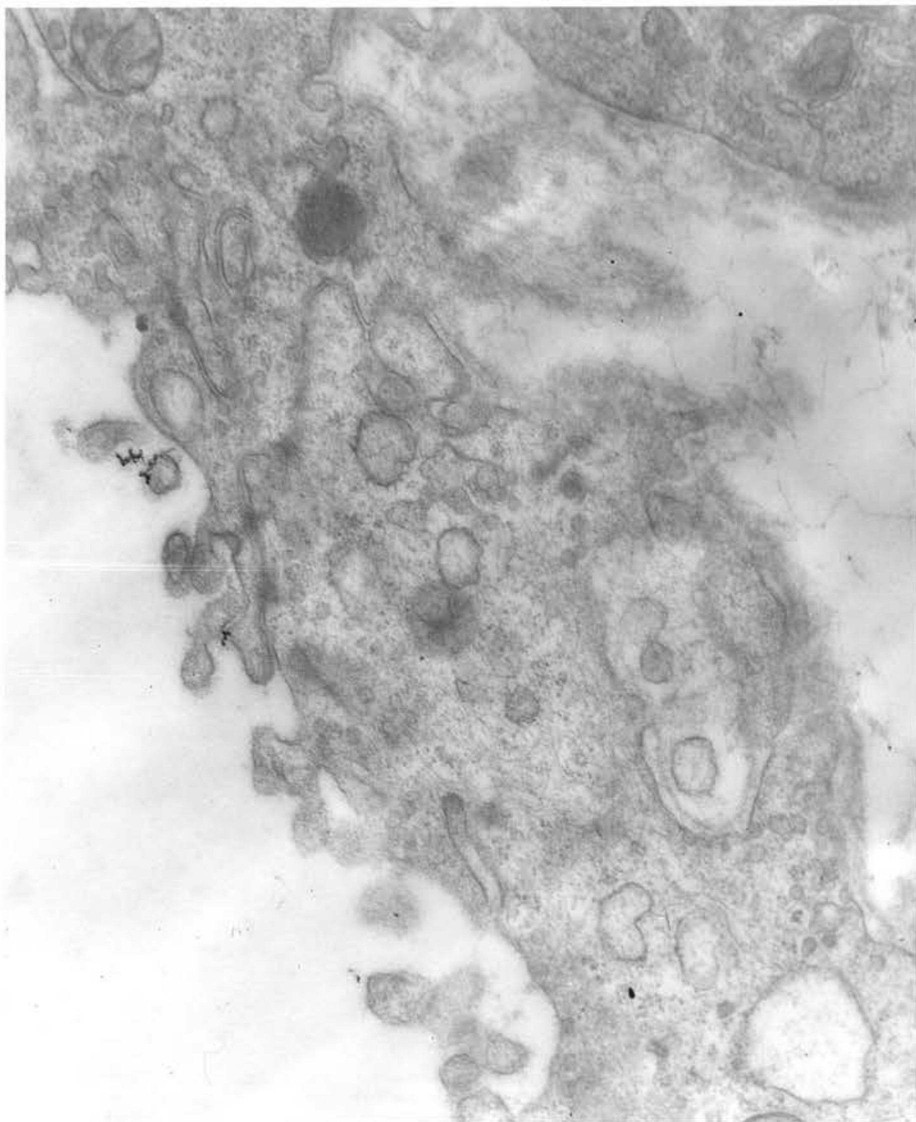

FIG. 77. High power electron micrograph of square seen in fig. 76. Particles of thorium (to) seen adhering to the plasma membrane of a lining cell of the crypt. (x 70,000)

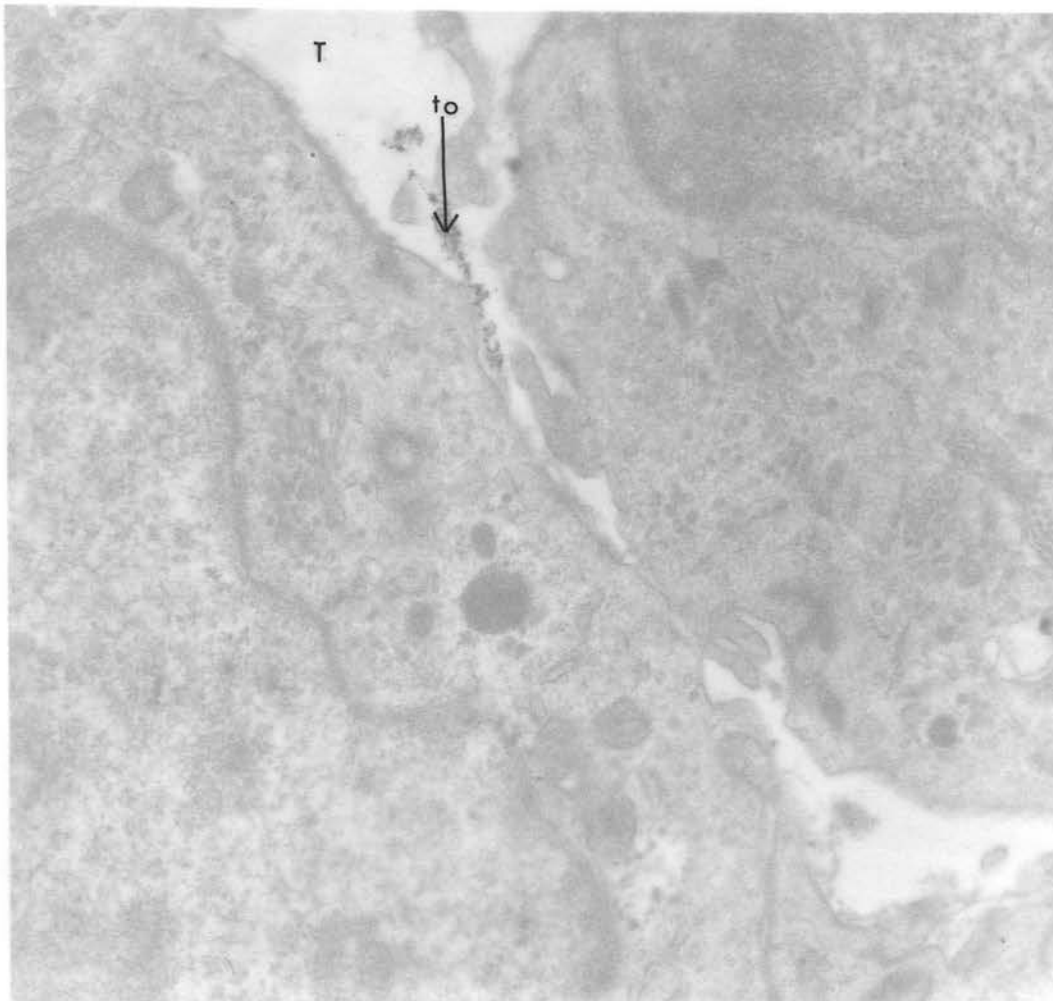

FIG. 78. Electron micrograph showing thorium particles(to) between the lining cells of a tubule in sheep arachnoid granulation, ½ hour after injection of thorium into subarachnoid space. (x 56,000)

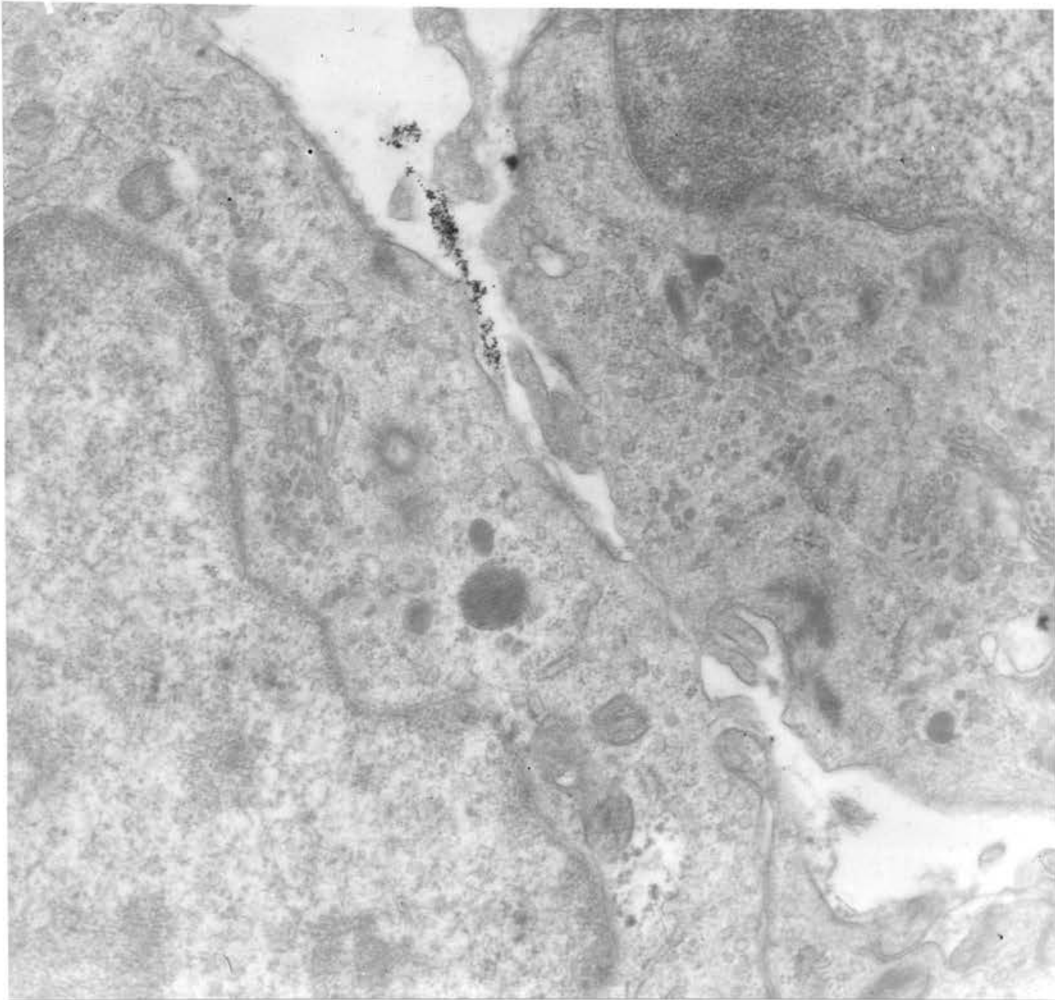

FIG. 78. Electron micrograph showing thorium particles(to) between the lining cells of a tubule in sheep arachnoid granulation, $\frac{1}{2}$ hour after injection of thorium into subarachnoid space. (x 56,000)

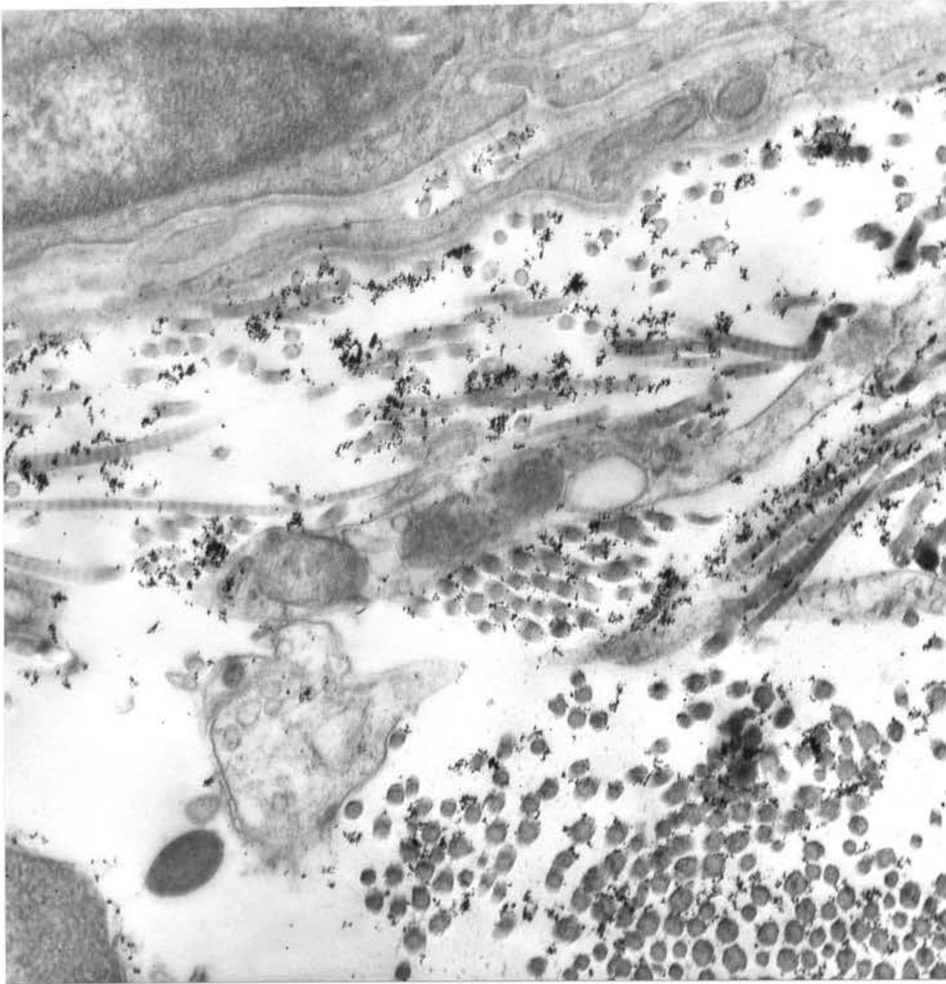

FIG. 79. Electron micrograph showing thorium particles surrounding collagen bundles and in the spaces between them, in the core of sheep granulation, ½ hour after injection. (x 56,000)

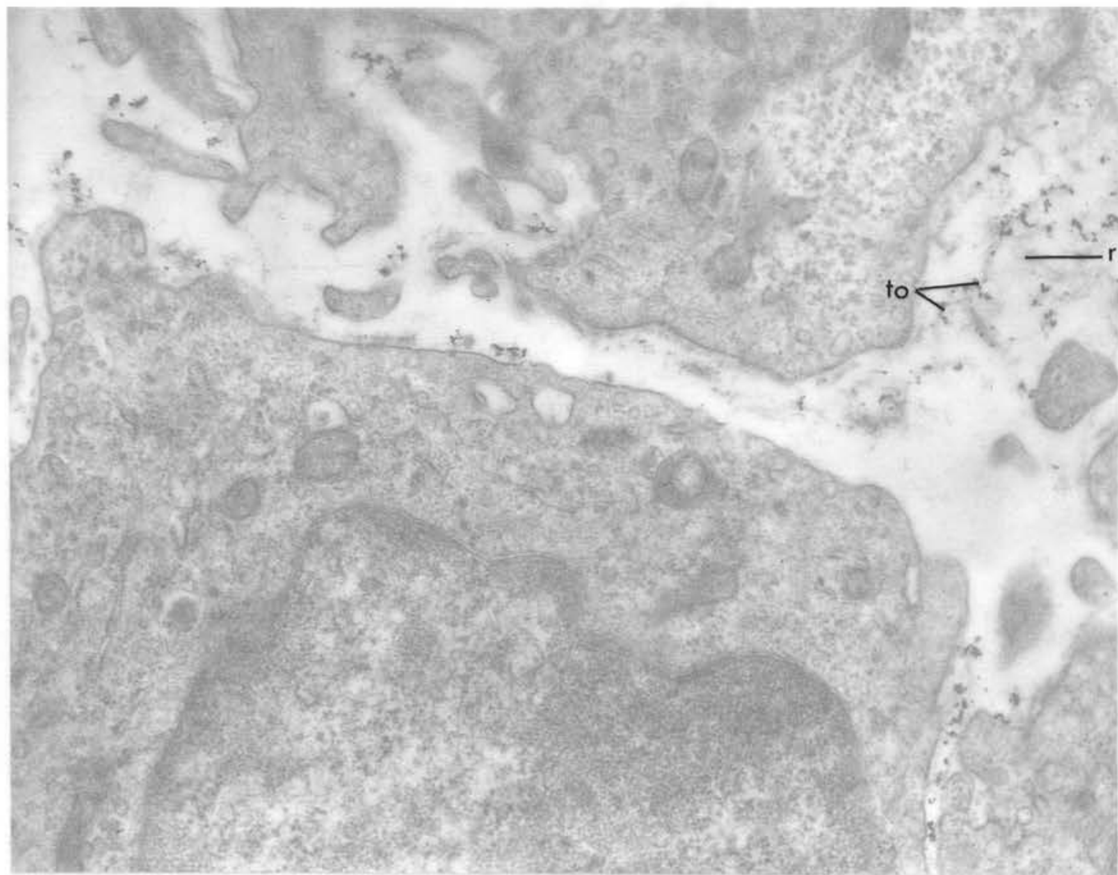

FIG. 80. Electron micrograph showing thorium particles (to) between reticulin fibres(r) in inter-cellular spaces of sheep granulation, ½ hour after injection. (x 56,000)

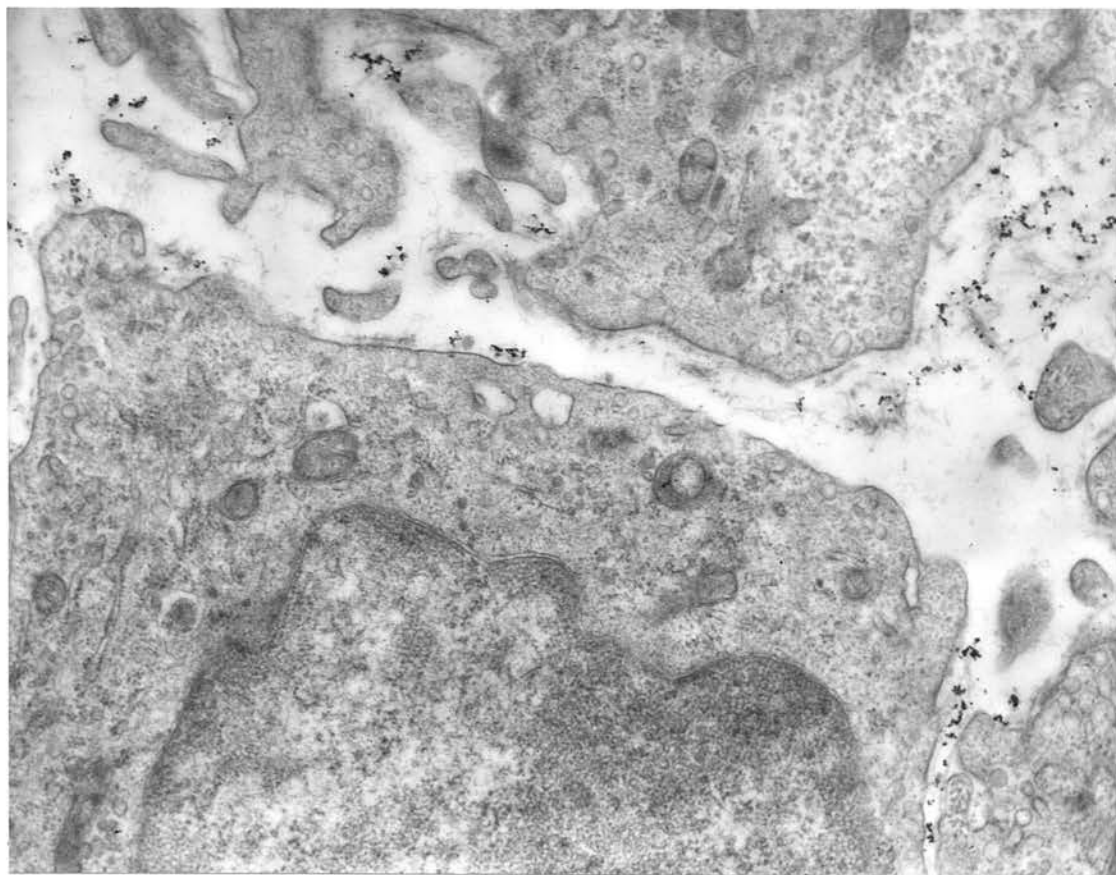

FIG. 80. Electron micrograph showing thorium particles (to) between reticulin fibres(r) in inter-cellular spaces of sheep granulation, ½ hour after injection. (x 56,000)

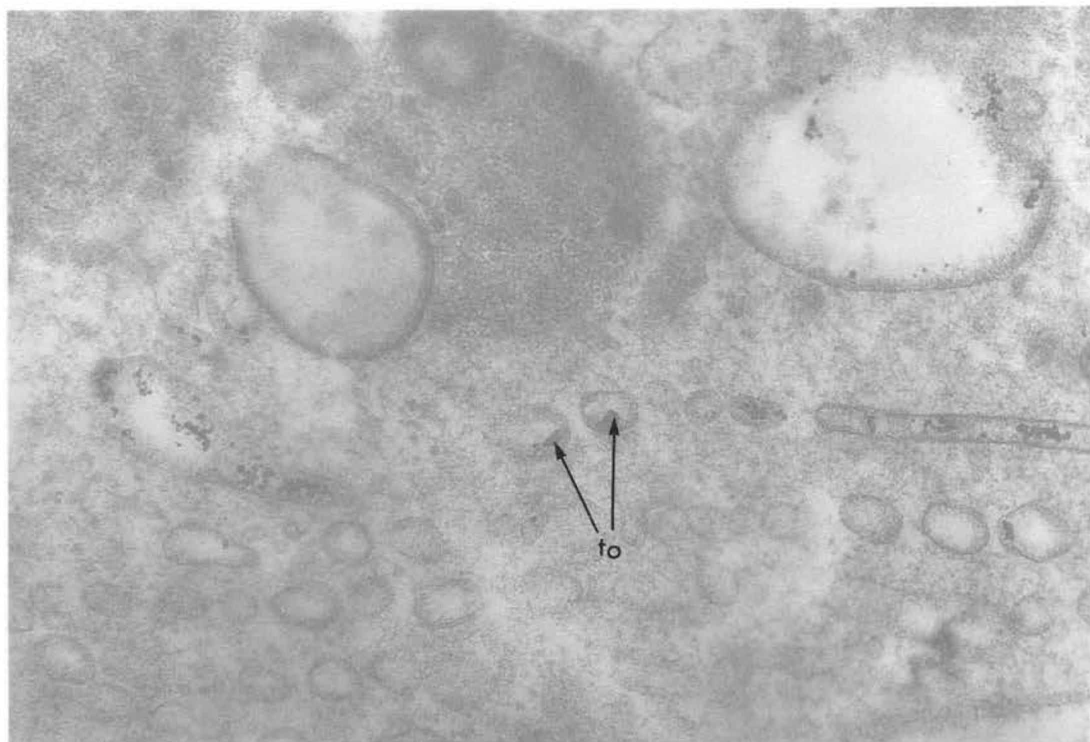

FIG. 81. Electron micrograph of type 1 macrophage in sheep granulation, ½ hour after after injection, showing thorium (to) in vesicles arranged in a linear fashion. (x 140,000)

