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MORPHOLOGIC STUDIES OF THE CAROTID RETE-CAVERNOUS SINUS COMPLEX AND ITS FUNCTIONAL SIGNIFICANCE IN SHEEP AND GOAT

Iowa State University

Ph.D. 1983

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Morphologic studies of the carotid rete-cavernous sinus complex and its functional significance in sheep and goat

by

Wael Abdul Hameed Khamas

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Veterinary Anatomy

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Iowa State University
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1983
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DEDICATION

This work is dedicated to all the special people in my life:

To my brothers and sisters for their continuous support, especially my elder brother Essam A. H. Khamas who encouraged me to continue in the School of Veterinary Medicine.

To my wife, Afaf M. Khadim, and children, Ashraf and Halla.

And finally, to my best friend, Mehdi S. Ahmed.
ABSTRACT

The carotid rete-cavernous sinus complex of sheep and goat (adult and feti) was investigated. Intra-vascular injection materials (Latex, Batson's #17 and Decopour) were employed to describe the blood vessels of this structure. Further, paraffin embedded sections were stained with Harris' hematoxylin and eosin, Van Gieson's, Gomori one-step trichrome and Weigert. After maceration, some injected vessels were used to study the carotid rete-cavernous sinus complex with scanning electron microscope (SEM). In addition, aldehyde fixed, cryofractured complexes were studied with the SEM. The complex was sectioned with the cryostat and several laboratory procedures were performed to detect the presence of alkaline phosphatase (A.P.), 5'-nucleotidase (5'-N) and adenosine triphosphatase (ATP-ase) enzymes.

Gross study revealed that the carotid rete was supplied with blood by one caudal rete branch, and two to four rostral rete branches which were normally found. The extracranial portion of the internal carotid artery was postnataally absent in both sheep and goat specimens. Light microscopic examination showed that the carotid rete branches were medium-sized, muscular arteries. These arterial branches shared a common tunica adventitia with the cavernous sinus. The carotid body cells were observed in an aberrant position (intracranially in the wall of the internal carotid artery) due to regression of the extracranial portion of the internal carotid artery after birth. The cavernous sinus, on the other hand, showed variations in thickness of the wall from region to region. In
some areas, the cavernous sinus had smooth muscle cells in the tunica media while in other areas only connective tissue and endothelial cells were noticed.

The carotid rete-cavernous sinus complex of sheep was easily filled with Batson's #17 and Decopour as seen by SEM. Craters were observed in the endothelial lining of the carotid rete branches but not of the cavernous sinus.

Finally, a minute amount of A.P. enzyme was demonstrated in the walls of the carotid rete-cavernous sinus complex. But, 5^-N was found in large quantities in the endothelial cells and the tunica adventitia of both the branches of the carotid rete and the cavernous sinus. It was, however, absent in the tunica media of the carotid rete branches. Varying quantities of ATP-ase was demonstrated in all layers of carotid rete branches and the cavernous sinus. The amount of this enzyme appeared larger towards the lumen and gradually decreased towards the peripheries in the arterial branches as well as the cavernous sinus.
INTRODUCTION

The carotid rete (Rete mirabile epidurale rostrale) (ICVAN, 1973) of sheep and goat is a complex structure resulting from the anastomosis of several medium-sized, muscular arteries which originate from the maxillary artery. It has several anastomoses with the adjacent blood vessels and lies intracranially within the cavernous sinus (Sinus cavernosus) at the base of the brain. Balankura (1954) states that the parahypophyseal portion of the internal carotid artery begins to break up into smaller vessels in the sheep embryo (30 mm long). Simultaneously, the maxillary artery sends branches through the oval foramen and the foramen orbitorotundum into the cranial cavity resulting in anastomoses between these branches and those of the internal carotid artery. The resulting structure out of such anastomoses is the carotid rete. The extracranial portion of the internal carotid artery gradually decreases in size once the anastomoses are established. Finally, the latter segment of the internal carotid artery either degenerates completely or remains as a fibrous cord (Balankura, 1954).

In the absence of the caudal epidurale rete mirabile (ICVAN, 1973) in both sheep and goat (Anderson and Jewell, 1956; Baldwin, 1964; Daniel, et al., 1953; Getty, 1975; McGrath, 1977) the term carotid rete will be used throughout the text instead of the rete mirabile epidurale rostrale. Baldwin (1964) states that the internal carotid artery in sheep disappears at about nine months of age. Further, Baldwin (1964) and Godynicki and Frackowiak (1979) mention that the blood to the carotid rete of ruminants is almost entirely derived from the maxillary artery via
the arteria anastomotica and the ramus anastomoticus (corresponding to the cranial and caudal rete branches, respectively of other authors). Moreover, Daniel et al. (1953) describe a well-developed carotid rete in the cat, sheep, goat, ox, pig and a rudimentary form in the dog, while in the rat and rabbit the rete is absent. The carotid rete is situated intracranially in all species mentioned above, except in the cat where it lies extracranially. In both instances, i.e., whether it is intra- or extra-cranially situated, the carotid rete lies within the cavernous sinus. Uehara et al. (1978) report that the blood supply to the carotid rete of the calf is from four different sources viz., the maxillary artery via the proximal and distal rete branches (corresponding to cranial and caudal rete branches, respectively), the basi-occipital arterial plexus, and from the internal carotid artery, which is poorly developed. They describe the presence of a V-shaped extension of the rete as a characteristic of the calf. Furthermore, Gillilan (1976) states that the proximal (extra-cranial) part of the internal carotid artery is small in the dog and non-functional in the cat. The maximum blood supply to the brains of these species comes over an anastomotic ramus leading to the formation of what he called the internal rete mirabile. Also, he reports that the proximal portion of the internal carotid artery is atrophied and nonfunctional in adult cats.

The nasal veins are connected to the cavernous sinus via the angularis oculi, dorsal ophthalmic veins and the ophthalmic plexus (Baker and Hayward, 1968b; Godynicki and Frackowiak, 1979; Khamas and Ghoshal, 1982a; Magilton and Swift, 1976). The blood in the above venous pathway flows in
either direction, i.e., towards the cavernous sinus against the gravity or towards the external jugular via the facial vein. Against the gravity, the blood flow is facilitated by the absence of valves in the angularis oculi vein and the presence of a thick tunica media in its wall as described by Magilton and Swift (1969) in the dog and Khamas and Ghoshal (1982b) in sheep. The smooth muscles in the tunica media of the angularis oculi vein is considered to be under autonomic nervous control.