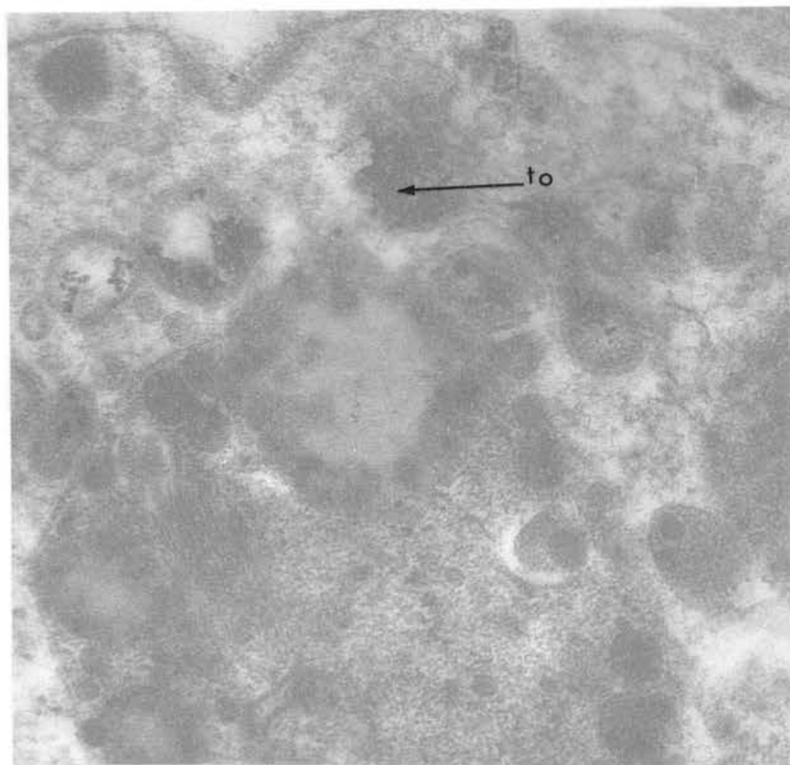

FIG. 82. Electron micrograph of type 1 macrophage in sheep granulation 4 hours after injection, showing high concentration of thorium in vesicles. (x 140,000)

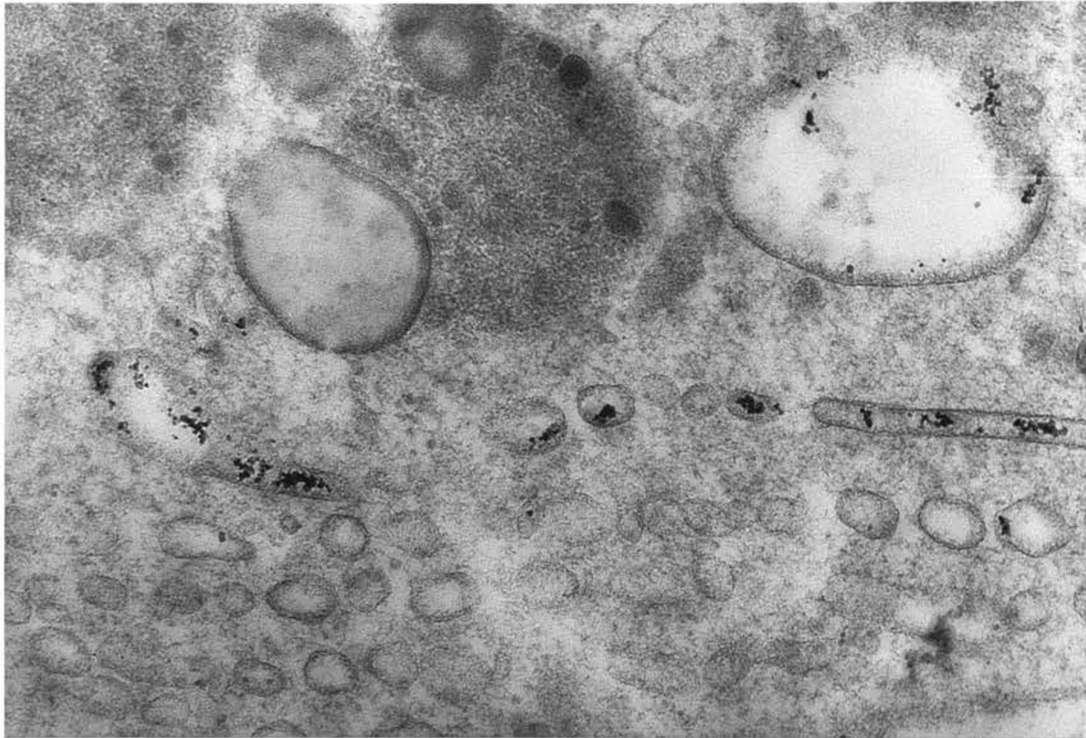

FIG. 81. Electron micrograph of type 1 macrophage in sheep granulation, $\frac{1}{2}$ hour after after injection, showing thorium (to) in vesicles arranged in a linear fashion. (x 140,000)

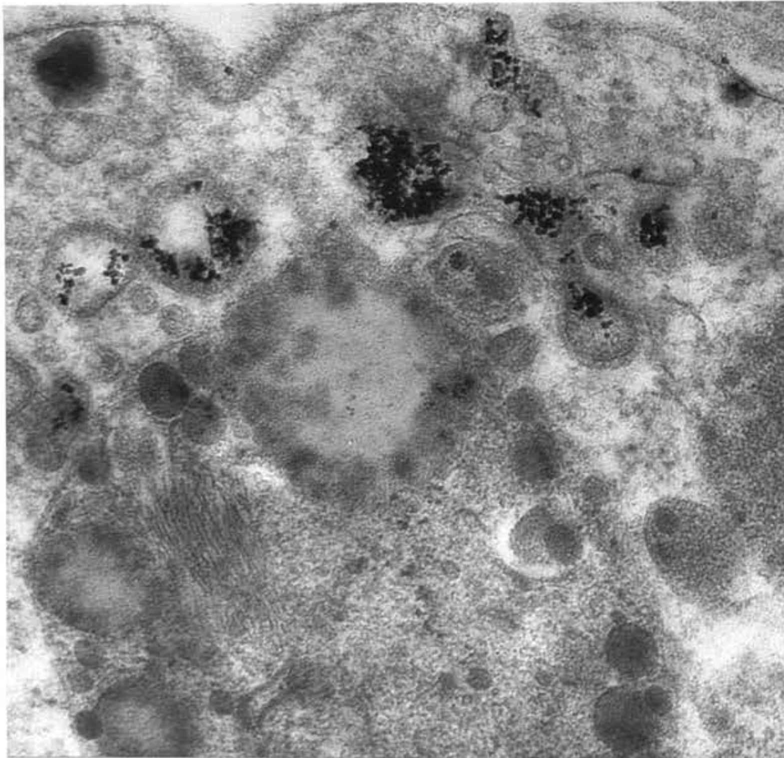

FIG. 82. Electron micrograph of type 1 macrophage in sheep granulation 4 hours after injection, showing high concentration of thorium in vesicles. (x 140,000)

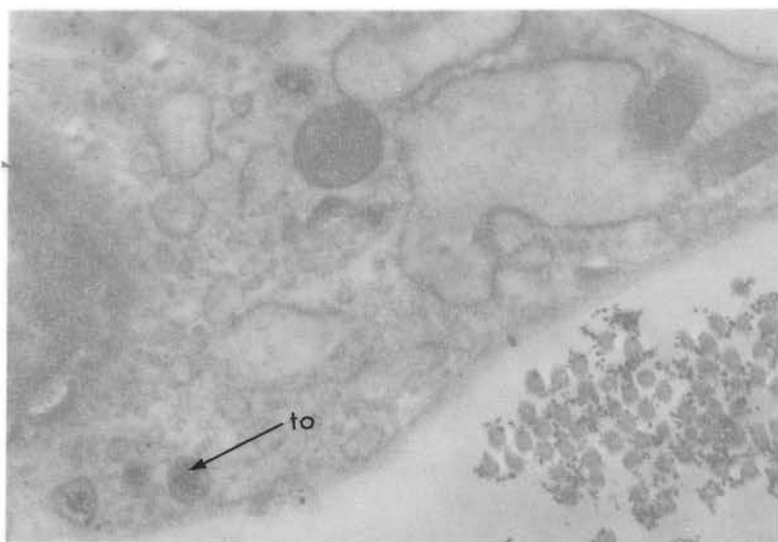

FIG. 83. Electron micrograph of arachnoid cell lining subdural space in sheep, showing thorium in vesicles, ½ hour after injection into subarachnoid space.
(x 56,000)

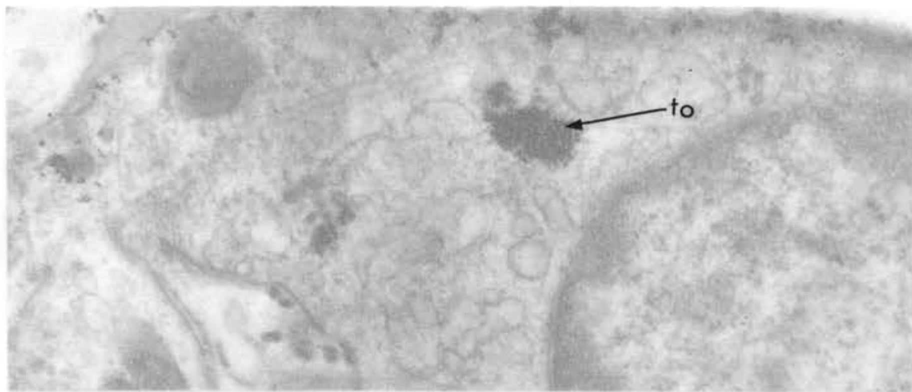

FIG. 84. Electron micrograph of arachnoid cell lining subdural space in sheep, showing greater proportion of thorium particles in vesicles, 4 hours after injection as compared to ½ hour specimen.
(56,000)

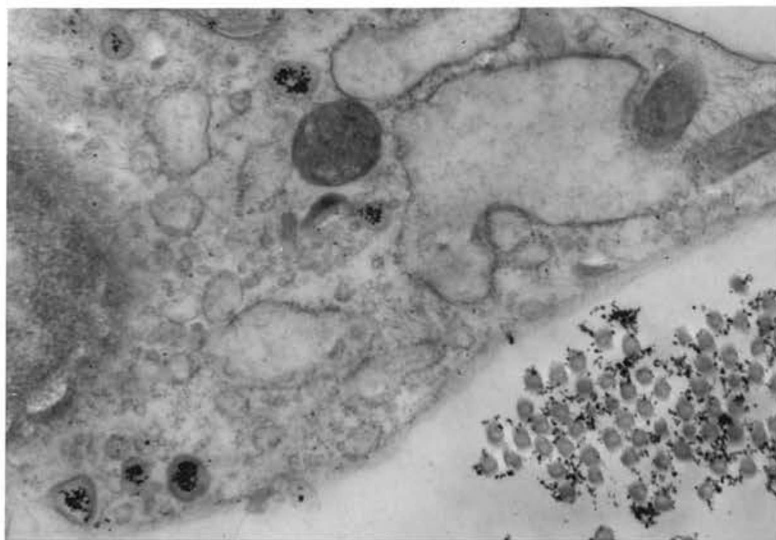

FIG. 83. Electron micrograph of arachnoid cell lining subdural space in sheep, showing thorium in vesicles, $\frac{1}{2}$ hour after injection into subarachnoid space.
(x 56,000)

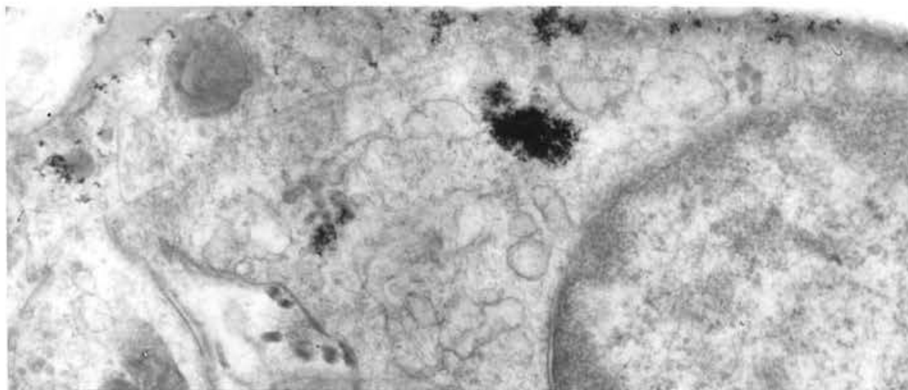

FIG. 84. Electron micrograph of arachnoid cell lining subdural space in sheep, showing greater proportion of thorium particles in vesicles, 4 hours after injection as compared to $\frac{1}{2}$ hour specimen.
(56,000)

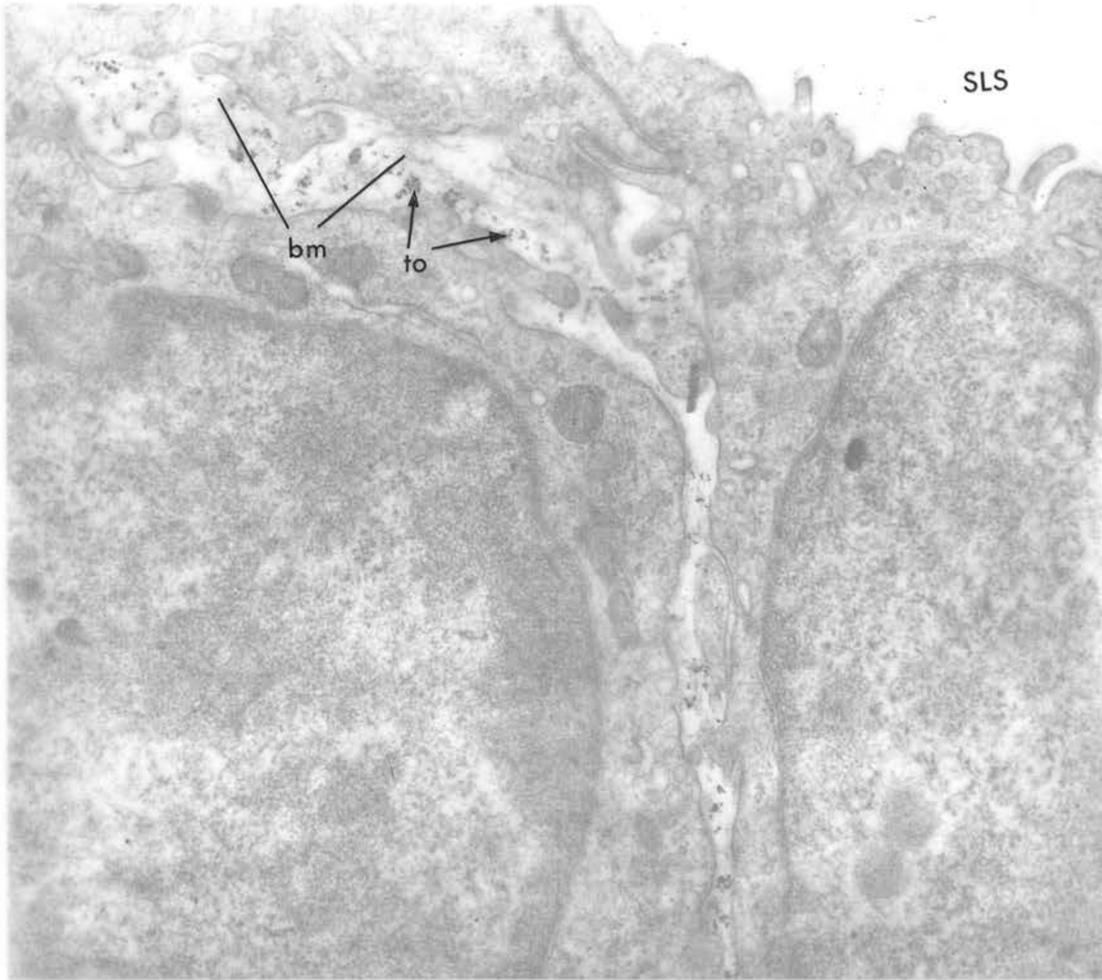

FIG. 85. Electron micrograph showing thorium particles(to) interspersed in basement membrane of surface cells of sheep granulation, 4 hours after injection. (x 56,000)

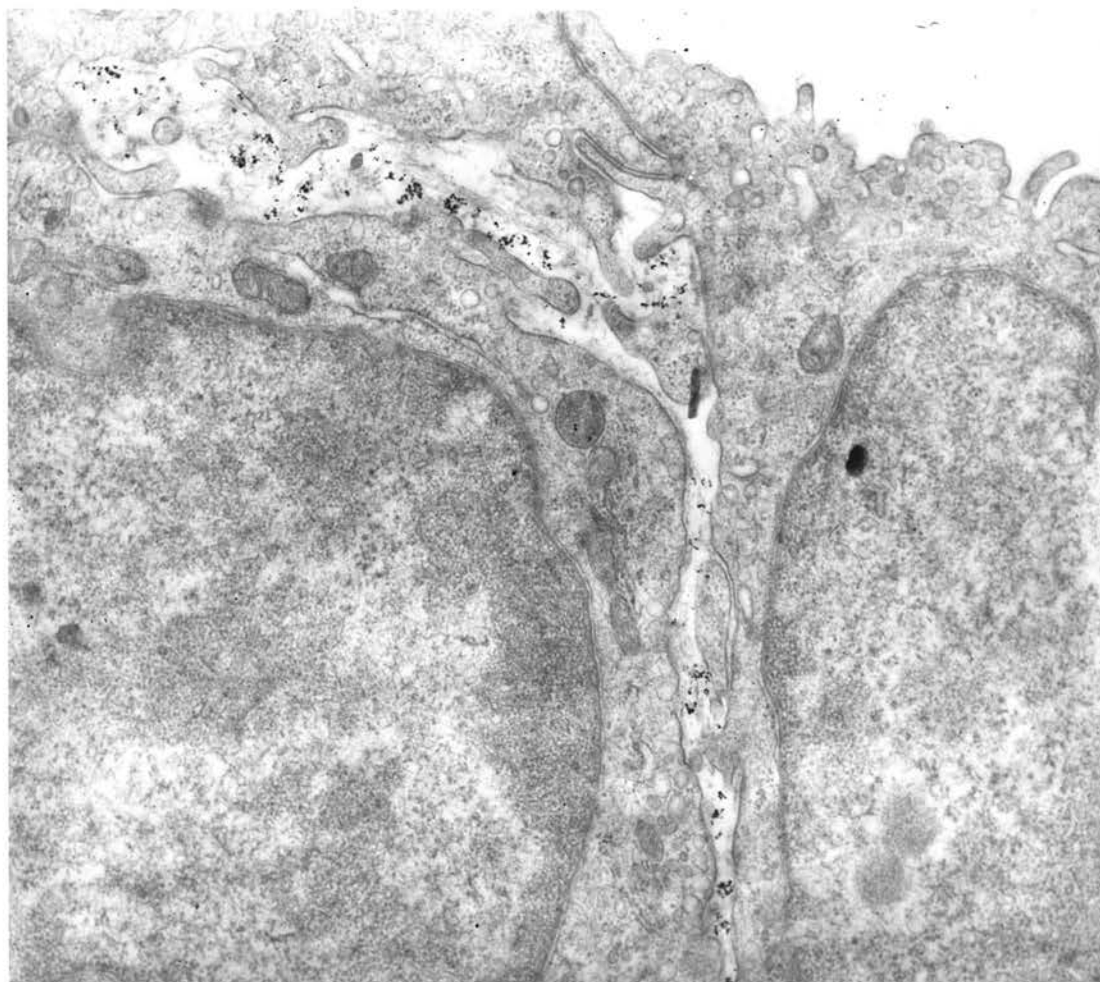

FIG. 85. Electron micrograph showing thorium particles(to) interspersed in basement membrane of surface cells of sheep granulation, 4 hours after injection. (x 56,000)

SURVEY OF ARACHNOID GRANULATIONS AND VILLI IN MAN AND OTHER MAMMALS.

MATERIAL AND METHOD.

(a) Man

4 adult human brains with meninges. Of these, one was from a man 30 years of age who died of malignant hypertension. The other 3 were from individuals over 60 years old and death was due to natural causes.

4 neonatal human brains with meninges. All died from asphyxia during birth.

(b) Other mammals

The brains and meninges of the following animals were examined. All the animals were fully grown. 10 dogs, 6 cats, 6 rabbits, 6 guinea pigs and 8 rats.

Human post-mortem material was obtained about 24 hours after death. Both adult and neonatal brains were perfused with 10% formol saline through the basilar artery and subsequently immersed in the same fixative for 7 days.

In the other animals, 10% formol saline was injected through the ascending aorta or common carotid arteries immediately after death. The vault of the cranium was then removed in all animals except in the rat, and the whole head immersed in formol saline, together with portions of the vertebral column of the rat containing the spinal cord.

In all specimens, i.e., both human and other mammals, the exposed intra-cranial venous sinuses were examined by naked eye and with a dissecting microscope. In those animals in which arachnoid granulations were macroscopically visible, they were removed in blocks of tissue which also contained dura mater, superior longitudinal sinus, the para-sagittal membranes and adjacent cortices. In animals where no arachnoid granulations/

granulations were macroscopically visible, the whole head was removed together with the meninges.