As the brain operates at a higher rate of metabolism in response to cognitive activity, it must be supplied with enough blood to bring oxygen and carry away waste products. At the same time, it produces a large amount of heat and the removal of this heat is a function of the flow rate and the temperature of the blood. Serota and Gerard (1988) and Baker (1979) suggested that the blood supply to the brain must be cooler than the brain tissue to enable it to remove some of the heat. This has been shown to be true in several mammalian species (Baker and Hayward, 1967; Baker et al., 1974; Hayward et al., 1966; Krabill, 1979). In addition, two countercurrent heat exchange systems are described in the dog (Magilton and Swift, 1968). The external countercurrent heat exchange system is between the ambient air and the alar fold venous lakes, while the internal one operates between the cavernous sinus, which contains relatively cooled venous blood from the facial and nasal areas, and the carotid rete carrying core-temperature blood. Scott (1954) states that the main blood supply to the cavernous sinus in the calf is the nasal venous blood which plays a role in selectively controlling brain temperature. By implantation of thermistors in the carotid rete arteries and the
cavernous sinus, Baker and Chapman (1977) observed fluctuation of temperature between the relaxed and the active state of the dog. Regardless of the physical state, the cavernous sinus blood is always cooler than the arterial blood entering the rete, which facilitates countercurrent heat exchange. Moreover, Baker and Hayward (1968c) demonstrate that the temperature in the cavernous sinus of the sheep drops markedly, while central arterial blood temperature falls only slightly after blowing cool air over the nasal and facial areas.

Different authors suggest many functions to the carotid rete-cavernous sinus complex viz., 1. the maintenance of adequate arterial blood flow in the event of interruption of flow in one of the carotid arteries ensured by extensive collateral anastomoses within the rete (Daniel et al., 1953; Edelman et al., 1972); 2. to protect the brain from unordinarily high perfusion pressures while allowing the augmentation of cerebral blood flow in physiological circumstances (Edelman et al., 1972); 3. to regulate blood flow to the brain under normal circumstances (Ask Upmark, 1935; Daniel et al., 1953); 4. to act as a capacitor which lowers high systolic pressures with a minimum effect on mean perfusion pressure, an ideal situation to the brain which is very sensitive to edema formation but requires relatively high blood flow for proper function (Edelman et al., 1972); 5. to assist in venous return from the cranium (Barnett and Marsen, 1961); 6. to regulate mechanically the cerebral circulation by diminishing the arterial pulsation (De Boissezon, 1941); and 7. finally, to act as a site for internal countercurrent heat exchange system to cool the blood destined for the brain and
to selectively regulate brain temperature. This function is found to be true in the dog (Baker and Chapman, 1977; Magilton and Swift, 1967, 1968, 1969), in the cat (Baker and Hayward, 1967) and in the sheep (Baker and Hayward, 1968a, b, c, d; Krabill, 1979; Young et al., 1976).

In the lamb, the luminal diameter of the carotid rete branches is variable, in contrast to capillaries, as in the former, the diameters are too large with a well-developed wall (De Boissezon, 1941). The periphery of these arteries in sheep and goat is covered by simple squamous epithelium which forms the lining endothelium of the cavernous sinus (Baldwin, 1964; De Boissezon, 1941; Godynicki et al., 1981). The carotid rete branches in sheep and goats have been described as medium-sized, muscular arteries (Anderson and Jewell, 1956; Baldwin, 1964; Daniel et al., 1953; McGrath, 1977). On the other hand, Edelman et al. (1972) found in acrylic polymer casts that the overall sectional area of the carotid rete branches in the goat is far greater than the cross sectional area of either its afferent or efferent vessels. In addition, Uehara et al. (1978), on the basis of gross and light microscopic observations, mention that the basilar venous plexus of sheep, goat and pig is either absent or does not show a distinct structure as in the calf. They conclude that the carotid rete in the above mentioned species and the calf is dependent on the form of the basilar venous plexus.

The extracranial arteries in rhesus monkeys, rats and hens, using light, scanning and electron microscopy, Dahl (1976) describes a well-developed, three layers or tunics in the wall of these vessels.
Godynicki et al. (1981) report that the cavernous sinus consists of large endothelial cells and an incomplete subendothelial connective tissue layer in sheep, and one or more layers of smooth muscle cells intervening between the endothelium and the internal elastic lamina of the carotid branches.

It has been found that following birth the mammalian arteries are composed of three discrete layers (viz., intima, media and adventitia). Striking changes are seen in the intima because of progressive decreases in the arterial lumen with advancing age. Besides, Stehbens (1960) describes the intimal thickenings or pads, as normal occurrences in all arteries of all sizes, having internal elastic laminae in human fetsi and infants, while Dahl (1976) concludes that the intimal thickening and cushion pads may represent a developmental defect. Nanda and Getty (1972) report the presence of intimal cushions in the cerebral arteries at their branching sites in the dog and pig of different age groups. Using tubes lined with silicon puffy, which is deformable with pressure changes, Rodbard (1956) inferred that the proliferation of the tunica intima is due to the changes in the pressure and fluid flow. These changes in fluid pressure resulted in intimal thickenings or pads in addition to other changes.

In sheep and steers, Stehbens (1965) describes the presence of fenestrated internal elastic lamina and intimal proliferations forming thickenings or pads. The latter consist predominantly of smooth muscle cells, elastic tissue and collagen fibers. The presence of lipid deposition in sheep, especially near the zone of the intimal thickenings has been described. Less amount of lipid has been reported in the steer
than in sheep vessels. The presence of the pads is either physiological or pathological as a site of early atherosclerosis in animals. Fourman and Moffat (1961) describe the presence of the intimal cushions in the rat and suggested a function of cell skimming because of their situation at the branching site of the vessels.

The presence of valves in the cerebral arteries of some mammals is described by several investigators and different functions are suggested. Shanklin and Azzam (1963) notice that the valves are arranged in a way to impede the movement of the blood, not like the valves in the veins to prevent backward movements. In addition, Rosen (1967) reports the presence of valves in cerebral arteries in the rat and describes their origin as entirely from the tunica intima possessing a complete endothelial lining. He concludes that by mechanical impedance the valves act to adjust the blood flow in small arteries, and in larger arteries they act in conjunction with wall contraction.

The presence of biogenic amines and other neurotransmitters in the cerebral arteries has been extensively studied in different species. In cat, dog and rat, Matakas et al. (1976) demonstrate the presence of adrenergic nerve fibers in small arteries and arterioles of the arachnoid, cerebral cortex and the brain stem. However, Bevan (1979) states that the smooth musculature of the cerebral blood vessels in rabbits is relatively insensitive to sympathomimetic drugs compared with that of systemic vessels. He suggests that the differences in their responses are due to the differences in embryonic origin of the intracranial and extracranial vessels.
Finally, Gillilan and Markesbery (1963) demonstrate by light microscopy the presence of major arteriovenous anastomoses in the cat, rat, guinea pig, rabbit, dog and to a lesser extent in the monkey. Such anastomoses are reported to be absent in the pig, calf, sheep and horse carotid rete-cavernous sinus complex (Gillilan, 1974).

The scanning electron microscopic (SEM) study of the blood vessels was possible after the development of some intravascular casts and after modification of those plastic materials already in use for gross anatomical investigations. This technique has been employed for microvascular studies, when the transmission electron microscopy (TEM) was already in use. According to Nopanitaya et al. (1979), for producing reliable corrosion casts of the microvascular system for SEM one is likely to experience two problems. They are: 1. using a too-high viscosity medium not readily permeable through the entire microvascular system and 2. using a too-soft cast not withstanding an electron beam bombardment.

Batson's #17 is diluted in Sevriton (a monomeric methyl methacrylate used as a binding agent in dental work) to produce an improved mixture for corrosion cast studies of microvascular systems of various organs. For instance, Nopanitaya et al. (1979) used this mixture to study the gastrointestinal microvascular system of rabbits, albino rats and albino mice as it yielded lower viscosity and provided exceptional details as well as reproducible casts. For studying the intrahepatic microcirculation of albino mice and albino rats, Nopanitaya et al. (1978) used chilled Batson's #17 to delay its polymerization until fine capillaries
were filled. Further, Nopanitaya (1980) injected a mixture containing three parts of Batson's #17 and one part of Sevriton to study the glomerular microcirculation. Kardon and Kessel (1979) obtained a mixture with lower working viscosity by increasing the ratio of catalyst to monomer base of Batson's methyl methacrylate.

In the common carotid artery of the rabbit, Kawamura et al. (1974) described the presence of numerous crater-like structures as well as out-pocketing or balloons after the occlusion of the right carotid artery for five minutes. By using new perfusion and fixation methods, Edanaga (1974) observes microvillous projections, crater-like grooves and unknown single cilium of the endothelial cells of the aorta of the rabbit.

Moffat (1959 and 1969) describes three types of cushions in the wall of the blood vessels: 1. intimal cushions protruding into the lumen of the vessel; 2. paired leaflets with a valve-like appearance; and 3. polypoid cushions which have a single flap-like intraluminal projection.

Takayanagi et al. (1972) in the intracranial arteries of the cat find typical cushions covered by endothelial cells and internal elastic laminae without any fenestrations in the endothelial cells.

Changes in vessels of different age groups are described in human intracranial arteries (Flora et al., 1967). Movat and Fernando (1963) state that the terminal vascular bed of the rabbit omentum, mesentery and dermis has interruption or fenestra in its internal elastic membrane. Further, Pease and Paule (1960) mention that the basement membrane of the endothelial layer tends to break up and disappear in larger arteries, while it is a conspicuous feature of small blood vessels. In the cat,
dog and rat the tunica media of the arterioles consists of smooth muscle cells held together by myomyal-tight junctions which are similar to the myoendothelial-tight junctions; whereas, the arteries have a complete internal elastic laminae which are either missing or incomplete in arterioles (Roggendorf et al., 1976).

The availability as well as diversity of enzymes in the animal body has drawn attention from a number of researchers. Though the difficulty of obtaining a sample from the animal and preserving it to save all or most of the enzymic activity have been the problem until now. In addition, movements of enzymes from their actual sites after death, either by absorption, degradation or other means, have been reported in the literature. Lansing (1959) states that the effect of age on the overall metabolic activities and the concentration of specific enzymes of arterial tissue closely resemble the pattern of changes occurring in other tissues.

To avoid some of the above mentioned problems, different techniques have been developed for detection of specific enzymes. Here then, emphasis is on the detection of the phosphatase enzymes. Gomori (1939) reports the presence of large amounts of these enzymes in the adventitia of medium-sized arteries and in the endothelium of capillaries of renal cortex in dogs.

In the dog, the adenosine triphosphatase (ATP-ase) activity of the vascular system greatly varies. The venous system, however, is devoid of the enzymic activity. The aorta has greater ATP-ase activity than the coronary, carotid and renal arteries, which seemingly share equal amounts (Carr et al., 1952). Further, Balo et al. (1948) report
the presence of soluble ATP-ase in the aortic tissue which is different from the ATP-ase of the myosin of the muscle. On the other hand, Eisenberg and Suddith (1979), by autoradiography, report that the activities of the sodium-potassium (Na^+–K^+)‐dependent ATP-ase are similar in microvessels of the brain and the choroid plexus of rats.

The presence of the ouabain sensitive potassium‐dependent phosphatase in the renal tubules of the rat is linked to the brush border where active transport of sodium ions takes place (Firth, 1974). Guth and Albers (1974) state that (Na^+–K^+)‐activated ATP-ase activity in the central nervous system and kidney of rats and guinea pigs is associated with the active transport system. Ernst (1972) using transmission electron microscopy finds ouabain‐sensitive, K^+‐dependent phosphatase activity in the secretory epithelium of the avian salt gland. He states that the distribution of reaction product is restricted exclusively to the plasma membrane which forms the extended basal and lateral compartments of the secretory cells. The significance of the ATP-ase activity is considered in relation to possible movements of membranes and to active transport in kidneys of several mammalian species, including man (Spater et al., 1958).

In albino rats, the ATP-ase enzyme is localized in basement membranes of the brain capillaries, while alkaline phosphatase (A.P.) is distributed uniformly in the cytoplasm of the endothelial cells (Jancso et al., 1975). Gomori (1941) finds that acetone is a good preservative of both alkaline and acid phosphatase enzymes. In general, he states that ATP is present chiefly in the walls of capillaries
although some may be found in the glandular epithelium.