In human neonatuses and in rats, parts of the vault of the skull were also excised along with brain and meninges, since in these the dura mater was firmly adherent to the overlying vault. These specimens and the vertebral columns of the rats were decalcified either in N/10 nitric acid or in a solution containing 5% formic acid and 20% sodium citrate. Complete decalcification was determined radiographically or by calcium oxalate precipitation tests. After decalcification the tissues were neutralized with 5% sodium sulphate. The tissues were then left to wash for 12 hours, doubly embedded and sectioned in the manner described on page 33. The same staining techniques were employed as for sheep paraffin sections.

As optic nerves are surrounded by arachnoid membrane, and there is evidence for the passage of foreign material from the subarachnoid space into the orbital tissues (Field and Brierley, 1949), adult human optic nerves were also studied. This material was made available to me by Dr. B.A. Bembridge, of the Department of Ophthalmology, Royal Infirmary of Edinburgh.

RESULTS.

HUMAN ADULT.

Two types of arachnoid granulations were observed in human adults. Type 1 project into cerebral venous channels, and are usually observed at the sites of openings of cerebral veins into venous sinuses as well as in the lateral lacunae (fig. 86). Type 1 granulations, although being most common along the superior longitudinal sinus, are also present in the transverse and cavernous sinuses (fig. 88).
Le Gros Clark (1920) observed that free arachnoid granulations occur in the floor of the anterior end of the superior longitudinal sinus, and the present observations confirm this. Arachnoid granulations of the transverse sinus are those that protrude through the floor of the sinus, from the arachnoid membrane covering the superior surface of the cerebellum. Again, they were most marked at the sites of openings of veins into the sinus.

Type 2 arachnoid granulations project into the subdural space and were observed over the surface of the cerebral cortex, especially about $\frac{1}{2}$ to 1 inch from the supero-medial border (fig. 87). These were not granulations lying in the lateral lacunae.
Type 2 granulations are numerically fewer but a little larger in size than type 1 granulations. Macroscopically both types of arachnoid granulations are similar; they are rounded, pearly white bodies with diameters varying from 1 to 3 mm. Type 1 and type 2 granulations elevate and or occasionally pierce the dura mater causing indentations of the overlying cranial bones. Thus, the orientation of the arachnoid granulations in human adults is very similar to sheep except that sheep granulations are always found in relation to venous channels. In the 30 year old/

old man, who died of malignant hypertension there were over 200 arachnoid granulations related to the brain and they were larger and more numerous than the arachnoid granulations observed in the other three who had died of natural causes.

Microscopic.

Type 1 arachnoid granulations are simple prolongations of the arachnoid membrane and subarachnoid tissue into the venous channels; type 2 had a similar structure, they do not invade the venous channels but project into the subdural space, and often lie embedded in the dura (fig. 89). Structures resembling granulations have been seen microscopically: the arachnoid villi. Except for the difference in size, arachnoid villi have a similar morphology to arachnoid granulations, so that, the descriptions of arachnoid granulations apply equally well to the arachnoid villi.

Human arachnoid granulations possess a body and neck, through which they communicate with the subarachnoid space. Unlike in the sheep where the body of a granulation is always associated with one neck, in the human, the bodies of two granulations may have a common neck (fig. 90).

The mean diameter of the human arachnoid granulations that have been examined was 1.5 mm; the mean height of the body measured 1.2 mm and the mean length of the neck was 0.5 mm. Type 2 granulations are somewhat larger and have a mean diameter of 2 mm.

Tightly packed type 1 arachnoid granulations occupy the entire laternal lacunae in the human adult, so that it appears as though the granulations are separated from the venous sinus by dura (fig. 90). Actually, the granulations are in contact with the blood of the lateral lacunae. As all type 1 granulations/

granulations lie in venous channels, they are covered by a layer of cells continuous with those lining the lumen of the venous channels. Microscopically, these cells are fusiform in shape with oval nuclei, and resemble the endothelial cells of the venous channel; they are also similar to the arachnoid epithelial cells lining the subdural space. Type 2 granulations on the other hand, have a covering of arachnoid epithelial cells as they are in no way associated with a venous channel. It is likely therefore that the covering cells of type 1 arachnoid granulations are arachnoid epithelial cells that have now taken on the rôle of endothelial cells.

Both types of arachnoid granulations have cell aggregations of the surface epithelium at their summits. These result in 'epithelial cell caps', (figs. 90, 92 and 93) that are formed by a collection of round or oval cells similar to the cell aggregations seen in the arachnoid membrane (fig. 94). Collections of cells similar to the 'epithelial cell caps' are observed in the core of the granulation, but they are continuous with the cell collections at the summit. Similar observations have been made by Turner (1957).

Both types of granulations have crypts arising from the surface, that are lined by flattened cells and are similar to the crypts observed in sheep (figs. 91 and 92). When serial horizontal (tangential) sections of these crypts are studied, they appear to be continuous with tubules in the core of the granulation (figs. 95 and 96). These findings correspond with those of Trolard (1870) and Schaltenbrand (1955), who described these tubules as cavernous spaces, lined by flattened, endothelial-like cells.

The core of the granulation is made up of closely/

closely packed collagen bundles with narrow spaces between them (fig. 97). Some of these spaces are lined by flattened mesothelial cells, but the honeycombed appearance found in sheep has not been observed in human arachnoid granulations. In the human granulations, as in the sheep, the spaces between the collagen bundles communicate with similar spaces in the subarachnoid space, and scattered in the collagen meshwork of the granulation are red blood cells, lymphocytes, polymorphs and plasma cells.

Arachnoid granulations are supplied by blood vessels, (figs. 98 and 99) which arise from the dural collar at the neck of each granulation, as well as from the dural fibres which Turner (1958) described as "transfixing" the granulations. By perfusion of the external carotid artery with gelatine, coloured by the addition of India ink, Kolesnikov (1940) demonstrated that these blood vessels are derived from the middle meningeal artery.

In view of the difficulty of obtaining adequate fixation of the human material so long after death, silver staining techniques give unreliable results for nervous tissue. It is particularly difficult to recognize fine nerve fibres in a meshwork of collagen bundles, as both take up the silver stain. Nevertheless, fine fibres resembling the nerve fibres of the cerebral cortex and giving a bluish-black stain were observed in human adult granulations and these may be nerve fibres, since they stained somewhat differently to the collagen fibres, which appeared to stain a brownish-black (fig. 100).

Calcareous degenerations, also called corpora amylacea or psammoma bodies were commonly observed in the arachnoid granulations of the human adult (fig. 99) and similar changes occur in the cell aggregations/

aggregations of the arachnoid membrane, a finding similar to that of Cushing and Weed (1915).

Optic nerve.

There are aggregations of cells in the arachnoid membrane surrounding human optic nerves (figs. 101 and 102). They measure about 200 to 300 µ in width and are about 150 µ high. These aggregations resemble villi and project into the subdural space surrounding the optic nerve. Morphologically they resemble arachnoid villi but are unrelated to venous channels, a respect in which they correspond to the type 2 arachnoid granulations in the human adult brain.

HUMAN NEONATUS.

Macroscopic.

No arachnoid granulations were observed either by naked eye examination or with a dissecting microscope, in relation to any venous channels.

Microscopic.

Serial sections of the superior longitudinal sinus have been examined microscopically, and show arachnoid villi possessing the same structure as the arachnoid granulations and villi of adults, in the lateral lacunae of the superior longitudinal sinus (fig. 103). They vary in width from 100 to 300 µ and many villi arise from a common neck, through which there is communication with the subarachnoid space. The surface epithelial cells are similar to those present in the adult and in many instances, crypts have been found arising from the surface of the villi. 'Epithelial cell caps' have been observed at the summits of the villi which have a similar appearance to the clusters of aggregated cells that occur within the arachnoid membrane (fig. 104). Such clusters of cells were also noticed in the roof of the lateral lacunae/

lacunae and according to Schmidt (1902) these are the primary foci of meningiomas. The cores of the villi are similar to those of in the adult, except that the spaces between the collagen bundles are narrow, due to the extreme cellularity, a finding which confirms the observations of Luschka (1852) and Meyer (1860).
Even in the neonatal specimens, fine tubules lined by flattened cells are present in the cores of the villi. Blood vessels are present but silver stained sections gave no conclusive evidence of the presence of nerve fibres in human neonatal villi.

Corpora amylacea have been observed in 2 of the infants examined, although according to Weed (1920b) calcareous degeneration is a process of ageing. Most of the infants had died at or during birth, and further investigations are necessary before any definite conclusions can be reached about the relations between corpora amylacea and the "process of ageing".

OTHER MAMMALS.

Detailed microscopic examination of these animals were not carried out in the same way as in man and sheep, as the survey was for the sole purpose of determining the presence or absence of arachnoid granulations and villi in those animals. Consequently, individual descriptions of the microscopic appearance will not be given but the structure will be compared with that of man and sheep.

DOG.

Naked eye examination of the superior longitudinal sinus in dogs shows, pearly white projections in the cerebral veins near their openings into the sinus (fig. 105). The projections are small and just visible to the eye, but on examination with a dissecting microscope, it is apparent that they have a similar/

similar appearance to the arachnoid granulations in sheep. Arachnoid granulations in the dog are common in the veins opening into the middle and caudal parts of the superior longitudinal sinus, and though they are not observed in the tranverse sinus they are present in the cavernous sinus.

An attempt was again made to relate the weights of brains and choroid plexuses to the number of arachnoid granulations present in the animal, as in sheep, but no correlation could be found in observations on 10 animals.
The following table gives the results.

Brain weight	Weight of choroid plexus	No. of granulations.
111.0 G	0.13G	6
103.0 "	0.14"	2
99.5 "	0.12"	2
98.4 "	0.13"	6
97.7 "	0.11"	2
93.5 "	0.13"	2
92.6 "	0.13"	3
90.7 "	0.09"	5
90.0 "	0.10"	2
88.5 "	0.15"	2

Microscopically, the arachnoid granulations in the dog do not project directly into the superior longitudinal sinus as in the sheep, but are confined to the cerebral veins (fig. 106). The average measurements are: width 400 µ, length 500 µ and height 600 µ. The surface epithelial cells of the granulation in the dog are similar to those of sheep and man. No 'epithelial cell caps' or crypts have been observed on the surface of the granulations whose core closely resembles that of the sheep. There are tubule-like spaces present in the core (fig. 107), but whether they have/

have the same structure as the tubules observed in man and sheep is uncertain. At the junction between the granulations and subarachnoid tissue, the subarachnoid space forms truncated dilatations (fig. 106), which Cooper (1961), also working on dogs, called 'cisterns'. The rest of the subarachnoid space over the cerebral cortex shows no dilatations so that it is unlikely that these 'cisterns' are artifacts produced in tissue preparation. Large veins, which drain into the superior longitudinal sinus, occur at the base of the 'cisterns'.

CAT.

No arachnoid granulations were seen macroscopically in any of the venous sinuses in the cats examined.

When serial sections of the cat brain are examined microscopically, arachnoid villi are observed in the central part of the superior longitudinal sinus where it is joined by veins (fig. 108). The microscopic structure of the arachnoid villi in the cat resembles that of the dog arachnoid granulation, except that the surface epithelial cells of the cat villus are aggregated to give a suggestion of 'epithelial cell caps' (fig. 109). No crypts have been observed on the surface of the villi and the core resembles that in the granulations of sheep, except that there are no tubules in the villi of the cat that could be recognized. 'Cisterns' similar to those occurring in the arachnoid granulations of dogs are present in the villi of cats. The average width of cat villus is 150 µ and its height is 300 µ.

RABBIT AND GUINEA PIG.

Macroscopically there were no arachnoid granulations visible either in rabbit or guinea pig venous sinuses.

In serial sections of the superior longitudinal/

longitudinal sinus in both rabbits and guinea pigs, arachnoid villi have been observed microscopically at the caudal end of the sinus, especially in the region where the sinus is related to the pineal body (figs. 110 and 112). The average width of the villus in the rabbit is 200 μ and in the guinea pig it is 100 μ; the height in the rabbit villus is 140 μ and is 100 μ in the guinea pig. The villi of the rabbit project directly into the sinus (fig. 111) while in the guinea pig they are adjacent to the dura mater that forms the lateral walls of the superior longitudinal sinus (fig. 113). In both these rodents the construction of the villi is similar and resembles that of the granulations of the sheep and of the dog. In both rodents, the pineal body is surrounded by a condensation of arachnoid tissue and the villi arise from this layer, immediately adjacent to the superior longitudinal sinus. The surface cells resemble those present in the granulations of man, sheep and dog, as do the cores of the villi, although in the core of the rabbit villus, spaces lined by flattened cells and containing red blood cells can be observed to extend into the subarachnoid space (fig. 111).

RAT.

No arachnoid granulations or villi are present in the venous sinuses of the rat.

In the spinal cord, however, there are proliferations of arachnoid cells surrounding the emerging spinal nerves, but they bear no relationship to venous channels (fig. 114). They resemble the arachnoid cell proliferations seen around the spinal nerves of sheep and are probably the villi described by Woollam and Millen (1958) in the spinal region of the rat.

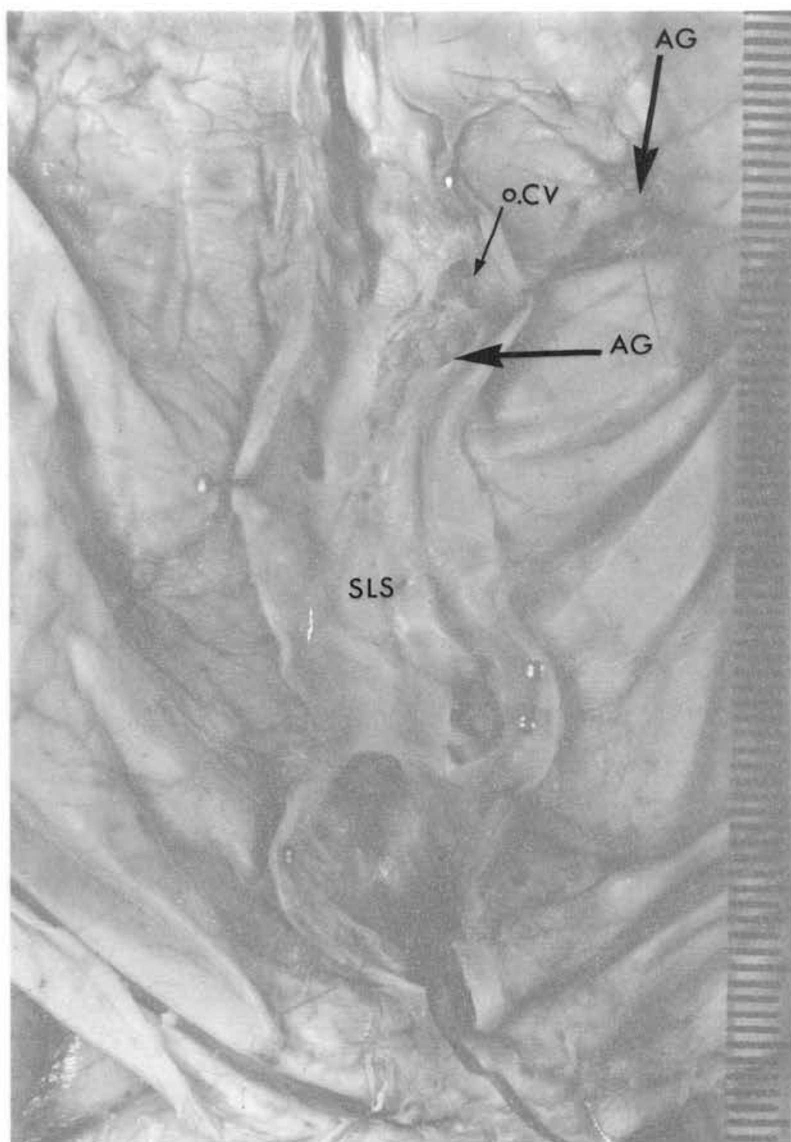

FIG. 86. Photograph of superior longitudinal sinus in the adult human showing arachnoid granulations.
(x 1)

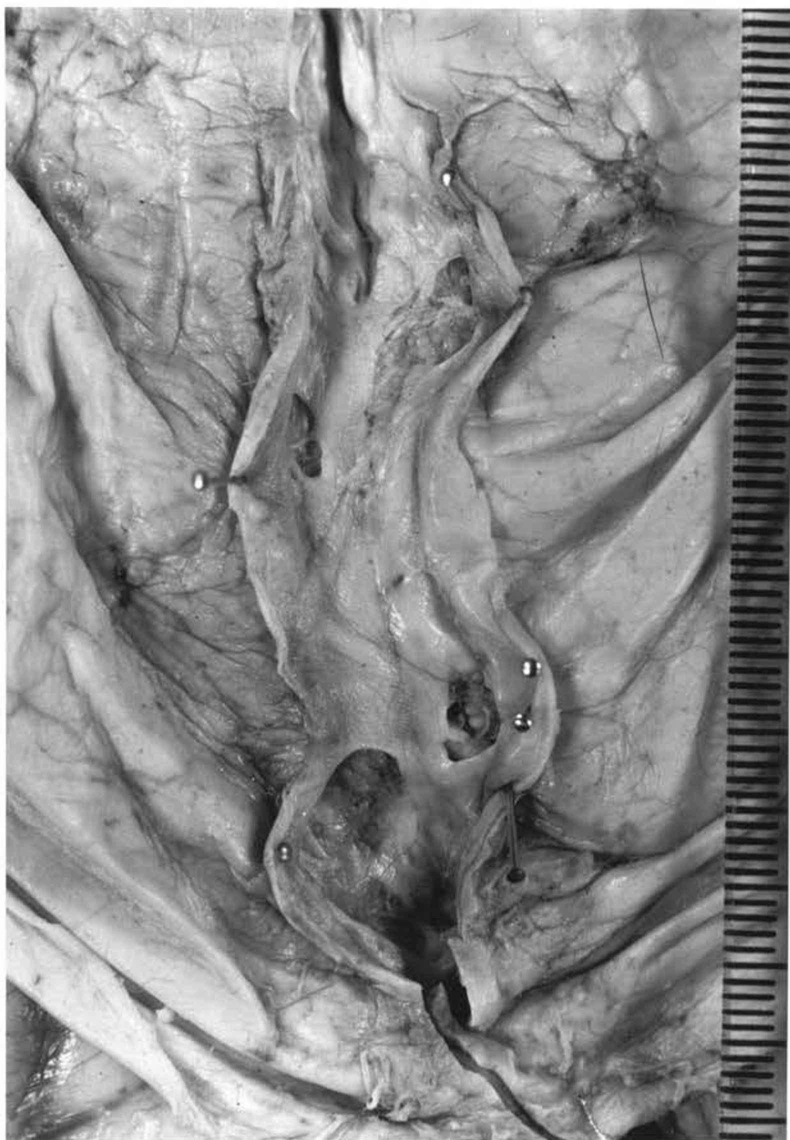

FIG. 86. Photograph of superior longitudinal sinus in the adult human showing arachnoid granulations.
(x 1)

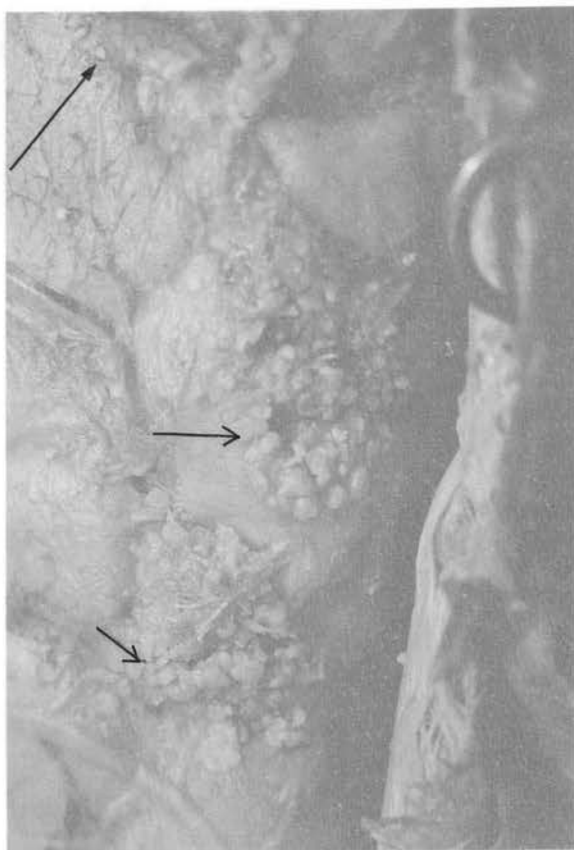

FIG. 87. Photograph showing arachnoid granulations(arrows) in human adult, on supero-medial border of cerebral hemispheres unrelated to venous channels. (x 1.5)

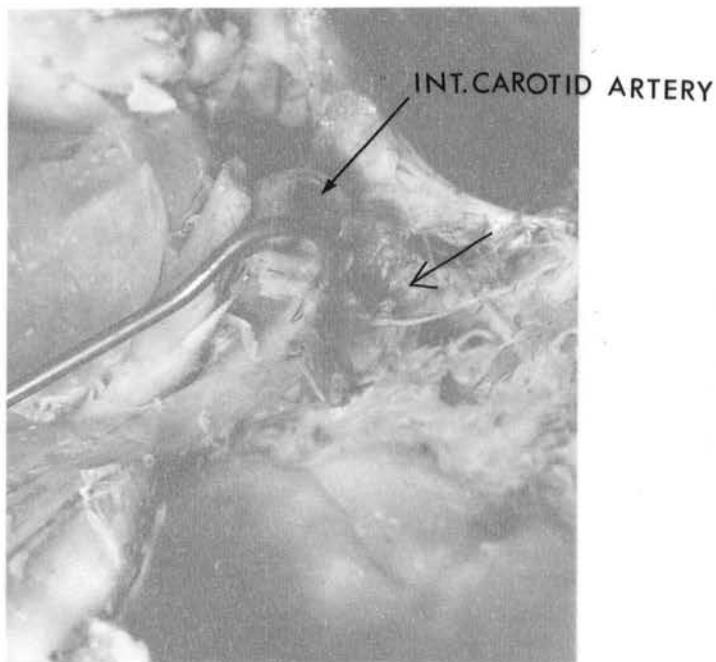

FIG. 88. Arachnoid granulations(arrow) in cavernous sinus in adult human. (x 1)