The enzyme 5'-nucleotidase catalyzes the dephosphorylation of mono­nucleotides. It is widely distributed in mammalian tissues but is generally absent from rapidly growing tissues. The presence of this enzyme is correlated with functional competence, which resulted in the hypothesis that the enzyme has a catabolic function in the cell (Hardenk and Kondstaaal, 1968). In addition, Goldberg (1973) states that the 5'-nucleotidase enzyme has a role of degrading residual nucleo­tides. The enzyme has been found to have moderate activity in the vascu­lar wall of portal vessels and central veins in the goat liver (Nanda et al., 1979). Further, Novikoff (1958) states that the functions of this enzyme are similar to those reported for the ATP-ase in active trans­port of metabolites across the plasma membrane.

The purposes of this investigation are to accomplish the following:

1. To describe grossly the carotid rete-cavernous sinus complex of sheep and goat by using different intravascular injection materials;

2. To describe the structure of the above mentioned vascular complex by light microscopy (viz., the tunics, smooth muscle cells, collagen fibers, elastic fibers, arteriovenous anastomoses, valves and intimal thickenings or pads, etc.);

3. To describe the carotid rete-cavernous sinus complex by using intravascular injection techniques (Batson's #17 and Decopour) and freeze-fractured tissue with the aid of scanning electron microscope;

4. To detect the presence or absence of alkaline phosphatase, 5'-nucleotidase, and adenosine triphosphatase enzymes.
5. Finally, to evaluate the current literature on the basis of the present study.
Gross

As early as the nineteenth century conflicting descriptions about the carotid rete of sheep exist in the literature, and the description considerably varies depending on the approach and the technique used to reveal this structure. With the availability of improved as well as intra-vascular injection materials, preparation quality of better carotid rete casts is now possible. This provides a precise description of both afferent vessels contributing to its formation.

The carotid rete has been described to be present in several species (Tandler, 1899) and, according to Daniel et al. (1953), could either be intracranial as in sheep, goat, ox, pig and dog, or extracranial as in the cat. A detailed account of the carotid rete as well as the carotid system in the cat has been presented by Davis and Story (1943). The blood supply to the carotid rete in different species may come from several sources, viz., the maxillary artery (via the rostral and caudal rete branches); branches of the occipital artery or the basioccipital plexus; the internal carotid artery; and the ascending pharyngeal artery (Daniel et al., 1953; Godynicki and Frackowiak, 1979; and Uehara et al., 1978). According to Daniel et al. (1953), the maxillary artery is the main vessel contributing to the carotid rete in sheep, goat, ox and cat, and the ascending pharyngeal artery in the pig. In the giraffe, the caudal rete branch may come from either the maxillary or the external ophthalmic artery. The caudal carotid rete of the giraffe is supplied by branches of the condylar and vertebral arteries and it is connected with
the corresponding rostral rete (Godynicki and Frackowiak, 1979). Following bilateral ligation of the carotid and vertebral arteries in the dog, Whisnant et al. (1956) observe collateral circulation between branches of the costocervical and superficial cervical arteries and the muscular branches of the vertebral artery beyond the ligated points. But no anastomotic channels were seen in the carotid system.

In the absence of anatomical connection with either the internal or external carotid artery in the cat, Gillilan and Markesbery (1963) suggest rete mirabile conjugation or geminum for the plexus of arteries and veins in that region instead of the carotid rete of other species. Further, Gillilan (1976) mentions that the proximal part (extracranial portion) of the internal carotid artery is small in the dog and non-functional in the cat. The main blood supply to the brain of these animals is from the anastomotic ramus from the extracranial rete. In addition, the brain is supplied by the first cervical branches of the vertebral arteries in the cat and the occipital in the dog. The external rete mirabile is supplied by a number of small rami from the maxillary artery which gives in turn an anastomotic branch to join the intracranial rete (Gillilan, 1976).

The two retia (i.e., left and right) are connected by several anastomotic branches and their numbers vary between species. In the goat, Daniel et al. (1953) state that the vessels connecting the retia of both side, in the caudal part of the pituitary fossa, are more numerous and plexiform in comparison to those of sheep. In the pig, McGrath (1977) reports that the anastomosis between the retia is very
extensive giving them the appearance of one single structure. In the

dog, intercarotid anastomoses between two internal carotid arteries are
grossly found in all cases dissected, but they are not seen in angio-
graphs of same specimens (De La Torre et al., 1959). These anastomoses
usually represent several small branches crossing the midline at the
caudal part of the sella turcica, thus resulting in the formation of the
posterior hypophyseal vessel as described by Jewell and Verney (1957).

They believe that the maxillo-carotid anastomotic artery (ramus anasto-
moticus) is embryologically a branch of the internal carotid artery and
develops from the primitive maxillary artery (De La Torre et al., 1959;
Jewell, 1952). Further, De La Torre et al. demonstrate the presence of
anastomoses between the internal carotid artery and the ascending
pharyngeal artery in one dissected specimen but not in the angiographs.

Intracranially or extracranially situated rete is enmeshed by a
venous plexus or lake (Daniel et al., 1953; Gillilan, 1974). The extra-
cranial carotid rete, which is present only in the cat, is surrounded by
the pterygoid plexus, while the intracranial rete is surrounded by the
cavernous sinus. Man, rhesus monkey, pig and cat have internal jugular
veins, while the dog, horse, rabbit, sheep and ox have dorsal (superior)
cephalic veins leaving the cranial cavity through the temporal foramen
as the major cerebral venous outflow. The internal jugular vein in the
latter group arises from the pharyngeal, thyroid and esophageal veins
and has infrequent and very small or no drainage of cerebral blood from
the cerebral sinuses (Ngedus and Shackelford, 1965). Further, in sheep
the superficial temporal vein carrying the extracranial blood joins the
large dorsal cerebral vein within a short bony canal before the latter
vein leaves the skull. Moreover, Uehara et al. (1978) state that the
basilar venous plexus of sheep, goat and pig is either absent or does
not show a well-developed structural wall as in the calf. They con­
clude that the development of the carotid rete in these species are
dependent on the form of the basilar system of the dural sinuses.

The carotid rete is intercalated in the course of the internal
carotid artery and it is invaginated into the cavernous sinus of the
dura mater (Godynicki et al., 1981). Uehara et al. (1978) and
Godynicki and Frackowiak (1979) describe the anatomical differences in
the venous system of ruminants. For instance, in the calf there are
rostral and caudal intercavernous sinuses which resulted in the formation
of the circular sinus, while in sheep and goat only the caudal inter­
cavernous sinus is present. Scott (1954) mentions that the main source
of blood supply to the cavernous sinus in the calf is the nasal venous
blood which carries relatively cool blood and selectively regulates the
brain temperature. Moreover, by injection of latex in dead sheep via one
of the nasal veins, Baker and Hayward (1968c) demonstrated connection
between the nasal veins and the cavernous sinus of the same side. By
venography, Khamas and Ghoshal (1982a) have shown the existence of the
above venous connection in live sheep (via the dorsal nasal vein →
angularis oculi vein → dorsal external ophthalmic vein + ophthalmic plexus
→ emissary vein of the foramen orbitotundum + cavernous sinus). In
addition, Schmidt-Nielsen et al. (1970) demonstrate that the stressed dog
inspires air through the nose and exhales it through the mouth which they
consider as an important mechanism for regulating the amount of heat dissipated in panting. In the dog, the body and the head may be thermally viewed as separate heat reservoirs which are vascularly connected. The anatomic proximity of the cerebral arterial circle and the extracranial cerebral vasculature to the naso-oral cavity provides in situ heat exchanger (Brown et al., 1964). Jessen and Pongratz (1979) state that the function of the carotid rete as heat exchanger in the goat is restricted only to heat stressed animals when the temperatures of both hypothalamus and respiratory surfaces are coupled, while in cold stress the hypothalamic temperature is uncoupled from the temperature of the respiratory surfaces and in this case presented as undistorted body core temperature.

Light Microscopy (LM)

Investigators have given attention to the blood vascular system because of its importance in regulation of several mechanisms in the body. The description of the blood vessel wall and the classification of vessels vary considerably among researchers. Structural classification of vessels is based upon whether smooth muscle cells or elastic fibers predominate the tunica media and accordingly called muscular or elastic arteries, respectively. Others classify the blood vessels depending on their sizes or luminal diameters into large, medium, and small vessels. Still some vessels do not fit in the above two classifications and a large number of vessels is intermediate between two or more categories.

Generally, the arteries are composed of the following layers or tunics: the intima, media and adventitia. The tunica intima is composed
of endothelium, which is simple squamous epithelium, the internal elastic lamina, and the subendothelial connective tissue which differs among vessels as well as between species. The tunica media of large arteries consists of either smooth muscle cells or elastic fibers. Sometimes this tunic is surrounded by an external elastic lamina which separates the tunica media from the tunica adventitia. The peripheral layer is the tunica adventitia, which consists mainly of collagen and some elastic fibers.

In sheep, Godynicki et al. (1981) state that the tunica media of carotid rete branches has up to seven layers of smooth muscle cells. In addition, they describe the presence of one or more layers of smooth muscle cells between the endothelium and the internal elastic lamina. The internal elastic lamina is fenestrated. They further add, that the cavernous sinus consists of large endothelial connective tissue layer. They conclude that the surrounding fluid developmentally may influence the structure of the vessel wall. On the other hand, Stehbens (1960) describes the cerebral arteries of human feti and infants to have an intima consisting of the endothelial layer and internal elastic lamina with no intermediate layer. Further, he mentions that intimal thickenings are found at the branching sites of the arterial tree in which the pads consist primarily of smooth muscle fibers with very fine elastic fibrils, and longitudinally arranged smooth muscle cells which are less dense than the tunica media.

Piffer et al. (1980) state that the cervical segment of the human internal carotid artery has clearly defined external and internal elastic
lamina, while in the petrosal (intracranial) portion only the internal elastic lamina is present which is sometimes double. Moreover, Bevan (1979) finds that the smooth muscle cells of cerebral blood vessels in rabbits are insensitive to sympathomimetic stimulation compared with those of the systemic vessels, and the sensitivity of the smooth muscles in both vertebral and internal carotid arteries decreases abruptly as they approach the brain.

Pease and Paule (1960) using transmission electron microscopy state that the tunica media of the thoracic aorta of the rat lacks innervation and conclude that its functional activity must depend upon intrinsic activation rather than upon nervous stimulation. On the other hand, Owman et al. (1974) find that both adrenergic and cholinergic nerve terminals in the adventitia reaching the superficial layer of the tunica media in the extra- and intracranial arteries, but they never penetrate between the muscle cells of the tunica media. They concluded that penetration of nerves into the tunica media is not a prerequisite for a vasomotor innervation and that smaller extra- and intracranial arterioles are supplied with autonomic fibers.

Peerless and Kendall (1976) using fluorescence microscopy show that in the rat, rabbit and man adrenergic innervation is very dense in the superficial cerebral arteries. All smaller arteries and arterioles arising from the circle of Willis carry one or more adrenergic fibers; most densely innervated vessels supplying the anterior hypothalamus. Also, autoregulation as well as neuronal effect has been described in
conjunction with the cerebral circulation. Neurotransmitters of different kinds are being implicated in one area or more of the cerebral blood vessels. In cerebral arteries of the cow, rat and rabbit, Head et al. (1980) state that norepinephrine is present, and in the rabbit it is greater than that of the rat, which, in turn, is greater than that of the cow. Moreover, Edvinsson and Mackenzie (1977), Edvinsson et al. (1977), and Jarrott et al. (1979) report the presence of biogenic amine in one form or another in selected cerebral blood vessels. Edelman et al. (1972) find that the carotid rete of the goat responds to carbon dioxide and catecholamine in a manner similar to that of the peripheral arteries, but is different from that of the cerebral vessels.

The carotid body is located dorsal to the bifurcation of the common carotid artery, and it is easier to find than the aortic body. It has been the subject of extensive studies in both light and electron microscopy. This structure is thought to be involved in triggering reflexes primarily in respiratory and cardiovascular systems and responds to changes in the concentrations of blood gases.