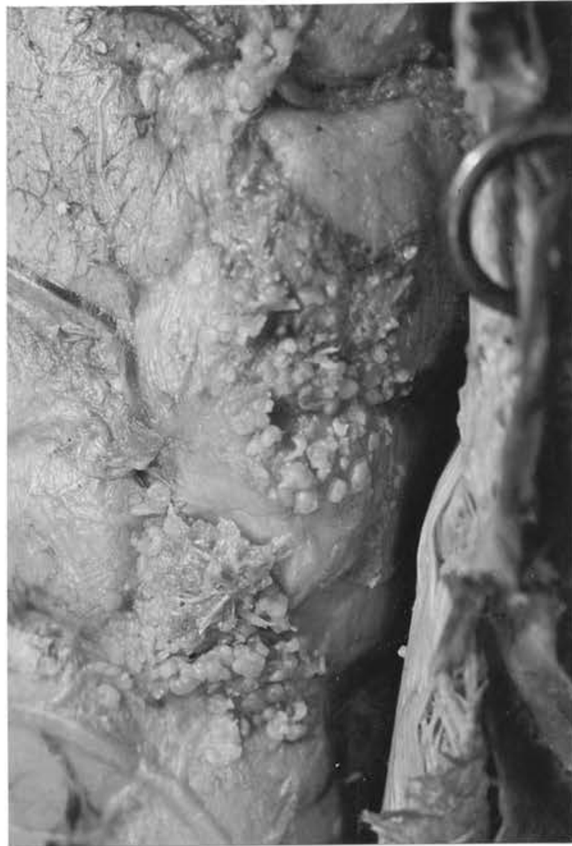

FIG. 87. Photograph showing arachnoid granulations(arrows) in human adult, on supero-medial border of cerebral hemispheres unrelated to venous channels.
(x 1.5)

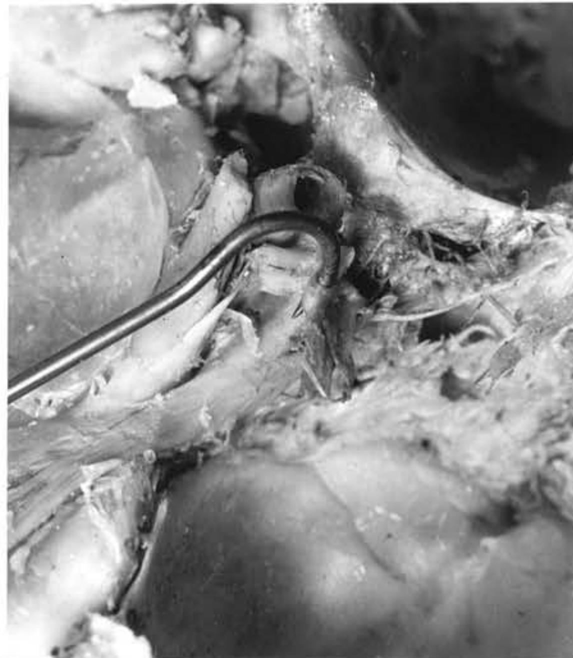

FIG. 88. Arachnoid granulations(arrow) in cavernous sinus in adult human.
(x 1)

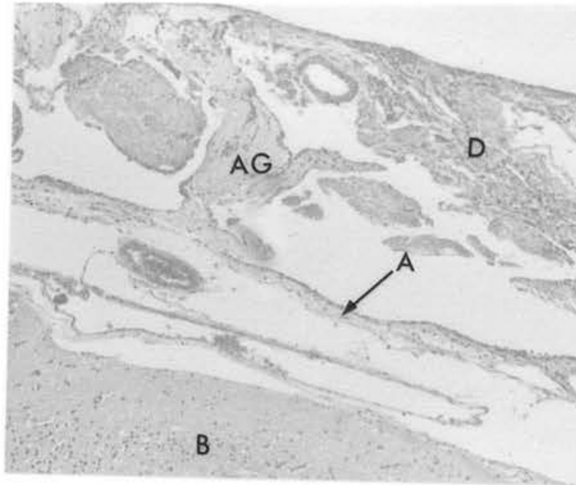

FIG. 89. Photomicrograph of arachnoid granulation in the adult human, unrelated to venous channel.
(paraffin embedded, H & E, 54)

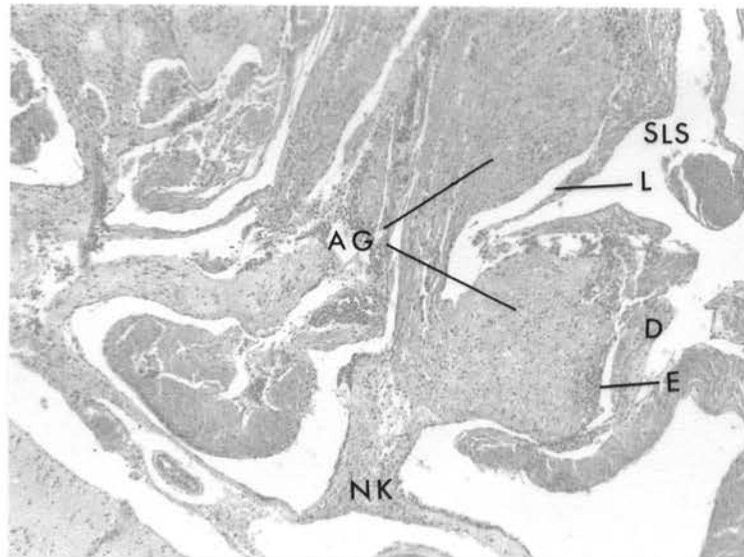

FIG. 90. Photomicrograph of two arachnoid granulations with a common neck(NK), in lateral lacunae(L) : adult human.
(paraffin embedded, H & E, x 54)

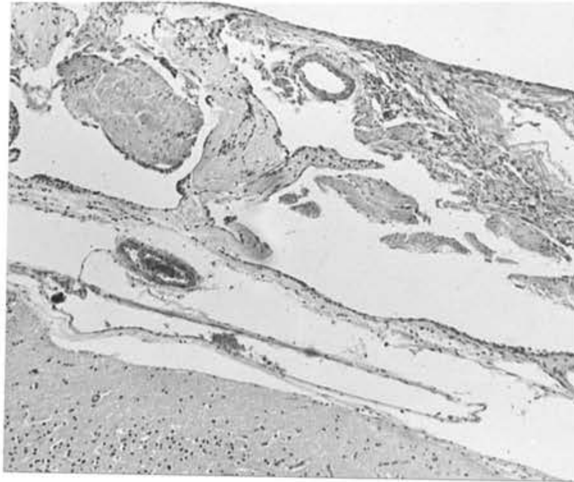

FIG. 89. Photomicrograph of arachnoid granulation in the adult human, unrelated to venous channel.
(paraffin embedded, H & E, 54)

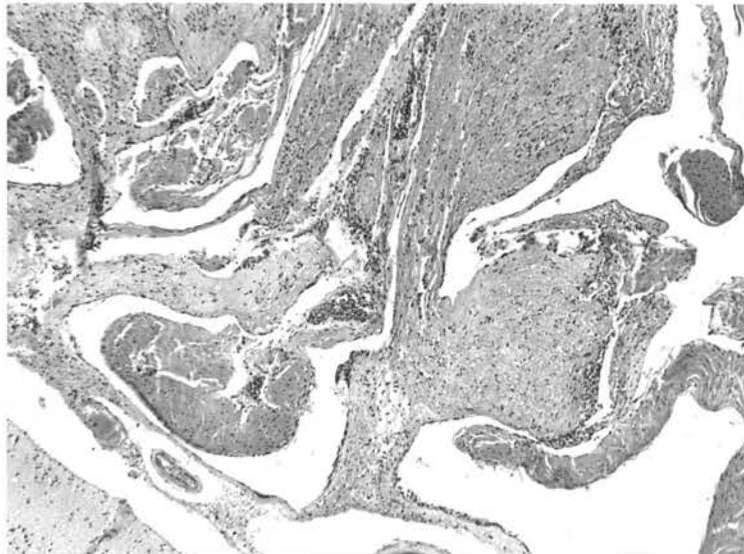

FIG. 90. Photomicrograph of two arachnoid granulations with a common neck(NK), in lateral lacunae(L) : adult human.
(paraffin embedded, H & E, x 54)

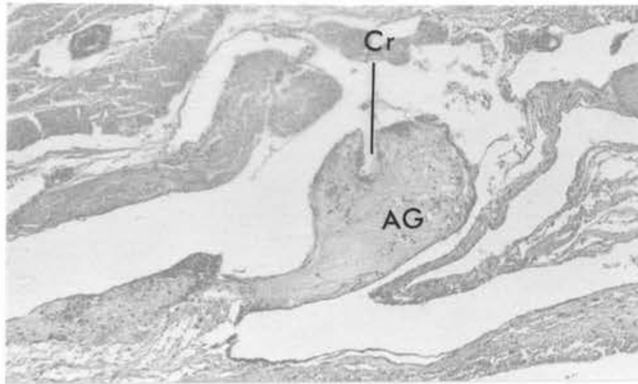

FIG. 91. Photomicrograph of a adult human arachnoid granulation showing a crypt.
(paraffin embedded, H & E, x 54)

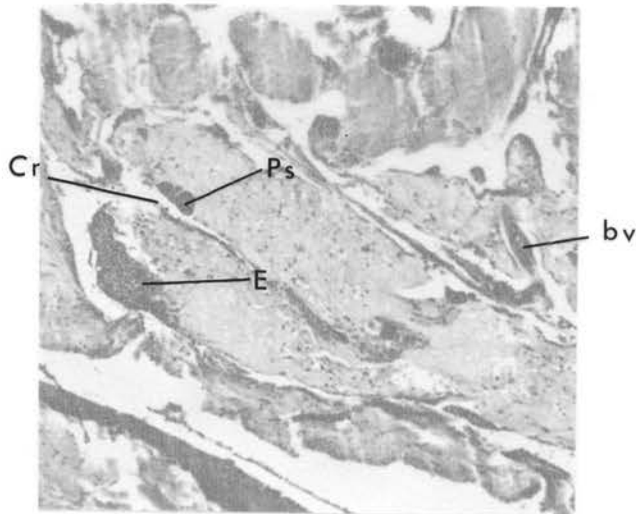

FIG. 92. Photomicrograph of an adult human granulation showing crypt, 'epithelial cell cap' (E) and psammoma bodies(Ps).
(paraffin embedded, H & E, x 54)

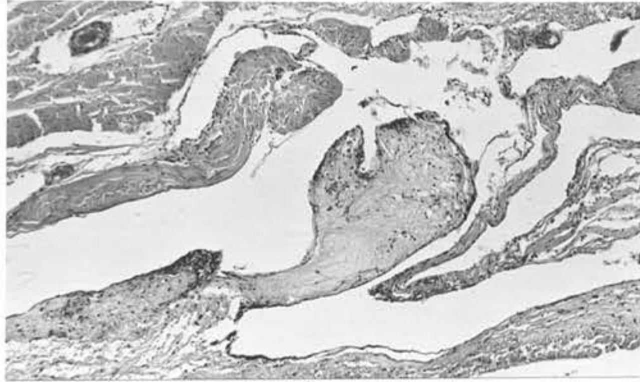

FIG. 91. Photomicrograph of a adult human arachnoid granulation showing a crypt.
(paraffin embedded, H & E, x 54)

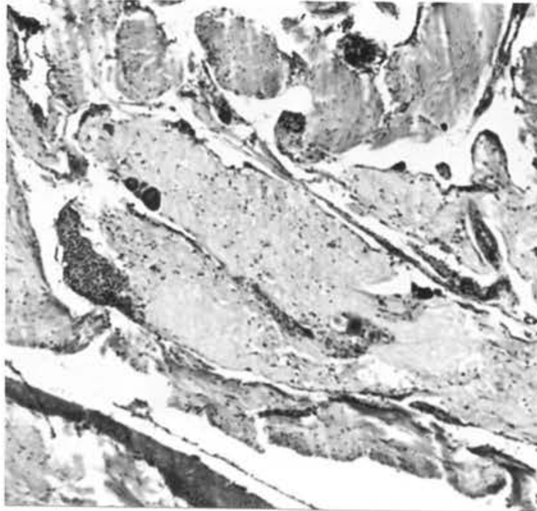

FIG. 92. Photomicrograph of an adult human granulation showing crypt, 'epithelial cell cap' (E) and psammoma bodies(Ps).
(paraffin embedded, H & E, x 54)

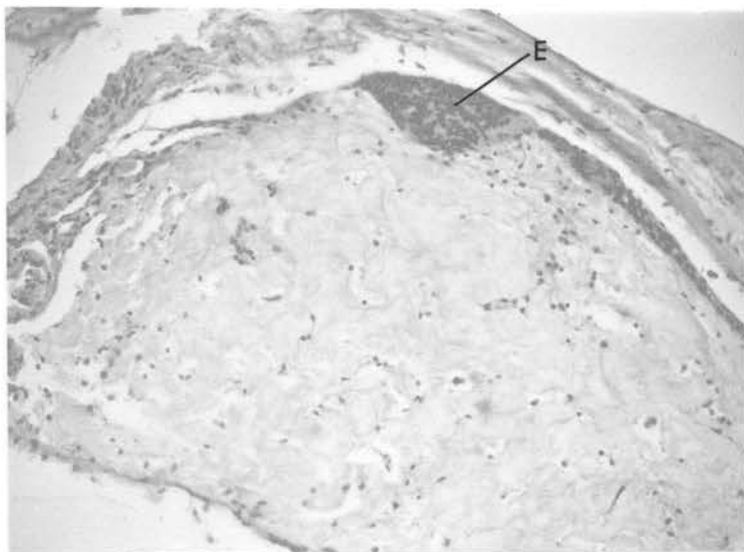

FIG. 93. Photomicrograph of adult human granulation showing 'epithelial cell cap'.
(paraffin embedded, H & E, x 140)

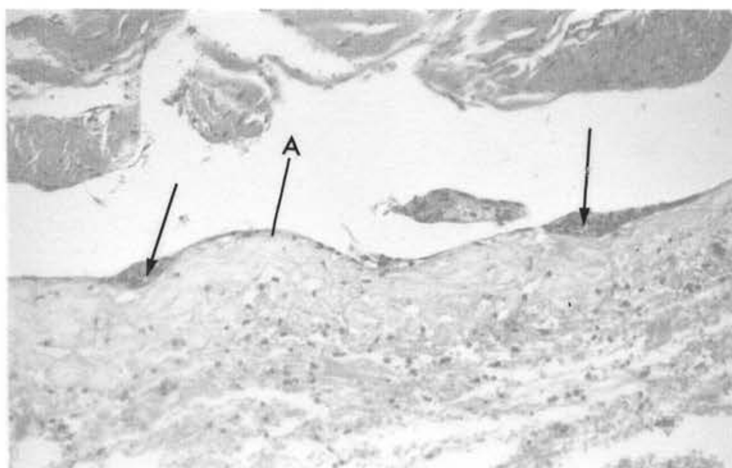

FIG. 94. Photomicrograph of arachnoid membrane in human adult. Arrow shows cell aggregations.
(paraffin embedded, H & E, x 140)

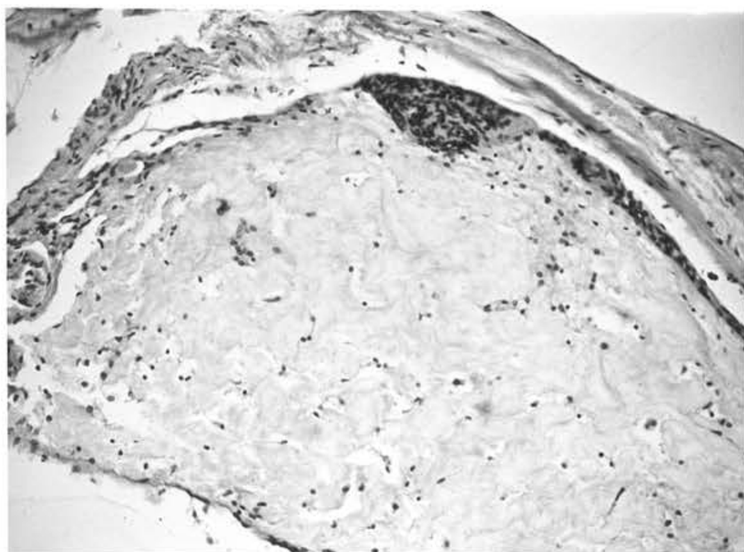

FIG. 93. Photomicrograph of adult human granulation showing 'epithelial cell cap'.
(paraffin embedded, H & E, x 140)

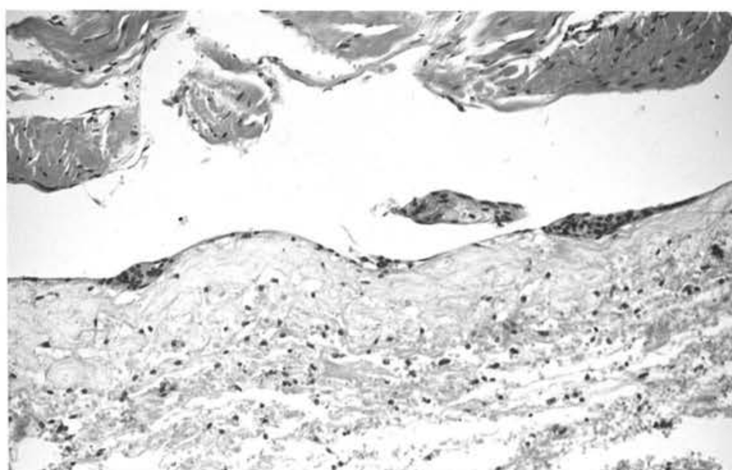

FIG. 94. Photomicrograph of arachnoid membrane in human adult. Arrow shows cell aggregations.
(paraffin embedded, H & E, x 140)

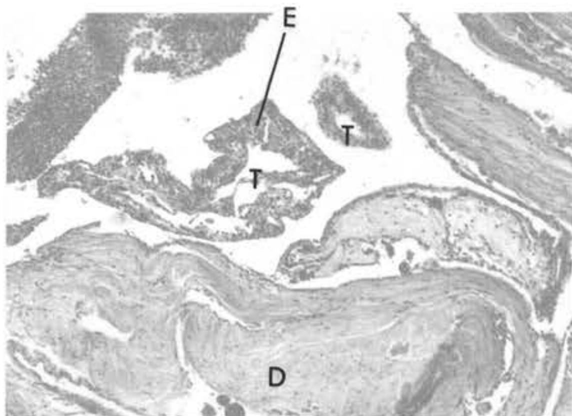

FIG. 95. Photomicrograph of a horizontal (tangential) section of adult human granulation at the summit, showing opening of crypt forming tubule, and 'epithelial cell cap'(E). (paraffin embedded, H & E, H & E, x 54)

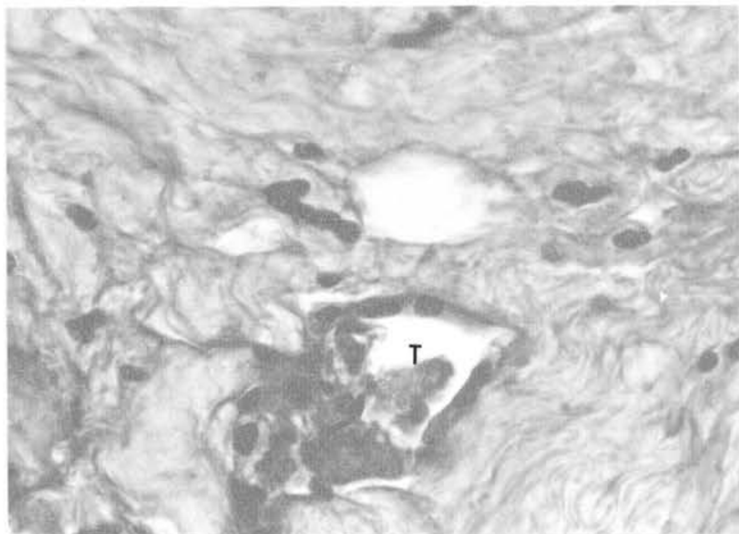

FIG. 96. Photomicrograph of an adult human arachnoid granulation showing tubule in the core. (paraffin embedded, H & E, x 530)

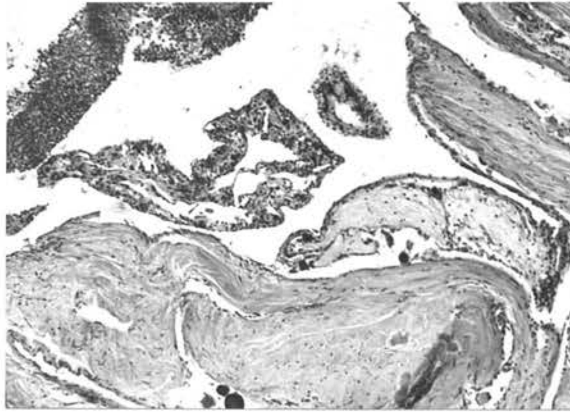

FIG. 95. Photomicrograph of a horizontal (tangential) section of adult human granulation at the summit, showing opening of crypt forming tubule, and 'epithelial cell cap'(E). (paraffin embedded, H & E, H & E, x 54)

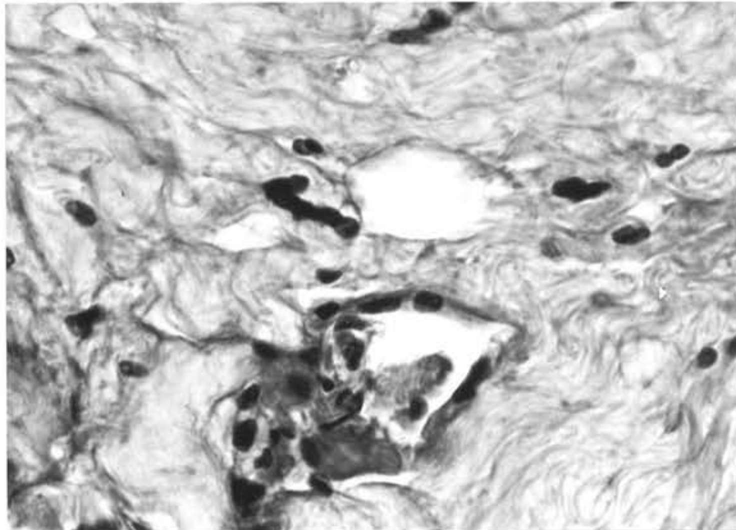

FIG. 96. Photomicrograph of an adult human arachnoid granulation showing tubule in the core. (paraffin embedded, H & E, x 530)