On the basis of their nuclear shape, two types of cells in the carotid body have been described (Gomez, 1970 and 1980). In addition, Goormaghtigh and Pannier (1939) and Meijling (1938) describe another type cell which lies outside the groups of the glomus cells and they call it interstitial cell. Biscoe (1971) states that the notion of the nerve fibers terminating inside the carotid body cells is found to be false under the transmission electron microscope, while
Eyzaguirre and Uchizono (1961) describe both myelinated and nonmyelinated fibers in the carotid sinus and the sympathetic nerve to the carotid body. In addition, Hollinshead and Sawyer (1945) have found cholinesterase enzyme in the carotid body. Later, several investigators confirmed the presence of the cholinesterase enzyme in type I cells (Becker et al., 1967; Fillenz and Woods, 1966; Koelle, 1950 and 1951; Pryse-Davies et al., 1964; Ross, 1957), while Serafini-Francassini and Frasson (1966) and Biscoe and Silver (1966) think that the cholinesterase enzyme is outside type I cells.

Several changes in the structure of the blood vessels in general, and especially in the cerebral vessels have been reported. These changes include proliferation of the smooth muscle cells in the tunica media and development of intimal thickenings or pads (Dahl, 1976; Elias and Pauly, 1966; Hassler, 1961; Legait, 1947 and 1949; Moffat, 1969; Serban and Ovula, 1961; Stehbens, 1965); presence of valves or valve-like structures (Rosen, 1967); splitting, doubling, or perforation of the internal elastic lamina (Godynicki et al., 1981); and the presence of arteriovenous anastomoses (Gillilan and Markesbery, 1963; Gillilan, 1974). The presence or absence of the above changes has been attributed to their certain physiological or pathological significance. Rodbard (1956) states that the pressure of the blood flow compresses the endothelial cells preventing proliferation of the intimal cells and the underlying connective tissue to form a pad or thickening. Thus, any alteration in blood pressure or flow may result in a change in the structure of the vessel.
Scanning Electron Microscopy (SEM)

In several mammalian species and human, changes in the blood vessel wall have been reported and linked to physiological or pathological events. These changes include intimal thickenings or cushions, intra-arterial bolsters, thickening, splitting or fenestration of the internal elastic lamina, and the like. Physiological changes are described because of a pressure change, blood flow and other metabolic effects in the organ. Age related changes in human intracranial arteries involve the ground substances as well as the aggregation of smooth muscle cells in the tunica intima (Flora et al., 1967). Moreover, in the rat and albino rabbit aorta Björkerud et al. (1972) report the presence of highly differentiated subcellular microvalves with a possible role to regulate the transfer of materials between the blood stream and the subendothelial arterial tissue. They further state that their presence most likely involve altering the permeability of certain serum constituents, which is the cause of therogenesis in aorta.

Various types of intraarterial cushions or thickenings have been described in the literature (Moffat, 1959, 1969; Takayanagi et al., 1972; Khamas and Ghoshal, 1982b). In the presence of an intimal cushion, the internal elastic lamina splits into several lamellae enclosing bundles of smooth muscle cells, and at the margin of the existing lamina they rejoin to form a single lamina. The smooth muscle cells in the cushion are surrounded by basement membrane (Takayanagi et al., 1972). Further, Takayanagi et al. state that the smooth muscle cells of the tunica media beneath the cushion show a thinning out and they are accompanied by a wedge-shape extension of
adventitial elements toward the lumen resulting in what is called medial defect. In addition, myointimal proliferations may result from injury, or nonspecific healing responses to injury, or as a feature of arteries subjected to long periods of perfusion (Fonkalsrud et al., 1976). Other changes, such as folding of the endothelium is correlated with the appearance and disappearance of the arterial blood pressure (Edanaga, 1974). Moreover, fenestrations of the internal elastic lamina are reported to be generally present in the rabbit omentum, mesentery and dermis (Movat and Fernando, 1963).

Myoendothelial-tight junctions have been described by Rhodin (1967) and Moore and Ruska (1957) in the heart, kidney and mesentery, while Dahl (1976) describes these junctions in the blood vessels of the central nervous system. Roggendorf et al. (1976) find that the tight junctions are rare in arteries and more numerous in vessels with smaller diameter similar to those described by Rhodin (1967). They attribute the following functions to these junctions: 1. cell-to-cell transmission of metabolic information; 2. transmission of excitatory impulses from cell to cell; and 3. transmission of excitatory impulses to endothelial cells. In arteries with an internal elastic lamina, the junctions between the endothelial cells are more complex than in small vessels as reported by Movat and Fernando (1963).

With the scanning electron microscope, several investigators observed changes in the wall of the blood vessel, which may be due to fixation or preparation procedures or as a part of the structures of the vessel wall. One of these changes is the crater (which is described as a small single
or multiple roundish depression of the cell surface) which is often associated with bulges or blebs and more diffuse surface swelling of the endothelium. The craters are frequently noticed at the branching site of the coronary and renal arteries of rats and rabbits and in the common carotid artery of the rabbit (Buss and Hollweg, 1977). Kawamura et al. (1974) state that their numbers are significantly increased in the ischemic arterial segment. Further, Shimamoto and Sunaga (1973) describe them in rabbit blood vessels maintained on a high cholesterol diet.

Different plastic injection materials are used to study the blood vessels. Their pliability, viscosity and the tendency to withstand electron beam bombardment are considered during selection (Nopanitaya et al., 1979). Modified Batson's #17 is used after altering the ratio between catalyst and monomer (Kardon and Kessel, 1979), chilled Batson or mixed with other substances such as Sevriton (a monomeric methyl methacrylate) (Nopanitaya, 1980). Most of these modifications result in decreasing the viscosity of the Batson's, facilitating better filling of the small blood vessels.

Clark and Glagov (1976) claim that artefacts, such as changes in the internal elastic lamina and the folds and ridges are reduced or absent in the tunica intima when perfusion is carried at physiological pressure. Further, they point out that in specimens fixed by perfusion at physiological distending pressure, edges of endothelial cells form thin flaps extending to overlap the edges of adjacent "down stream" endothelial cells, i.e., endothelial cells immediately distal with regard to the direction of the blood flow. But, according to Buss and Hollweg (1977),
this can occur at both directions. No overlapping of endothelial cells is obtained, however in the cerebral arteries and veins. Further, they describe linear folds, arranged parallel to the long axis of the vessel, in the tunica intima of the main renal arterial branches and carotid arteries. They are absent in the ascending aorta, in cerebral arteries and in main branches of coronary arteries, but they are irregularly present in the descending aorta.

Histochemistry

Enzymes are proteins or glycoproteins varied in structure and distribution from one region to another in the animal body. Most enzymes have been given a function or two according to their localization, while other enzymes, which do not show consistency in their distribution, cannot readily be assigned a function.

Alkaline phosphatase (A.P.) enzyme as found in fresh tissue has the chemical characteristics of a glycoprotein with a molecular weight of 100,000-200,000 (Moss, 1974). Based on kinetics, response to inhibitors, stability and electrophoretic motility, three categories of A.P. enzyme have been described:

1) Placental A. P., which occurs in human placenta but generally not in adult tissues;
2) Intestinal A.P., which sometimes resembles the placental enzyme;
3) A.P., which occurs in bone, kidney, liver and other tissues.

High concentrations of A.P. are often associated with: a) absorptive cells, especially brush borders of the intestinal mucosa and proximal convoluted tubules of the kidney; and b) calcification sites,
particularly in osteoblasts and chondrocytes.

Alkaline phosphatase enzyme activity has been localized in the cell membrane and can be removed by treatment with phospholipase C (Low and Finean, 1977). The enzyme is capable of hydrolyzing monophosphates, pyrophosphates (Cox and Griffin, 1965) and adenosine triphosphatase (Moss and Walli, 1969). In the rat trabecular bone fixed by perfusion followed by additional fixation at 4°C, 60% of the original A.P. activity is preserved and the morphological preservation is excellent (Doty, 1980). Doty adds that 60-80% of the original activity is preserved by immersion in 4% paraformaldehyde for eight hours, while 40% of the activity is preserved with 6% glutaraldehyde for three hours. Furthermore, he demonstrated the presence of the enzyme in the endothelial cells of the capillaries and in cartilage cells. On the other hand, Gomori (1941) states that acetone is a good preservative of both A.P. and acid phosphatase enzymes in human and animal tissues under normal and pathological conditions. Further, he reports that walls of capillaries are rich in A.P. Jancso et al. (1975) demonstrate the presence of A.P. in the capillary of albino rats which is uniformly distributed in the cytoplasm of the endothelial cells. The activity of the A.P. enzyme is demonstrated by Nanda et al. (1979) in hepatic cells of the goat. The enzyme is found concentrated along the bile canaliculi margin, while the vascular wall of the portal vessels and central vein have moderate activity. Danielli (1953) suggests that the A.P. enzyme acts in a transport of materials across the plasma membrane and intracellular fluid.
The adenosine triphosphatase (ATP-ase) enzyme is a collection of different families of enzymes and these being linked as they metabolize the same substrate (Firth, 1980). The sodium-potassium \((Na^+\text{-}K^+)\)-dependent ATP-ase enzyme is one of this family which is difficult to demonstrate by the conventional method of histochemistry, though several methods have been developed to localize it in recent years.

Carr et al. (1952) demonstrate the presence of ATP-ase enzyme in the dog, frog, chicken, guinea pig, and rabbit aorta. In addition, Balo et al. (1948) report that the ATP-ase of the myosin from the skeletal muscle is different from that in the aortic tissue in which the former hydrolizes one mole of phosphoric acid from ATP, while the latter hydrolizes two moles. Due to the presence of \((Na^+\text{-}K^+)\)-dependent ATP-ase activity within the cell membrane and in areas where active transport of sodium ions \((Na^+)\) takes place, several functions have been given to this enzyme. First, the generation of membrane potential because of its ability to extrude \(Na^+\), and this resulting in an increase in potassium ions \((K^+)\) within the cell (Firth, 1974; Guth and Albers, 1974). Secondly, regulation of cell volume; as the \(Na^+\) is extruded water will follow to the outside thus preventing distention (Ernst, 1972; Guth and Albers, 1974). Finally, regulation of electrolyte compositions within the cells and within the interstitial space (Guth and Albers, 1974). It has also been found that the capacity to actively transport \(Na^+\) or \(K^+\) is directly proportional to the activity of the \((Na^+\text{-}K^+)\)-ATP-ase enzyme in the tissue. The ratio of cation exchange transported to actively measured enzyme is nearly equal in six different tissues studied (Bonting and

In support of the role in ion transport, ATP-ase has been described in several epithelia noted for active transport of electrolytes using both light microscopy and transmission electron microscopy. For instance, ATP-ase has been demonstrated in the kidney tubules (Kaplan and Novikoff, 1959), frog skin, toad bladder (Bartoszewicz and Barnett, 1964; Keller, 1963), gall bladder and ciliary epithelium (Cole, 1964; Kaye and Tice, 1964).

Tormey (1966) states that this specific enzyme is histochemically distinguishable from other forms of ATP-ase by the following criteria: 1) Na\(^+\), Mg\(^{++}\) and in many cases K\(^+\) are absolutely necessary for its activation; 2) in no case is the enzyme activated by K\(^+\) alone in the absence of Na\(^+\); 3) it is inhibited by Ca\(^{++}\) and low concentration of glycosides such as ouabain. On the basis of comparative observations of a variety of epithelia, both transporting and nontransporting, one might be justified in concluding that the histochemical ATP-ase activity is related to active transport. However, Ellis et al. (1963) conclude that there is no necessary relationship between histochemically demonstrated ATP-ase and transcellular transport because of the absence of ATP-ase activity in the secretory epithelium of the avian salt gland, in spite of the fact that such glands are notably engaged in electrolytes transport and contain high concentration of sodium pump (Hokin, 1963). In addition, Ernst (1972) using transmission electron microscopy finds that cytoplasmic as well as endothelial cells of capillaries surrounded the secretory epithelium of the avian salt gland and are generally free of
The venous system of the dog is devoid of ATP-ase activity while the amount of its activity varies greatly in the arterial system (Carr et al., 1952). Carr et al. think that the vasodilators inhibit ATP-ase activity and might have ATP-ase as their target enzyme and through this inhibition elicit their responses. Furthermore, Spater et al. (1958) state that ATP-ase activity shows considerable variations both in localization and intensity among six species investigated (dog, rat, man, mouse, monkey and opposum). In addition, Hori and Chang (1963) detect cytoplasmic ATP-ase in the liver, kidney, cardiac and skeletal muscles of the rat by using the section freeze-substitution technique. In the kidney, the cytoplasm of the ascending limb of the Henle's loop, the intertubular, glomerular capillaries, tunica intima and tunica media of the arterial wall are strongly stained. Freiman and Kaplan (1960) state that the major areas of activity of ATP-ase enzyme are the brush borders, cytoplasm of tubule cells and peritubular and glomerular capillaries as well as the media of larger blood vessels. Padykula and Herman (1955) demonstrate the presence of ATP-ase in smooth muscles and endothelium of large coronary blood vessels. They also suggest that the vascular activity demonstrated is due to a less specific organic phosphatase. Generally, endothelial cells of blood vessels have been demonstrated to possess ATP-ase activity, mainly in their surface caveolae (Hoff and Graf, 1966; Marchesì and Barnett, 1963).