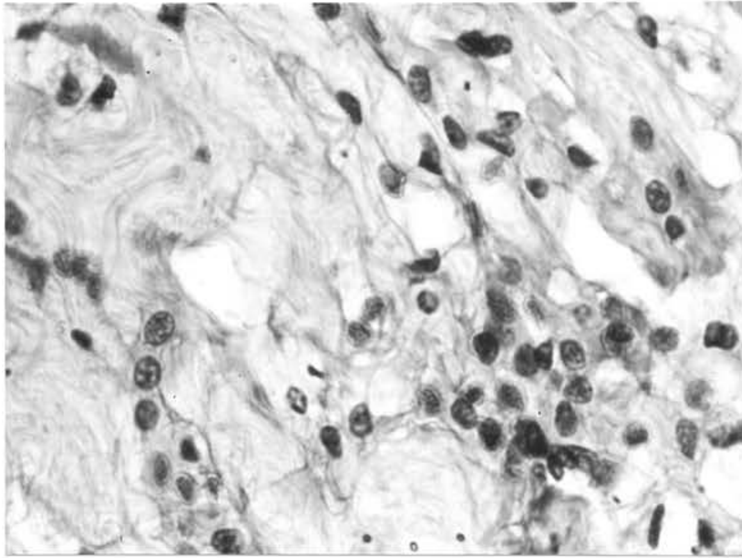

FIG. 97. Photomicrograph of the core of an adult human granulation.
(paraffin embedded, H & E, x 530)

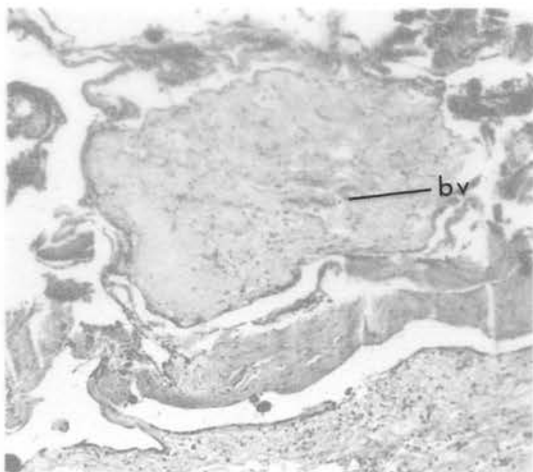

FIG. 98. Photomicrograph of arachnoid granulation in adult human showing blood vessels.
(paraffin embedded, H & E, x 54)

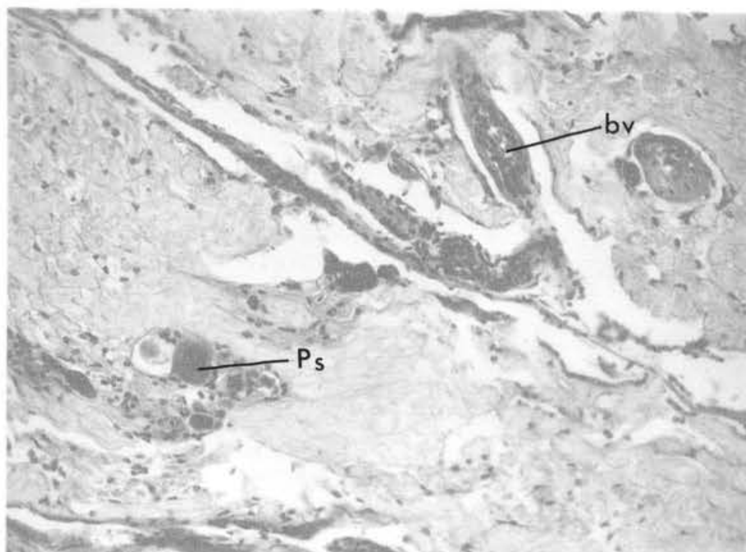

FIG. 99. Photomicrograph of granulation in the adult human showing blood vessels and psammoma bodies(Ps).
(paraffin embedded, H & E, x 140)

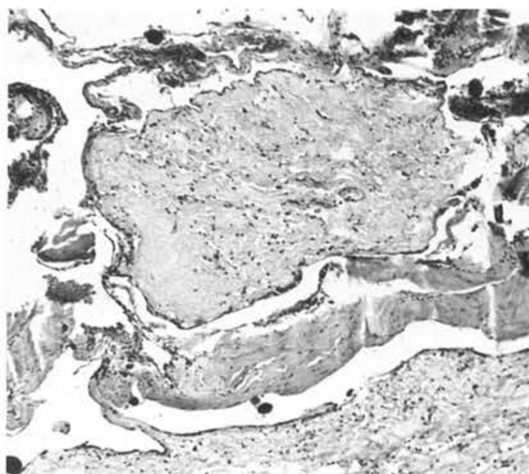

FIG. 98. Photomicrograph of arachnoid granulation in adult human showing blood vessels.
(paraffin embedded, H & E, x 54)

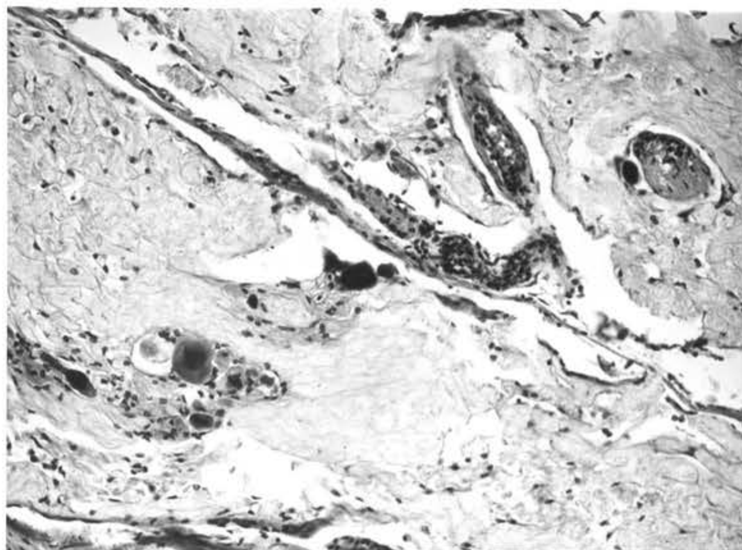

FIG. 99. Photomicrograph of granulation in the adult human showing blood vessels and psammoma bodies(Ps).
(paraffin embedded, H & E, x 140)

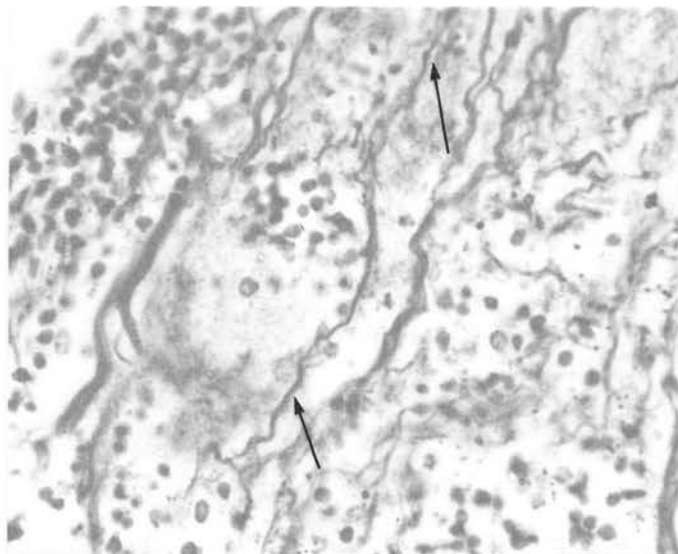

FIG. 100. Photomicrograph of granulation in human adult showing nerve fibres (arrows).
(Peters' silver-proteinate, x 530)

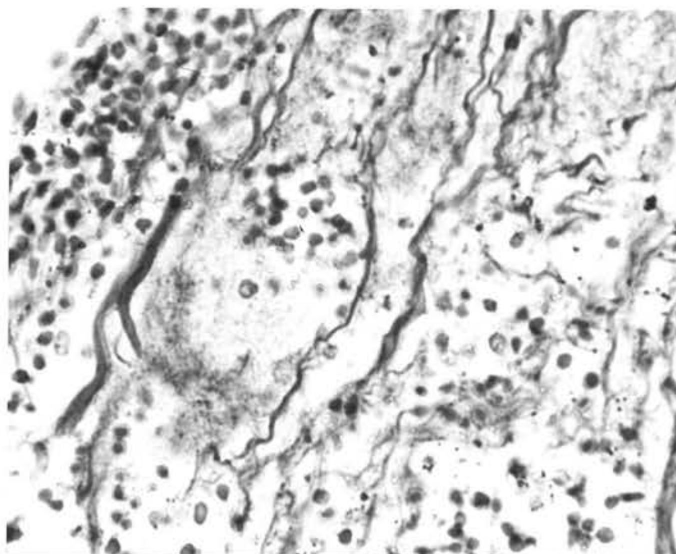

FIG. 100. Photomicrograph of granulation in human adult showing nerve fibres (arrows).
(Peters' silver-proteinate, x 530)

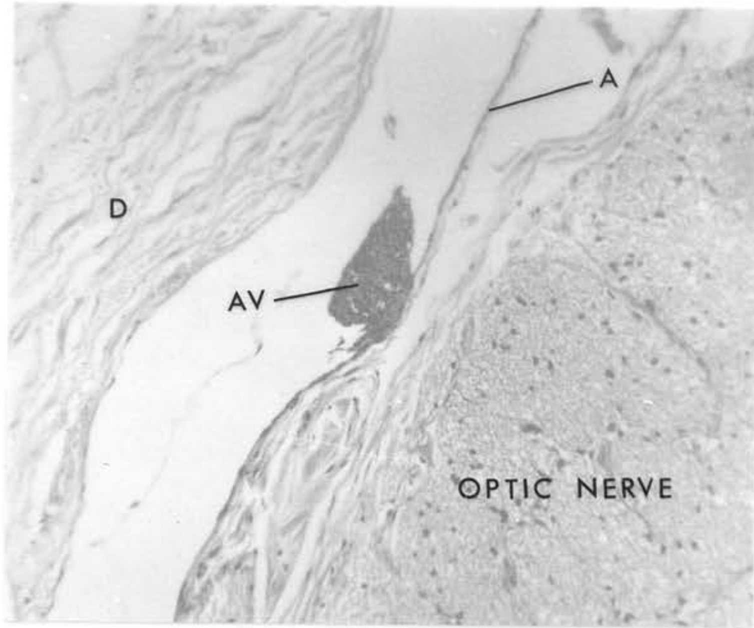

FIG. 101. Photomicrograph of arachnoid villus in a section of optic nerve. Adult human.
(paraffin embedded, H & E, x 140)

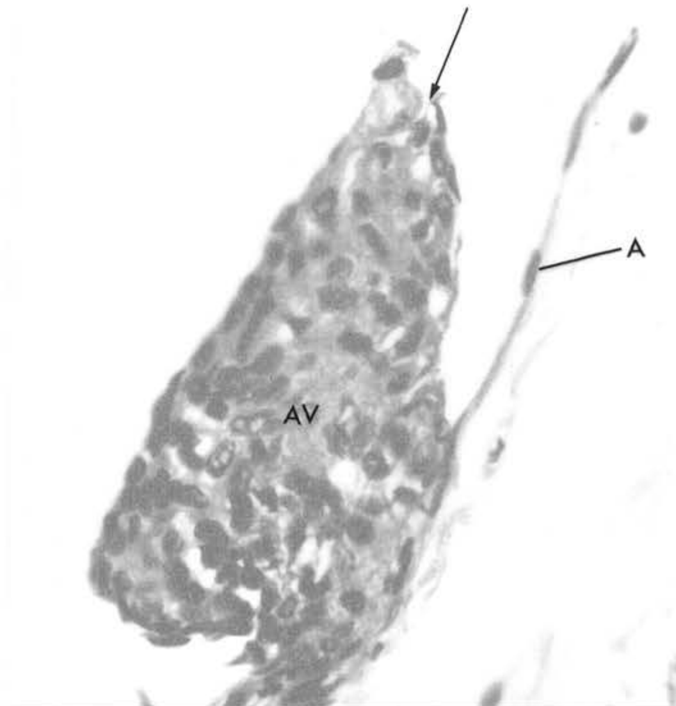

FIG. 102. Photomicrograph of a section of adult human optic nerve showing arachnoid villus projecting into the subdural space. Arrow shows invagination, probably artifact.
(paraffin embedded, H & E, x 800)

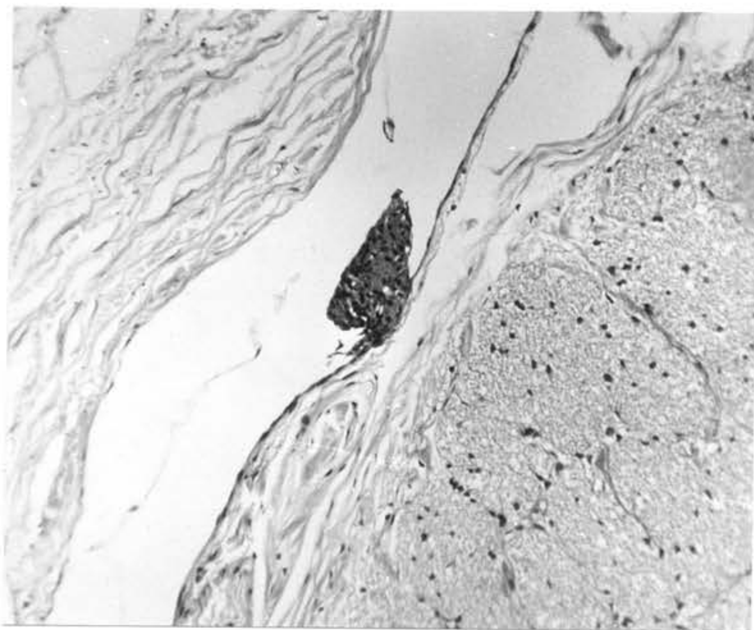

FIG. 101. Photomicrograph of arachnoid villus in a section of optic nerve. Adult human.
(paraffin embedded, H & E, x 140)

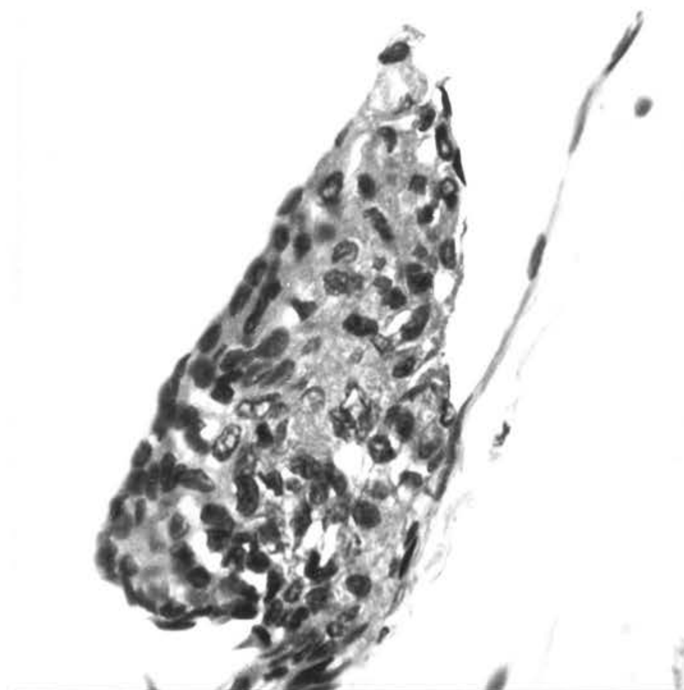

FIG. 102. Photomicrograph of a section of adult human optic nerve showing arachnoid villus projecting into the subdural space. Arrow shows invagination, probably artifact.
(paraffin embedded, H & E, x 800)

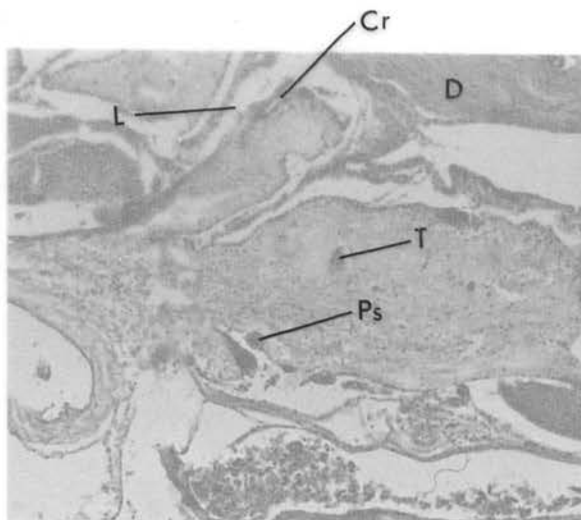

FIG. 103. Photomicrograph of a villus of human neonatus in lateral lacunae (L).
(paraffin embedded, H & E, x 54)

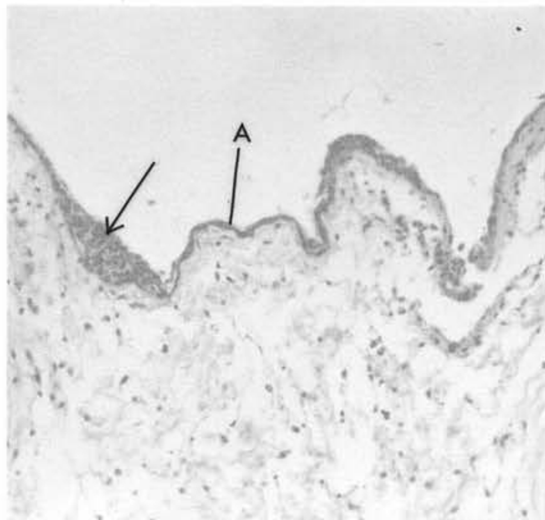

FIG. 104. Photomicrograph of arachnoid membrane in human neonatus showing cell aggregations marked by arrow.
(paraffin embedded, H & E, x 140)

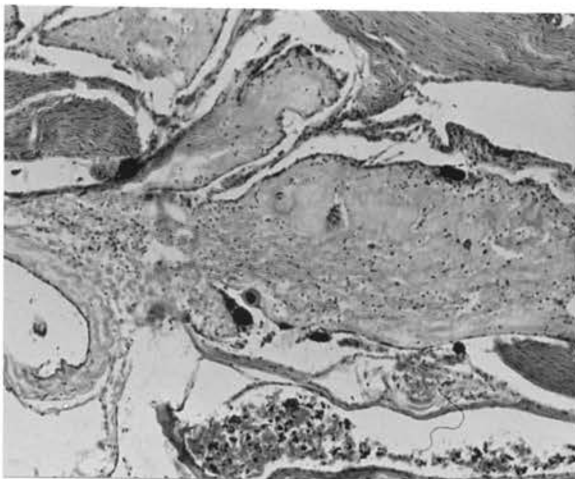

FIG. 103. Photomicrograph of a villus of human neonatus in lateral lacunae (L).
(paraffin embedded, H & E, x 54)

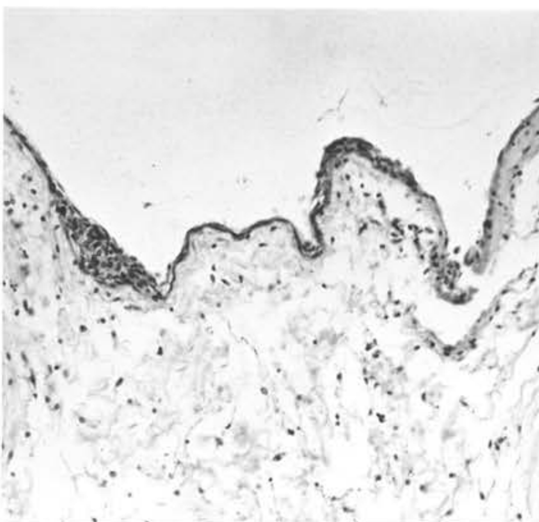

FIG. 104. Photomicrograph of arachnoid membrane in human neonatus showing cell aggregations marked by arrow.
(paraffin embedded, H & E, x 140)

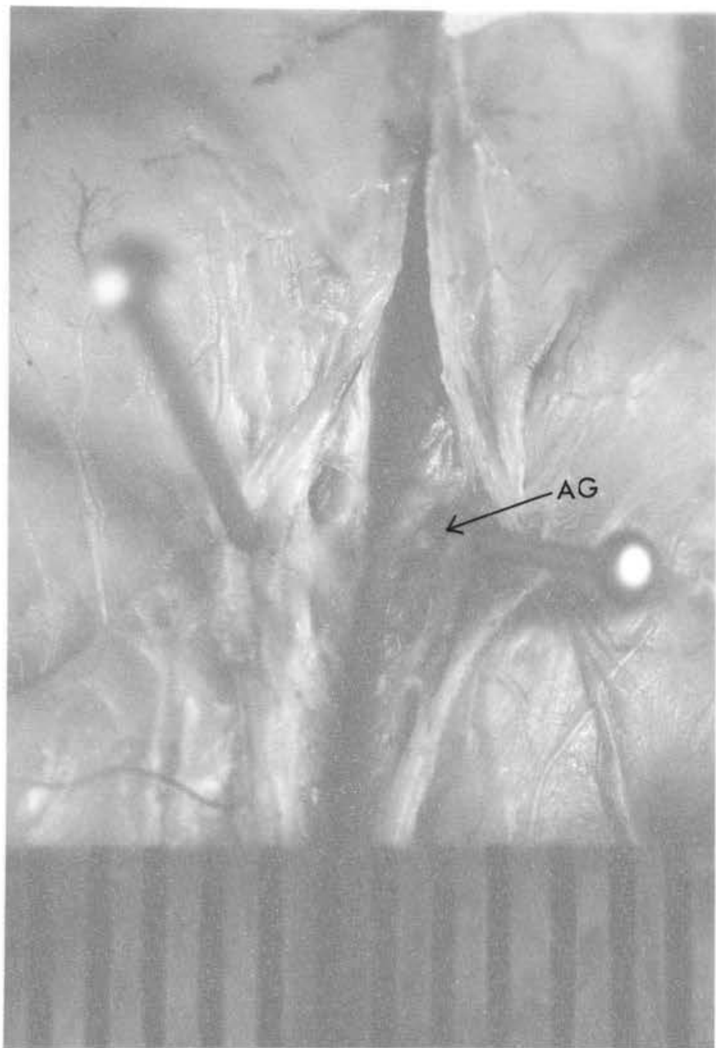

FIG. 105. Photograph of dog superior longitudinal sinus. (x 3.5)

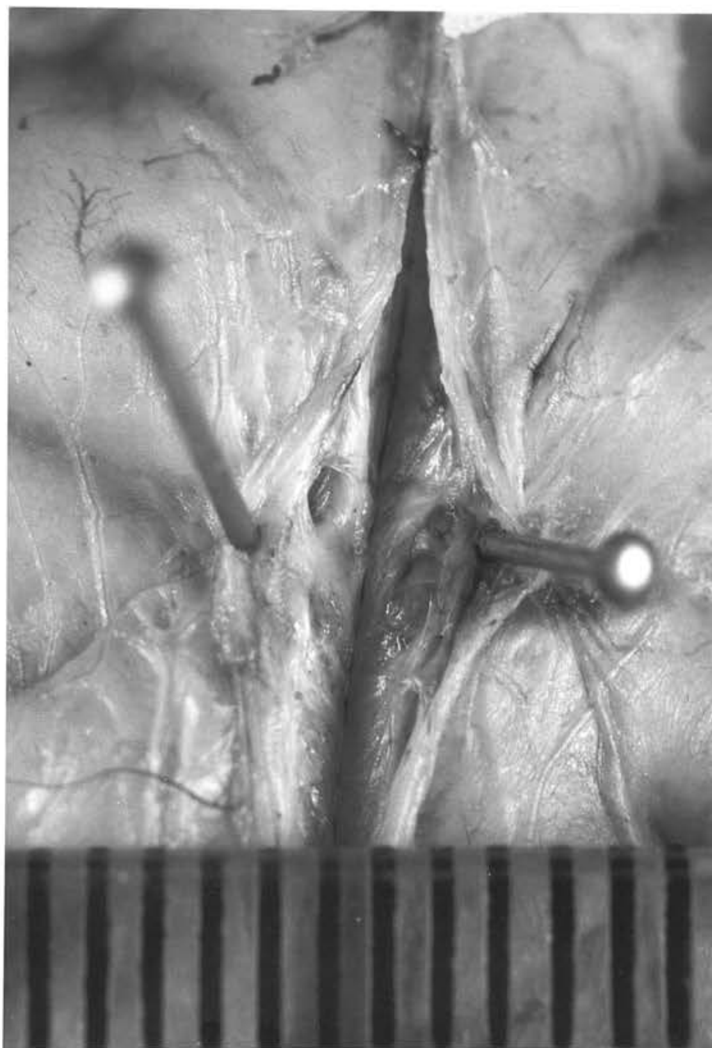

FIG. 105. Photograph of dog superior longitudinal sinus. (x 3.5)

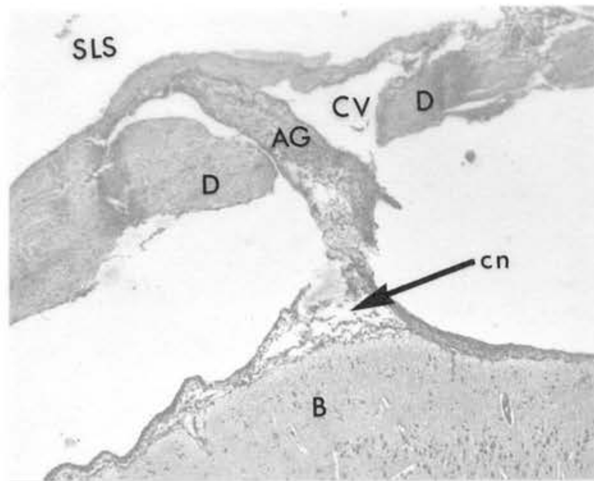

FIG. 106. Photomicrograph of arachnoid granulation of dog in cerebral vein.
(paraffin embedded, H & E, x 54)

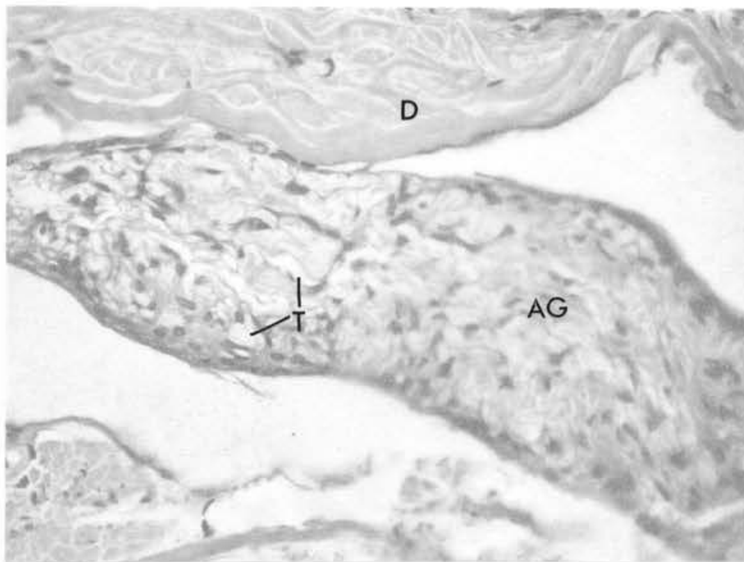

FIG. 107. Photomicrograph of dog granulation showing tubule-like structures.
(paraffin embedded, H & E, x 350)

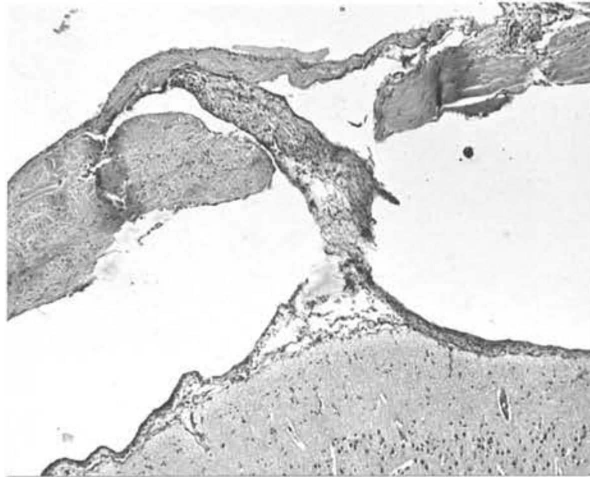

FIG. 106. Photomicrograph of arachnoid granulation of dog in cerebral vein.
(paraffin embedded, H & E, x 54)

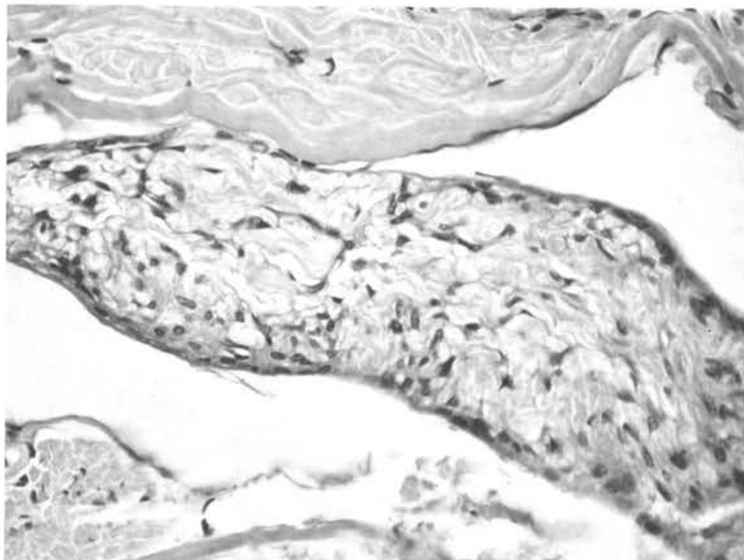

FIG. 107. Photomicrograph of dog granulation showing tubule-like structures.
(paraffin embedded, H & E, x 350)

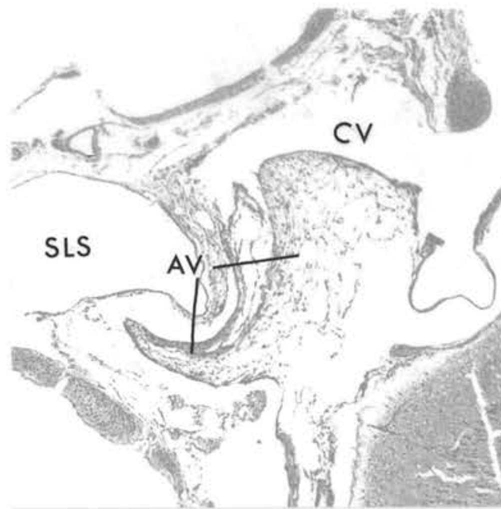

FIG. 108. Photomicrograph of arachnoid villus in cat.
(paraffin embedded, H & E, x 54)

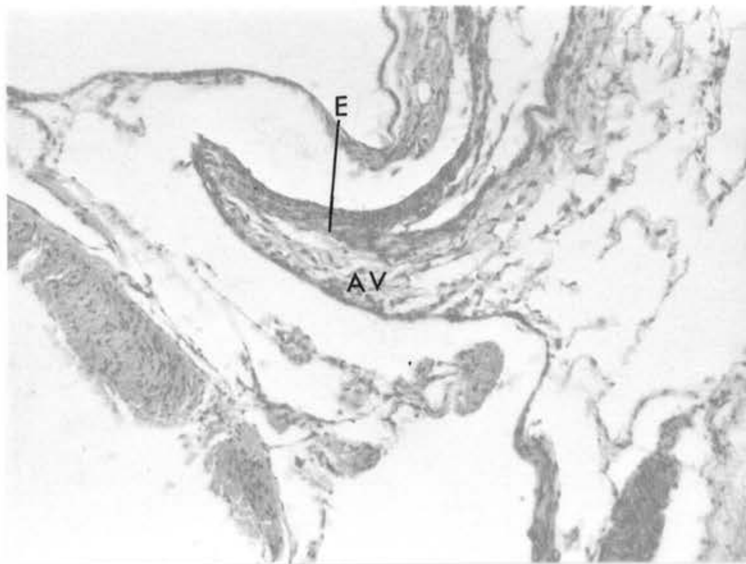

FIG. 109. Photomicrograph of a cat villus showing 'epithelial cell cap'.
(paraffin embedded, H & E, x 140)

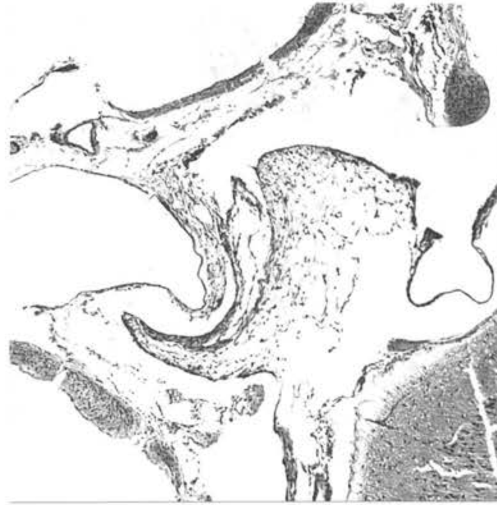

FIG. 108. Photomicrograph of arachnoid villus in cat.
(paraffin embedded, H & E, x 54)

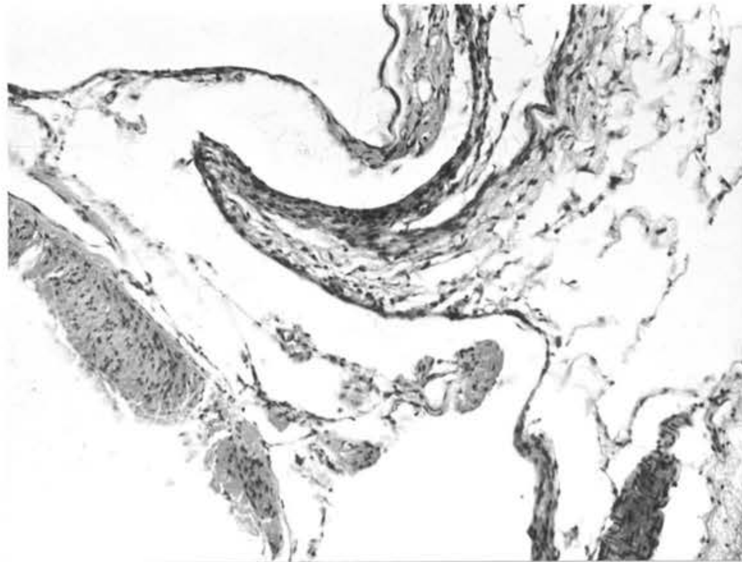

FIG. 109. Photomicrograph of a cat villus showing 'epithelial cell cap'.
(paraffin embedded, H & E, x 140)

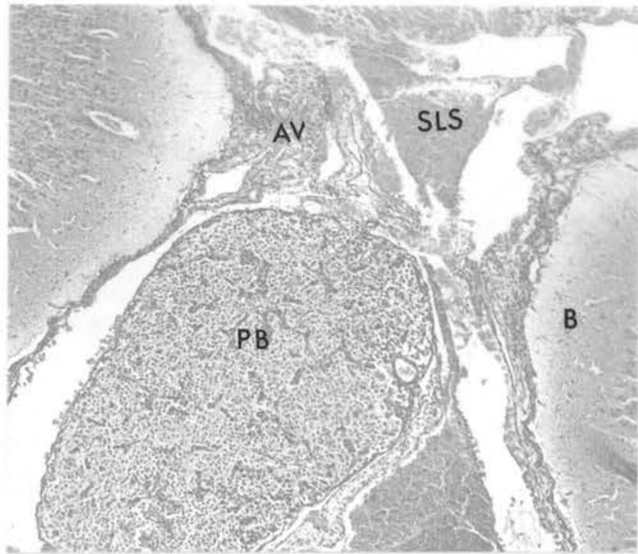

FIG. 110. Photomicrograph of rabbit arachnoid villus in relation to pineal body(PB).
(paraffin embedded, H & E, x 54)

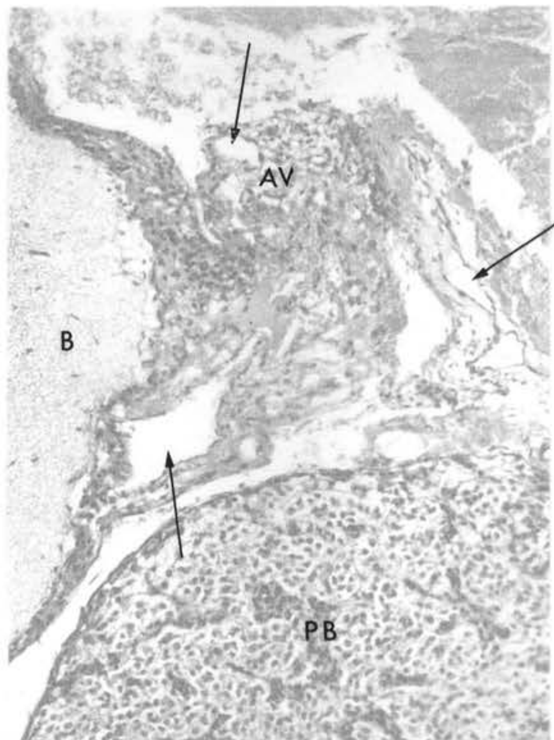

FIG. 111. Photomicrograph of rabbit villus showing spaces lined by flattened cells. One space extends into subarachnoid space.
(paraffin embedded, H & E, x 140)

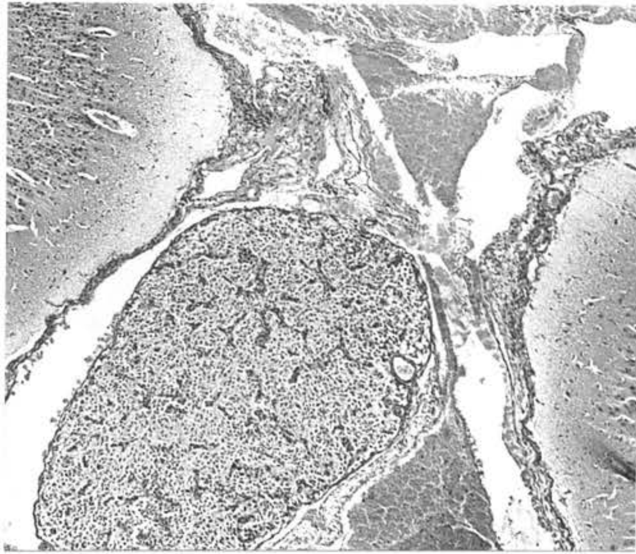

FIG. 110. Photomicrograph of rabbit arachnoid villus in relation to pineal body(PB).
(paraffin embedded, H & E, x 54)

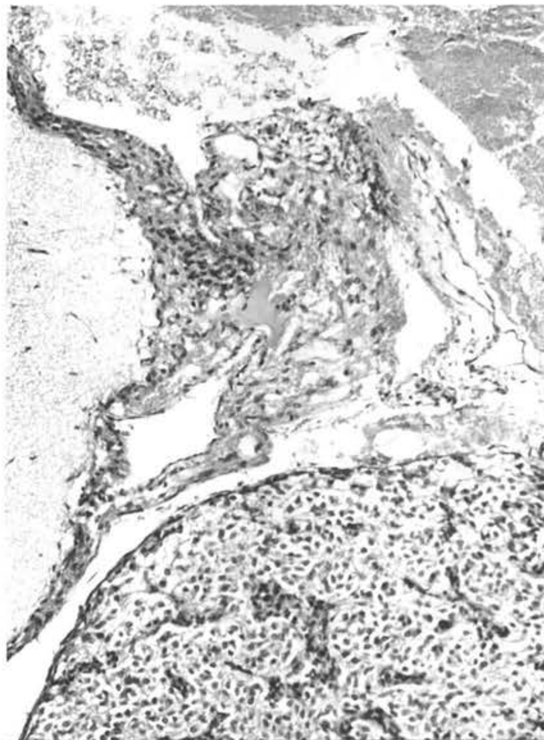

FIG. 111. Photomicrograph of rabbit villus showing spaces lined by flattened cells. One space extends into subarachnoid space.
(paraffin embedded, H & E, x 140)

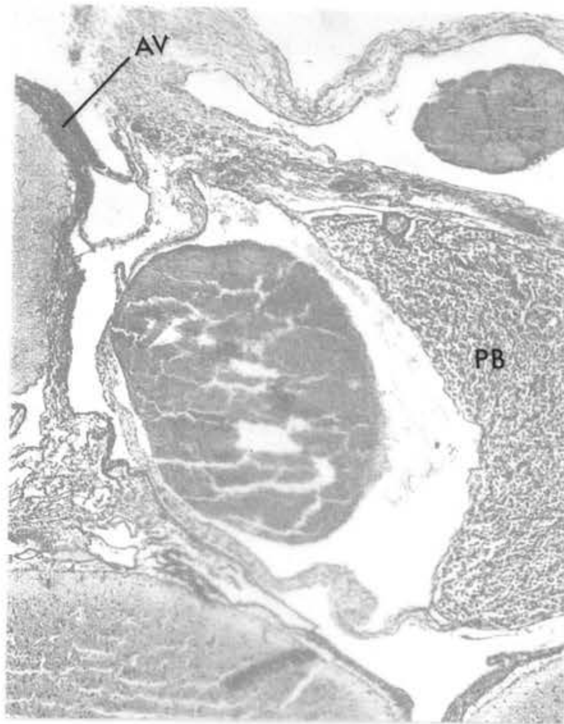

FIG. 112. Photomicrograph of guinea pig villus in relation to superior longitudinal sinus and pineal body(PB). (paraffin embedded, H & E, x 54)

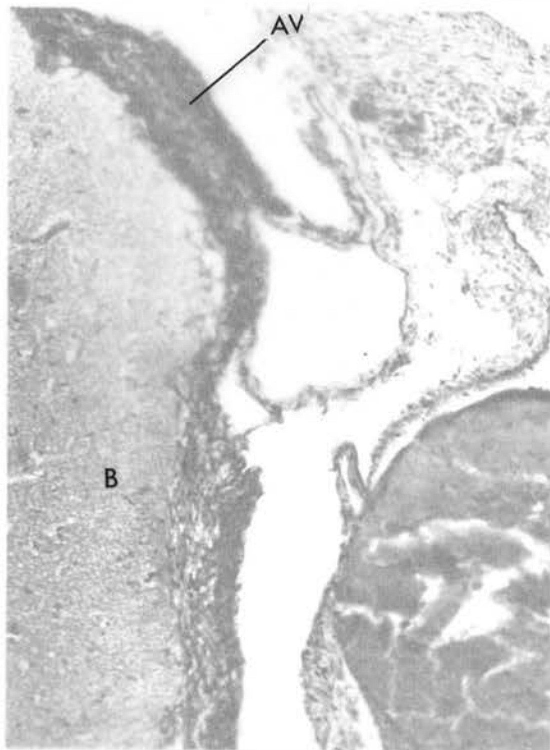

FIG. 113. Photomicrograph of guinea pig villus.

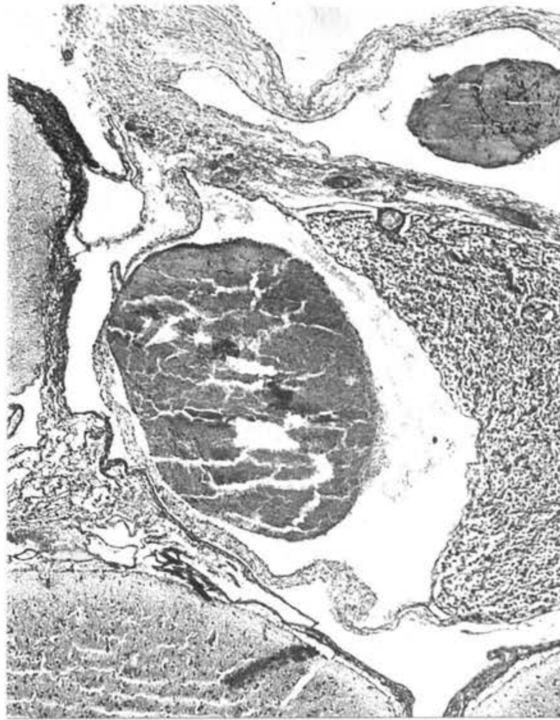

FIG. 112. Photomicrograph of guinea pig villus in relation to superior longitudinal sinus and pineal body(PB). (paraffin embedded, H & E, x 54)

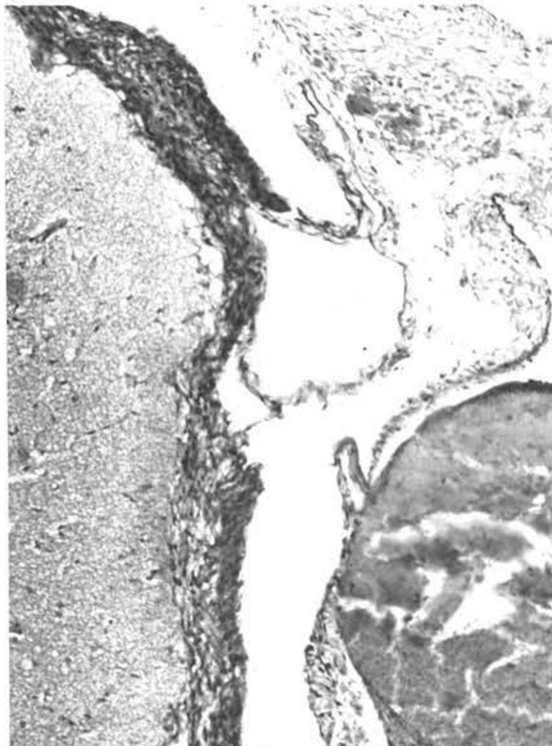

FIG. 113. Photomicrograph of guinea pig villus. (paraffin embedded, H & E, x 140)

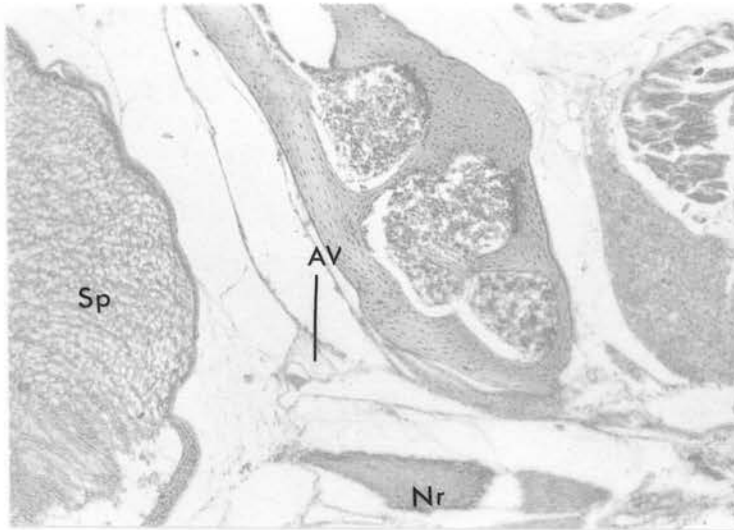

FIG. 114. Photomicrograph of inter-vertebral foramen in spinal region of rat, at emergence of ventral nerve root. (paraffin embedded, H & E, x 54)

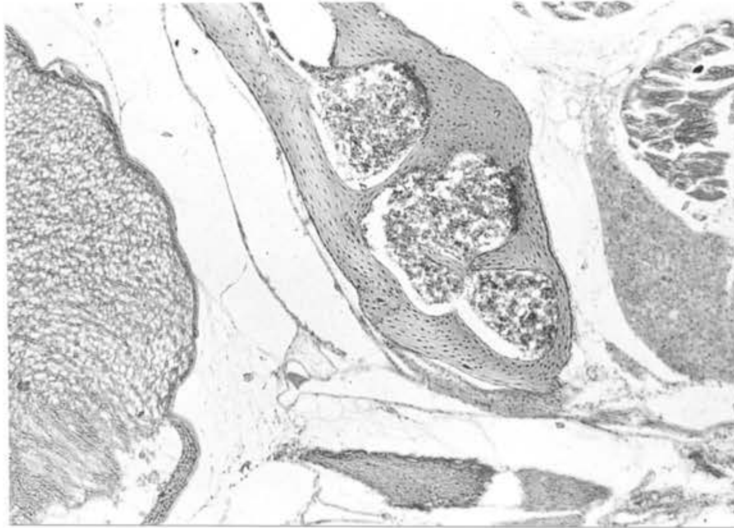

FIG. 114. Photomicrograph of inter-vertebral foramen in spinal region of rat, at emergence of ventral nerve root. (paraffin embedded, H & E, x 54)

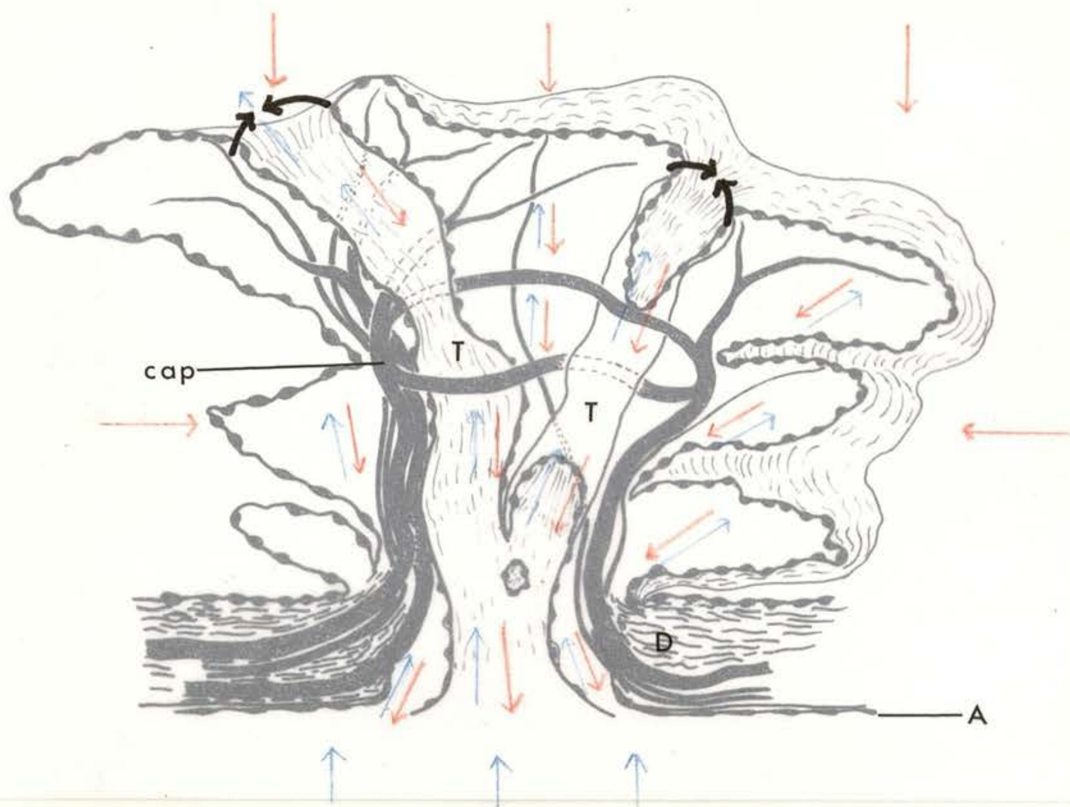

FIG. 115. Diagrammatic representation of valvular mechanism in arachnoid granulation. With increase of C.S.F pressure, fluid could go into the core of the granulation as well as into the tubules, marked by blue arrows. With increase of venous pressure, the fluid in the tubules and in the core would flow back into the subarachnoid space (marked by red arrows), thereby collapsing the granulation. This would cause closure of the tubules as indicated by the black arrows.

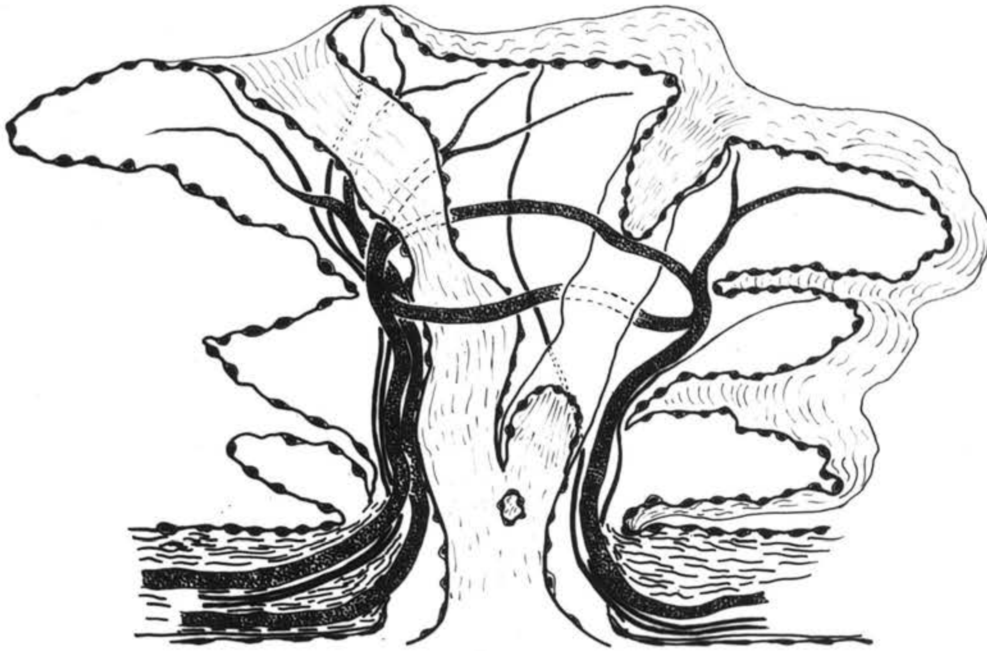

FIG. 115. Diagrammatic representation of valvular mechanism in arachnoid granulation. With increase of C.S.F pressure, fluid could go into the core of the granulation as well as into the tubules, marked by blue arrows. With increase of venous pressure, the fluid in the tubules and in the core would flow back into the subarachnoid space (marked by red arrows), thereby collapsing the granulation. This would cause closure of the tubules as indicated by the black arrows.

GENERAL DISCUSSION.

This study confirms the observations of Turner (1958) that arachnoid granulations and villi are herniations of the arachnoid membrane and sub-arachnoid tissue either into venous sinuses or into the subdural space. This is also supported by developmental anatomy, the granulations appearing to arise by proliferation of the arachnoid cells surrounding the cerebral veins at their entry into venous sinuses (figs. 57 and 58).

The significant finding in this investigation is the presence, in the core of arachnoid granulations of sheep, of tubules connecting the subarachnoid space with the venous sinus. The patency of these tubules has been demonstrated by the fact that following the injection of India ink and thorium particles into the subarachnoid space, these substances have been found both in the tubules as well as in the venous sinus, with no evidence of rupture of the granulation, as shown by the unbroken surface of the endothelial cells, lining the tubules with the surface endothelial cells of the granulation.

Similar tubules were also observed in the arachnoid granulations of man and dog. Arachnoid villi in the human neonatus contain fine tubule-like structures and they may also be present in the arachnoid villi of other animals. Further, it would appear that the tubules in man and dog in no way differ from those present in the granulations of the sheep.

The divergent views regarding the existence of an open communication between the subarachnoid space and the venous sinus have already been outlined, yet, the view of the American neurosurgeon Cushing is worth repeating here. In 1901, in his Mütter lecture he stated; "there exists very free communication between the cerebro-spinal space and the longitudinal sinus. The original hypothesis offered by Key and Retzius/

Retzius, that the Pacchionian granulations subserve some purpose of filtration, can hardly be entertained, for ... the points of exit of the fluid undoubtedly are in the situation of the lacunae laterales of the sinus, into which these granulations for the most part project... Whatever the nature of the openings may be or wherever their situation, they certainly are of considerable size and apparently possess a valvular action. The process is not a mere filtration through a membrane" (Cushing, 1902). Cushing observed experimentally that an injection of mercury into the lumbar meninges in the human cadaver may be easily made to pass from the cerebro-spinal space into the longitudinal sinus, and through the jugular veins into the right atrium. An injection in the opposite direction by way of the veins, never or only exceptionally entered the cerebro-spinal space. When Weed (1914b) failed to demonstrate any morphological evidence for the presence of valves and suggested that cerebro-spinal fluid was filtered through villi, even Cushing (1914) accepted this theory in preference to his previous view. For over forty years Weed's theory of the filtration of cerebro-spinal fluid by villi held sway, in spite of sporadic attempts by some workers to demonstrate other pathways of absorption. Recently, however, Welch and Friedman (1959 and 1960) and Welch and Pollay (1961) working on monkeys have produced good evidence in support of the existence of an open communication between subarachnoid space and venous sinus. These workers observed the passage of particulate matter (colloidal gold, polystrene spheres, yeast, erythrocytes) from the subarachnoid space to the venous sinus, through villi in monkeys, after these substances were injected into the subarachnoid space. Histologically, they found that this was not due to rupture caused by high pressure of/

of the injection; the pathway of communication being tubes connecting the subarachnoid space to venous sinus. Unfortunately, they mistook the spaces (lined incompletely by mesothelial cells), between the collagen bundles to be the tubes (See Welch and Friedman, 1960; plate 74, fig. 6). Consequently, they did not show the openings of the tubes onto the surface of the villi, as demonstrated in these investigations in sheep (see fig. 18 and figs. of reconstructions, 66 and 67).

The presence of thorium particles both in the tubules and on the surface of the surface epithelial cells of granulations in sheep, half an hour after injection into the subarachnoid space, suggests that the tubules are normally open, thereby allowing the passage of particles into the venous sinus. The cells lining the tubules and those on the surface of the granulation showed no evidence of thorium uptake in their cytoplasm. This may be due to the fact that these cells were not in contact with the particles long enough for any uptake to occur, as the particles were washed away either by cerebro-spinal fluid or by blood, although particles in the core of the granulation are taken up rapidly by the macrophages. The absence of particles in the tubules of the granulation in the 4 hour specimen, as compared with the ½ hour specimen, suggests that the number of therium particles in cerebro-spinal fluid had fallen due to phagocytosis by macrophages, flow through tubules and dilution by new formation of cerebro-spinal fluid with hardly any particles remaining suspended in the fluid.

It must be admitted, however, that as the samples of tissue taken for viewing by electron microscopy were small, many particles may have been missed, although had they been in any concentration, they would/

would certainly have been observed. The most likely interpretation is that the tubules were open and that most of the particles washed away.

Remarkably little information is available regarding the venous pressure of the cranial venous sinuses and the pressure of the cerebro-spinal fluid, over the cerebral hemispheres, in man. In the dog, however, Weed and Hughson (1921) and Bedford (1935) measured these pressures and came to the conclusion that the hydrostatic pressure of the cerebro-spinal fluid was higher than that of the sinus venous pressure. All of these workers measured the pressure of the cerebro-spinal fluid in the cisterna magna and arrived at a figure of 150 mm of water. Weed and Hughson (1921) found the venous pressure in the torcular Herophili; to be 5 to 50 mm below that of the cerebro-spinal fluid, while Bedford (1935) also measuring the pressure in the same situation, observed the difference to be 25 to 95 mm of water. The filling of the core of the granulation by cerebro-spinal fluid would cause the granulation to inflate, and the pressure difference under normal conditions would be expected to produce a flow of fluid through the tubules, into the sinus. Welch and Friedman (1959 and 1960) demonstrated that when the sinus venous pressure was increased, the pressure acting uniformly on the granulation, collapsed and flattened it, with obliteration of the spaces in the core of the granulation and thus forcing the fluid back into the subarachnoid space. The presence of thorium particles in the core of the granulation after injection into the subarachnoid space, in this investigation, suggests that they are carried in by the ebb and flow of cerebro-spinal fluid produced by this mechanism. For, if it were not so, the thorium particles/

particles could not enter the cul-de-sac like core of the granulation, if it was already filled with cerebro-spinal fluid, unless filtration was taking place through the core and surface epithelium. In this investigation, there is no good evidence to support or dispute whether filtration of cerebro-spinal fluid occurs through the surface epithelial cells of granulations and villi. However, in the specimen of the granulations, 4 hours after injection of thorium, collections of thorium particles were seen beneath the surface epithelial cells (fig. 85). This suggests a slow movement of cerebro-spinal fluid from the core of the granulation towards the periphery, and this may be due to a slow filtration of the fluid through the surface epithelial cells. Thus, it is possible, that cerebro-spinal fluid could drain into the venous sinus both by direct passage through the tubules as well by filtration.

A valve-like mechanism would explain the results of Weed (1914b), who, working on cats, increased the pressure in the venous sinus far above the cerebro-spinal fluid pressure by ligation of the veins of the neck, and failed to observe any red blood cells either in the villi or in the subarachnoid space. In such a case, the villi would be expected to collapse, thereby closing the tubules which would not open again until the cerebro-spinal fluid pressure increased, as it does soon after the venous pressure is raised (Bowsher, 1953). The valvular mechanism is summarised in fig. 115.

Conclusive proof of this valvular mechanism may well lie in the direct observation of the granulations, under varying cerebro-spinal fluid and venous pressures, although observation of the granulations without incision of the superior longitudinal sinus is almost impossible. Until the problem/

problem of viewing these granulations without upsetting the physiological conditions obtaining in the sinus is overcome, the evidence for the valvular mechanism must remain mainly circumstantial.

If it is true that granulations and villi act as physiological valves, it is interesting that the granulations of man, sheep, dog and the villi of other animals are mainly situated at the highest points of their neuraxes. Davson (1962) suggests that this is due to the fact, that at these sites the pressure of the venous blood is at its lowest value, so that this would favour the flow of fluid from the subarachnoid space to the venous sinus. The explanation seems reasonable though the evidence is not supported by good hydrostatic principles. Not only is the venous pressure in the dural sinuses at its lowest value but the cerebro-spinal fluid at those sites is also at its lowest hydrostatic pressure. Thus, there are two columns of fluid with a pressure difference of about 50 mm of water, acting along the entire length of both columns. There is, therefore, no sound mechanical reason why the flow from cerebro-spinal fluid to the blood should occur at the highest point of the two systems, unless there is some factor which causes upward movement of fluid in the cerebro-spinal fluid system with subsequent build up of pressure at the highest points. A suggestion of an upward movement of cerebro-spinal fluid was made by Eichler et al (1951). They injected radio-active sodium into the cerebro-spinal fluid of the lumbar region of horizontally placed, anaesthetized dogs, and found radio-activity in the thoracic region after 10 minutes, but not in the sacral region. By injecting radio-active sodium intravenously they found a considerable amount of radio-activity in the cerebro-spinal fluid of the lumbar/

lumbar region almost equal to that found in the cisterna magna. The level of radio-activity in the thoracic region was less than in the cervical and lumbar regions. This led them to postulate a lumbar source of fluid which caused this upward movement. These experiments are subject to criticism on the grounds that the radio-activity was determined with a Geiger-Muller counter, placed on the surface of the animal so that the activity of the lumbar region may have included activity in the kidneys and the pelvices of the ureters. Again, the volume of cerebro-spinal fluid in the lumbar region is greater than in the thoracic region, and therefore the comparative levels of radio-activity measured in this way in these two regions would not give a measure of the absolute radio-activity of the cerebro-spinal fluid in each region. They also did not demonstrate the anatomical site for the production of cerebro-spinal fluid in the lumbar region. Sachs et al (1930) also showed cranialward movement of trypan blue, when injected into the lumbar region of dogs. They concluded that this was by a process of diffusion caused by movements of the body, e.g. in respiration, movement of trunk, head etc. The evidence of Eichler et al and Sachs et al is unconvincing so that other evidence must be sought to explain the upward movement of cerebro-spinal fluid in the cranium.

Turner (1961) observed in postmortem material, that following bleeding into the subarachnoid space from vessels situated at the base of the brain, blood elements and exudate passed over the cerebral convexities and found their way into venous sinuses through arachnoid granulations. He concluded that cerebro-spinal fluid moved upwards towards the superior longitudinal sinus. Dott and Gillingham/

Gillingham (1958) suggested that there was a possibility of an active directional propelling mechanism upon cerebro-spinal fluid within the cranial subarachnoid space, brought about by the propelling action of pulse waves of cerebral arteries upon the fluid, in spaces around them (Bramwell et al, 1923). Dott and Gillingham (1958) reported that 'subarachnoid collections of C.S.F.' or 'pouches' formed by cicatrical adhesions within the subarachnoid space over the temporal region, were relieved completely by division of the adhesions. They also observed that cerebro-spinal fluid was discharged intermittently into the cavity of these 'pouches' from the inter-peduncular cistern and synchronously with systole of cerebral arteries. Further, Gillingham (1964) accidently deposited Myodil in the Sylvian fissure (lateral sulcus) while injecting it into the lateral ventricle. He recorded the rapid progression of Myodil laterally in the Sylvian fissure and over the lateral aspect of the cerebral hemispheres, in jerks along the trunk and branches of the middle cerebral artery, corresponding with the systole of these vessels. These observations certainly suggest a propelling movement of cerebro-spinal fluid towards the superior longitudinal sinus, with subsequent build up of pressure at this site, a feature which would make the situation of the granulations and villi at the highest point significant. If this interpret-ation is valid, it raises the question as to whether there is free communication throughout the cerebral subarachnoid space or whether a tubular system is present here also.

The sizes of the granulations may also be related either to posture, or to the size of the animal. In the adult human, a large animal with an upright posture, the granulations are large and situated/

situated mainly at the superior surface of the brain, while in the human neonatus, which is comparatively smaller, and does not have an upright posture, villi are present. Le Gros Clark (1920) observed that granulations are visible in children about the age of 18 months and this is about the time the child is able to walk. There seems to be some temporal relationship at least between the appearance of granulations and posture in case of children.

On the basis of an open communication between the subarachnoid space and the venous sinus, the results of some previous workers can be explained. In man, Key and Retzius (1875) found particulate matter in the venous sinus after subarachnoid injection and explained the passage of particles through 'stomata' (between cells of the surface epithelium) instead of through the tubules, of which they had no knowledge. Further, in the cat, Weed (1914 a and b) observed prussian blue granules in the venous sinuses after introducing potassium ferrocyanide and iron ammonium citrate into the subarachnoid space and subsequently fixing the animal in acidified formalin. He concluded that the ferrocyanide-citrate mixture had filtered through villi and was precipitated by the acidified formalin in the sinus. An alternative explanation is that the toxicity of the injected substances (see Howarth and Cooper, 1955), may have altered the permeability of the surface epithelium of the villus, thereby allowing free flow of the solutions into the venous sinus. Further, the prussian blue precipitate forms even without the presence of hydrogen ions (hydrogen ions only render the precipitate insoluble). The presence of the prussian blue in the sinus could also be explained by the open communication through the tubules into the venous sinus. In 1920 (a), Weed injected lamp black into/

into the subarachnoid and ventricular spaces of kittens and produced hydrocephalus. He attributed the formation of hydrocephalus to a blockage of the villi. What he failed to realize was that not only had he blocked the villi but had also interfered with the other pathways of drainage of cerebro-spinal fluid as suggested by Mott (1910), Dandy and Blackfan (1913 and 1914), Wislocki and Putnam (1921), Hassin (1924), Scholz and Ralston (1939) and Howarth and Cooper (1949 and 1955). Later, in 1952 and in 1953, Simmonds observed the passage of labelled red blood cells and plasma from the subarachnoid space to the venous sinus, and concluded that the passage occurred probably through the arachnoid villi in rabbits, the villi being considered as specific sites of protein absorption (see above p.25). Similarly, Sweet and Locksley (1953) and Turner (1961) were of the opinion that villi were sites of protein absorption. The labelled red cells and proteins could have passed through the tubules within the villi. Thus, the results of Key and Retzius, Weed, Howarth and Cooper, Simmonds, Sweet and Locksley and Turner together with the findings in the present investigation, suggest that the passage of cerebro-spinal fluid through granulations and villi does seem to occur by a direct communication through the tubules, rather than by filtration alone.

Welch and Friedman (1960) found that in the monkey, the passage of cerebro-spinal fluid occurred above a critical pressure of 20 to 50 mm of Ringer lactate solution. The passage of fluid through the villi at these pressures was determined by the movement of an air bubble in a solution contained in one of the glass tubes fixed on either side of the villus. (see page 28). The friction exerted by an air bubble on/

on the sides of a glass tube is considerable, and these pressures may not indicate the true pressures needed to cause flow of fluid through the villus. It is possible, however, that granulations act only as safety valves to permit unidirectional flow of fluid, when the cerebro-spinal fluid pressure is raised above normal.

On the premise that granulations act as valves, the function of granulations and villi unrelated to venous channels, for example over the cerebral cortex and around the optic nerves of man, can be explained. The fluid that leaks through them could pass into the subdural space, from where as Field and Brierley (1948 a and b, and 1949) suggest, the fluid may be drained away via the perineural lymphatics.

The observation of granulations at openings of cerebral veins into venous sinuses is also striking. In sheep (fig. 6) one side of the granulation is intimately attached to the dura while the other, unattached side, overhangs the opening of a cerebral vein. With an increase of sinus pressure it has been suggested that the granulations collapse and become flattened (Welch and Friedman, 1959 and 1960). Though there is no experimental evidence to support this mechanism, it is possible that being attached to the dura, the flattened granulation could act as a flap valve in preventing regurgitation of blood into cerebral veins, as was suggested by Bell in 1803 and Cooper in 1958, though the significance of this in relation to venous pressures is not understood.

Previous workers have speculated about the nature of the cells of the surface epithelium of the granulations in man. From this investigation in sheep, it is impossible even by electron microscopy to differentiate between endothelial, mesothelial and arachnoid/

arachnoid epithelial cells. All that can be stated is that the surface epithelial cells are endothelial cells, which are in contact with blood and form part of the wall of the venous sinus. Similarly, the lining cells of the tubules and the arachnoid epithelial cells lining the subdural space, are morphologically indistinguishable from the surface epithelial cells of the granulation. The terminology of these flattened cells must therefore depend on their situation e.g. cells lining venous channels as endothelial, those lining the subdural space as arachnoid epithelial cells, and those lining the tubules as endothelial, for they are continuous with the surface epithelial cells.

The 'epithelial cell caps' of granulations are also formed from the cells of the surface epithelium. The cell aggregations found in the arachnoid epithelium resemble the 'epithelial cell caps'. Essick (1920) and Weed (1920 b) believed that formation and calcification of arachnoid cell aggregations was a phenomenon associated with old age, but this was questioned by Watt (1962) by his finding of arachnoid cell aggregations in infants. Turner (1958) also considered corpora amylacea or psammoma body formations were a degenerative process, but these bodies have been observed in the villi of the neonatal human in this investigation. Chornyak (1948) found that under conditions of anoxaemia the cells of the arachnoid epithelium proliferate and form typical cell clusters. It is likely that 'epithelial cell caps' and arachnoid epithelial cell aggregations are produced by a similar condition, though the nature of the condition is one of conjecture at the present time, and falls outside the scope of this study.

The presence of blood vessels in sheep arachnoid granulations has been demonstrated by India ink/

ink injections through the common carotid arteries. A similar procedure was employed by Kolesnikov in 1940, when he studied the granulations in man. The present investigation clearly demonstrates that blood vessels are present in granulations and that they arise from dural blood vessels in the sheep. The demonstration of these blood vessels separately from the tubules, both of which are lined by endothelial cells allows of a clear indication of the presence of two such separate systems.

Medullated and non-medullated nerve fibres have been observed by both light and electron microscopy. Since no muscle fibres or myo-epithelial cells were seen in granulations, it must be concluded that these nerve fibres are sensory. It is possible that some of the non-medullated fibres are autonomic but in the absence of arterioles or venules, their function would be obscure. The nature of the medullated fibres is unknown, and in view of their relatively large diameters, it is surprising that no specialized nerve endings have been found. The exact termination of all the fibres requires further investigation.

The present investigation into the structure of the core of the granulations in man, sheep and other mammals confirms the findings of Key and Retzius (1875) in man, and also the findings of more recent workers. However, they are at variance with those of Turner (1958) who maintained that the spaces between the collagen bundles are artifacts. In the present investigation following the injection of thorium into the subarachnoid space in live sheep, particles were observed in such spaces. If spaces do not exist during life it is unlikely that the particles could have entered artificial spaces during specimen preparation. The presence of thorium particles in these spaces indicates that cerebro-spinal fluid is contained/

contained within them during life, as the thorium was injected into the cerebro-spinal fluid in the experiments. Similar spaces have been reported by Alksne (1962) in the villi of dogs and by Pease and Schultz (1958) in subarachnoid tissue of rats, by electron microscopy. Turner (1958), however, aligns himself with Arnold (1839), Tuke (1882), Middlemas and Robertson (1895) and Le Gros Clark (1920) in denying the existence of these spaces in the subarachnoid tissue in man.

Quincke (1872) observed the uptake of particulate matter by the cells of arachnoid granulations, and in 1920, Essick made a similar observation for the cells in arachnoid tissue. Russell (1950) suggested that macrophages arose from arachnoid cells, the granulations and subarachnoid tissue being thus a component of the reticulo-endothelial system. In the present investigation the cells described by Quincke, Essick and Russell were observed, these appear to be the macrophages present both as normal inhabitants (type 1) and those that appear in response to stimuli of foreign material (type 2). Under normal conditions the type 1 macrophage probably ingests both lipoid and protein remains of dead cells in the granulation as well as in the subarachnoid space. These are likely to be phagocytosed by the cell and retained in the large vesicles present in the cytoplasm, so that when the tissue is fixed by means of osmium, they show as the electron dense bodies; the fibrils in the matrix may represent a digested lipoid-protein complex while the small circular bodies may be akin to the lysosomes or lipofuscin pigment of liver cells (Palade and Siekevitz, 1956; Scheuer, Williams and Muir, 1962).

The absence of electron dense bodies in the cytoplasm/

cytoplasm of the type 2 macrophage can be explained on the basis of the postulate that these cells are not usually phagocytic but assume this rôle only during times of stress.

Sweet and Locksley (1953) stated that apart from the function of absorbing proteins from the cerebro-spinal fluid, arachnoid villi "serve a function in the C.S.F. system analogous to the lymphatics of the general circulation". Hastings (1953) further emphasized that the cerebro-spinal fluid was the "lymphatic fluid of the central nervous system". Morphologically, the tubules of the granulation appear to be similar to lymph vessels; functionally, the former convey cerebro-spinal fluid to the venous system while the latter carry lymph to veins. The activity of the cells of the granulation and subarachnoid space is also similar to those cells of a lymph node, and is supported by Russell's contention that granulations and subarachnoid tissue are part of the reticulo-endothelial system. These considerations certainly support the view of Sweet and Locksley, that villi and the subarachnoid space are analogous to the lymphatics of the general circulation. It is indeed a credit to the genius of those three anatomists, Willis, Pacchioni and Fantoni, that despite very limited facilities, they formulated a similar concept regarding the function of the arachnoid granulations or 'glands of Pacchioni'.

The story is by no means complete. Further work is necessary to ascertain the morphology of the granulations and villi under varying physiological conditions. Detailed study of the nature of the subarachnoid and subdural spaces is also necessary before a final conclusion could be made regarding the formation, circulation and 'absorption' of cerebro-spinal fluid. However, the belief that arachnoid granulations and villi are responsible for the

'transport'/

can/

'transport' of cerebro-spinal fluid to the venous sinuses, still holds good, and is entirely supported by these findings, though the extent of their contribution to the passage of cerebro-spinal fluid into the blood stream under different physiological conditions, is obscure.

SUMMARY.

The literature relating to arachnoid granulations and villi in man and in other animals has been reviewed. A survey of the occurrence and structure of granulations and villi was undertaken in a series of mammals, and as a result of this, sheep were finally chosen as the most suitable animal for a detailed study.

The morphology of the arachnoid granulations in 23 adult sheep was studied both by light and electron microscopy. The spinal meninges of sheep were also examined and at the sites of emergence of the spinal nerve roots from the dura, cell aggregations of the arachnoid membrane were noted, and identified as villi, since they resembled the structures previously described by other workers as villi. The development of arachnoid granulations in foetal sheep was studied in 40 day, 60 day, 90 day, 110 day, 120 day and 140 day foetuses.

A survey of arachnoid granulations and villi was made in 4 brains of adult human, 4 brains of neonatal human, 10 dogs, 6 cats, 6 rabbits, 6 guinea pigs and 8 rats, and the structure in each compared with those of the sheep.

Arachnoid granulations and villi are usually situated in relation to intra-cranial venous channels in all the material examined. In man, however, granulations unrelated to venous channels are present e.g. over the cerebral cortex away from the lateral lacunae and in the arachnoid membrane of the optic nerves, where they project into the subdural space.

Granulations and villi are herniations of the arachnoid membrane and subarachnoid tissue, either into venous sinuses or into the subdural space. This is supported by developmental anatomy, the granulations appearing to arise by proliferation of the arachnoid cells/

cells surrounding the cerebral veins at their entry into venous sinuses. An arachnoid granulation or a villus possesses a body and a neck, through which the core of their structure communicates with the main parts of the subarachnoid space.

The surface epithelium of granulations and villi is formed of endothelial cells, while in those granulations and villi unrelated to venous channels, the epithelium is formed of arachnoid epithelial cells. Morphologically, there is no difference between endothelial and arachnoid epithelial cells.

'Epithelial cell caps' are present in the granulations and villi of man, sheep and in the cat. They are derived from the surface epithelium of the granulations and resemble the cell aggregations of the arachnoid membrane.

The surface epithelium of granulations in man and the sheep, present invaginations into the granulations forming crypts. These crypts join with tubules in the core of the granulation. Both crypts and tubules are lined by endothelial cells. Spaces lined completely by flattened endothelial-like cells have been observed in the granulations of the dog and in the villi of the rabbit, but whether they are comparable to the tubules of man and sheep is uncertain.

Another set of channels lined by endothelial cells were also present in the arachnoid granulations of sheep. These had smaller diameters than the tubules and considered to be capillaries. In order to differentiate between capillaries and tubules, India ink was injected intravascularly in one set of experiments and into the subarachnoid space in another set. Intravascular injections blackened the capillaries but not the tubules. Conversely, subarachnoid/

subarachnoid injection blackened the tubules but not the capillaries. The two systems were found to be separate, and there was no evidence of communication between capillaries and the venous sinus.

Two graphic reconstructions were made from the intravascularly injected granulations of sheep. The tubules were seen to form a network in the centre of the granulation. From this network separate tubules arose which seemed to communicate with similar tubules in the subarachnoid tissue. The patency of the tubules and the existence of a communication between the subarachnoid space and the venous sinus was demonstrated by the fact that following the injection of India ink and thorium dioxide particles into the subarachnoid space of sheep, these substances have been found both in the tubules and in the venous sinus, with no evidence of rupture of the tubules or surface epithelium of the granulation.

The core of the granulation in sheep is formed of a meshwork of collagen bundles, between which are spaces lined incompletely by mesothelial cells. Morphologically mesothelial cells are similar to endothelial cells, and as a result, the spaces between the collagen bundles have an appearance akin to that of tubules, but are always capable of being differentiated from them by the incomplete lining. The cores of arachnoid granulations and villi of man and the other mammals surveyed, have a similar structure to those of sheep.

Scattered in the meshwork of collagen, a variety of cells such as lymphocytes, polymorphs, red blood cells, macrophages and an unclassified cell are found.

Two types of macrophages have been identified in electron micrographs of the arachnoid granulations of sheep. One is a normal inhabitant (type 1) while the/

the other is identified in the granulation, only after subarachnoid injection of thorium dioxide.

In electron micrographs of sheep arachnoid granulations, an unclassified cell is observed, containing electron dense polygonal bodies. The nature of the cell is obscure.

There are nerve fibres in the arachnoid granulations of man and sheep. In sheep, the presence of medullated and non-medullated nerve fibres are observed both by light and electron microscopy, though specialized nerve endings are not evident. The function of these nerve fibres is unknown.

Half an hour after injection of thorium into the subarachnoid space of live sheep, particles of thorium are found adhering to the plasma membrane of the surface epithelial cells towards the venous sinus, in the tubules, in the crypts, in the spaces between collagen bundles, in inter-cellular spaces, in macrophages and in the arachnoid epithelial cells. No particles were observed in the endothelial cells lining the tubules or of the surface epithelium. These results suggest that the tubules are normally open, the absence of particles in the endothelial cells of tubules and surface epithelium is probably due to the fact that the particles were washed away quickly by cerebro-spinal fluid or blood, so that uptake could not occur.

In the 4 hour specimen after injection of thorium, no particles were observed on the surface epithelial cells, in the tubules and crypts suggesting that a number of particles in cerebro-spinal fluid had probably fallen due to phagocytosis by macrophages, flow through tubules and dilution by new formation of cerebro-spinal fluid. This further suggests that the tubules were open and most of the particles washed away./

away. Few particles were observed in the core of the granulation but there were many particles interspersed in the basement membrane of the surface epithelial cells of the granulation, suggesting a slow filtration of cerebro-spinal fluid. The ratio of type 1 to type 2 macrophages was about 10:1, with increase in the number of particles in the vesicles of type 1 macrophages, as well as in the arachnoid epithelial cells.

The hydrostatic pressure of cerebro-spinal fluid is higher than that of the sinus venous pressure. The structural details of the granulations are commensurate with the following functional analysis:- The filling of the core of the granulation by cerebro-spinal fluid causes the granulation to inflate, and the pressure difference causes flow of cerebro-spinal fluid through the tubules, into the venous sinus. When the venous pressure increases, the fluid within the granulation is forced back into the subarachnoid space, with collapse of the granulation and closure of the tubules. This prevents sinus blood from flowing into the subarachnoid space. The granulations, thus act as physiological valves.

The situation of granulations and villi at the highest points of the neuraxes of animals is also significant. This is not entirely due to the fact that at these sites the venous pressure is at its lowest pressure, for the cerebro-spinal fluid pressure is also at its lowest value, so that there is no mechanical advantage for arachnoid granulations and villi to be situated at these highest points. However, there is evidence that there is a build up of cerebro-spinal fluid pressure at the vertex of the cerebral hemispheres, due to an upward propelling movement of cerebro-spinal fluid caused by the systole of/

of cerebral arteries.

There is also a possibility that the sizes of granulations may be related to either the posture or size of the animal. The adult human possesses granulations while neonatal humans have villi. It may be significant that granulations appear in children at a time when the child is able to walk.

On the basis of an open communication between the subarachnoid space and venous sinus, the results of previous workers, together with the findings in the present investigation, suggest that the passage of cerebro-spinal fluid through arachnoid granulations and villi does seem to occur by a direct communication through the tubules, as well as by filtration.

The experiments of Welch and Friedman (1960) also suggest that arachnoid granulations and villi could act as safety valves, permitting unidirectional flow of fluid, when the cerebro-spinal fluid pressure is raised above normal. On this premise, it is probable that the fluid that leaks out of the granulations and villi unrelated to venous channels, for example over the cerebral cortex and around the optic nerves of man, could pass into the subdural space and drain into the orbital tissues via the perineural lymphatics (Field and Brierley, 1949).

Due to the fact that some granulations are attached to the dura on one side of the opening of a cerebral vein into the venous sinus, it is possible that when the venous pressure increases the granulations collapse as suggested by Welch and Friedman (1959 and 1960), and act as a flap valve in preventing regurgitation of blood into cerebral veins.

The tubules of the granulation are morphologically similar to lymph vessels, functionally

tubules/

tubules convey cerebro-spinal fluid to blood while lymph vessels carry lymph to blood. These considerations and Russell's contention in 1950, that the arachnoid granulations, villi and subarachnoid tissue form a component of the reticulo-endothelial system support the view of Sweet and Locksley (1953) that granulations, villi and the subarachnoid tissue are "analogous to the lymphatics of the general circulation" a concept similar to that of Willis, Pacchioni and Fantoni, three pioneers in the study of the structure and function of arachnoid granulations.

CONCLUSIONS.

1. Arachnoid granulations are found in adult human, in sheep and in the dog. Arachnoid villi are present in the human neonatus, in the cat, in the rabbit and in the guinea pig. Cell aggregations of the arachnoid membrane resembling villi are also found in the spinal region of sheep and rats.
2. Usually arachnoid granulations and villi project into a venous sinus and the surface epithelium of these structures is formed of endothelial cells lining the wall of the sinus. The surface epithelium of the granulations and villi that project into the subdural space is made up of arachnoid epithelial cells.
3. Arachnoid granulations and villi are herniations of the arachnoid membrane and subarachnoid tissue, either into venous sinuses or into the subdural space, and they appear to arise by proliferation of the arachnoid cells.
4. The cores of granulations and villi communicate with the subarachnoid space, through the narrow necks of the granulations and villi where they pierce the dura.
5. Two sets of channels lined by endothelium are present in the arachnoid granulations of man and sheep. One set belongs to capillaries which are derived from dural blood vessels. The other set belong to the tubules that form a communication between the subarachnoid space and venous sinus.
6. The core of a granulation is formed of collagen bundles between which are spaces, lined incompletely by mesothelial cells. These are not artifact spaces and in life contain cerebrospinal fluid. The spaces within the granulation communicate with one another and with similar spaces of the subarachnoid tissue.

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7. Two types of macrophages are found in sheep arachnoid granulations. One is a normal inhabitant (type 1) while the other appears in response to injection of foreign material into the subarachnoid space. This is the type 2 macrophage. The number of type 2 macrophages diminish once the scavenging is done.
8. Both medullated and non-medullated nerve fibres are present in the arachnoid granulations of sheep. Nerve fibres are also present in the human granulation.
9. The granulations act as physiological valves permitting 'transport' of cerebro-spinal fluid from the subarachnoid space to the venous sinus. Under normal conditions, they allow unidirectional flow of fluid through the tubules into the venous sinus and prevent blood from entering the subarachnoid space when the venous pressure is raised above that of cerebro-spinal fluid. The passage of cerebro-spinal fluid from the subarachnoid space to blood seems to occur both by a direct communication through the tubules as well as by filtration.
10. Granulations and villi are situated at the highest points of the neuraxes of animals. There seems to be some evidence of a build up of cerebro-spinal fluid pressure at these sites, which would favour the flow of cerebro-spinal fluid to the venous blood.
11. Some granulations may act as venous valves.
12. Arachnoid granulations, villi and subarachnoid tissue are analogous to lymphatics of the general circulation.

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ABBREVIATIONS.

A	arachnoid membrane
AC	arachnoid cell
AG	arachnoid granulation
AV	arachnoid villus
B	cerebral cortex
Cr	crypt
CV	cerebral vein
D	dura mater
E	epithelial cell cap
EC	endothelial cell
ER	endoplasmic reticulum
G	Golgi apparatus
M	mitochondria
N	nucleus
Nb	nerve bundle
No	nucleolus
Nr	nerve root
Ps	psammoma body
SAS	subarachnoid space
SDS	subdural space
SLS	superior longitudinal sinus
T	tubule
V	large vesicle
bm	basement membrane
bv	blood vessel
cap	capillary
cb	collagen bundle
cn	cistern
f	fibril
fm	fibrillar material in matrix of E.D.B.
o.Cr	opening of crypt
o.CV	opening of cerebral vein
sbc	spaces between collagen bundles
t	terminal bar
to	thorium
v	small vesicle

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