The presence of 5'-nucleotidase enzyme is demonstrated in the liver of the rat, mouse, guinea pig and man (Wachstein and Meisel, 1957).
Wachstein and Meisel report that the periportal connective tissue and walls of arteries and veins react positively in rat and guinea pig, while in the mouse, dog, and man only the endothelium of the vessels react positively. Reis (1934) shows that 5'-nucleotidase enzyme does not hydrolize adenosine 3'-phosphate or adenosine triphosphate (Ahmed and Reis, 1958). Because 5'-nucleotidase has shown activity against a wide variety of nucleotidases, it has been suggested that there might be more than one 5'-nucleotidase (Novikoff, 1958; Scott, 1965). Scott (1965) demonstrates the presence of 5'-nucleotidase in the brain of the mouse and describes its specificity by using various fixatives. Suran (1974) using light microscope and transmission electron microscope demonstrates the presence of this enzyme in the spinal cords of the mouse and cat.

Several functions have been suggested for the 5'-nucleotidase enzyme viz., catabolic functions (Hardonk and Kondstaal, 1968), degrading residual nucleotidases (Goldberg, 1973), and a function similar to those reported for the ATP-ase (Novikoff, 1958).
MATERIALS AND METHODS

A total of fifty-six animals (48 sheep and eight goats) were used. Their ages, body weights, sexes, and breeds were not considered. The animals were killed either by decapitation or by bleeding after the injection of Rompun (Xylazine hydrochloride U.S.P.) intramuscularly at a dosage level of 10 mg/kg body weight.

Twenty-one animals (19 sheep and two goats) were injected with different intravascular plastic materials (Table 1).

Table 1. Materials injected and route of injection

<table>
<thead>
<tr>
<th>Number of animals used</th>
<th>Species</th>
<th>Material injected</th>
<th>Route of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>sheep (adult)</td>
<td>Batson's #17^a/or colored latex</td>
<td>Common carotid arteries and/or external jugular veins</td>
</tr>
<tr>
<td>11</td>
<td>sheep (feti)</td>
<td>Batson's #17/or colored latex</td>
<td>Common carotid arteries and/or external jugular veins</td>
</tr>
<tr>
<td>1</td>
<td>goat (adult)</td>
<td>Batson's #17</td>
<td>Common carotid arteries and external jugular veins</td>
</tr>
<tr>
<td>1</td>
<td>goat (adult)</td>
<td>Colored latex</td>
<td>Common carotid arteries</td>
</tr>
</tbody>
</table>

^Polysciences, Inc., Paul Valley Industrial Park, Warrington, PA.

Heads injected with Batson's #17 were macerated with 33% potassium hydroxide (KOH)^1 solution for two weeks. The heads were taken out of the solution twice a week and washed under running tap water for half an

^Mallinckrodt, Inc., Kentucky.
hour, then immersed again in the solution. At the end of two weeks, the casts were washed with running water and then placed in 5% hydrochloric acid (HCl) solution for half an hour to remove the remaining fat and tissue debris. Finally, the casts were washed again with running tap water and examined. The heads injected with colored latex were routinely dissected.

Ten animals (eight sheep and two goats) were used for light microscopic study, and the carotid rete-cavernous sinus complexes were collected by splitting the heads sagittally. They were fixed in 10% buffered neutral formalin (BNF) solution for 24 hours. Then, they were embedded in paraffin following standard histologic techniques (Table 2).

Table 2. Light microscopic study

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adult sheep&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Adult sheep</td>
</tr>
<tr>
<td>1</td>
<td>Sheep fetus - close to term</td>
</tr>
<tr>
<td>2</td>
<td>Adult goat</td>
</tr>
</tbody>
</table>

<sup>a</sup>Following serial sections, all of them were collected and examined.

The paraffin blocks were serially sectioned (5-8 μm thick) and sections were stained with the following stains:

a. Harris' hematoxylin and eosin;

b. Van Gieson's (collagen-red; tissue-yellow);

c. Gomori one-step trichrome (muscle cell-red; tissue-blue) after Gomori (1950); and
d. Weigert's method for elastic fibers (elastic fibers-black) after Luna (1968).

In addition, five sheep were used to examine the carotid retia after they were perfused with normal saline solution. Two sheep were injected with either Batson's #17 or Decopour \(^1\) via both common carotid arteries. The head was severed after two hours in the case of Batson's #17 and after 24 hours in the case of Decopour injection during this period, each head was kept immersed in cold water. Then, the heads were placed in 33% potassium hydroxide (KOH) solution at room temperature for about one week. During this period the heads were twice removed from the solution and washed under running tap water for half an hour or so and placed back in a fresh KOH solution. After one week, the vascular casts were removed, washed with water and placed in a 5% HCl solution for half an hour. Two vascular casts were prepared and, with careful dissection, the carotid retia were removed. They were glued with silver paint to a stub, sputter coated with palladium-gold for three minutes, using Polaron E5100 sputter coater, and were examined in a JSM-35 JEOL scanning electron microscope.

The remaining three animals were bled to death after intramuscular injection of Rompun solution. The heads were flushed with normal saline solution, followed by perfusion of 5% glutaraldehyde in molarity phosphate buffer (pH 7.2) via both common carotid arteries. The heads were severed and

\(^1\) Decopour, Flecto, Co., Inc., P.O. Box 12955, Oakland, California.
the carotid retia were carefully dissected out and placed in 5% glutaraldehyde overnight in a refrigerator (4°C). Osmication and dehydration of the retia were followed as hereunder:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>phosphate buffer plus 4% sucrose</td>
<td>3 changes</td>
</tr>
<tr>
<td>2</td>
<td>phosphate buffer</td>
<td>3 x 15 minutes</td>
</tr>
<tr>
<td>3</td>
<td>osmium tetroxide</td>
<td>1 hour (1% OsO₄ in phosphate buffer)</td>
</tr>
<tr>
<td>4</td>
<td>distilled water</td>
<td>5 minutes</td>
</tr>
<tr>
<td>5</td>
<td>70% ETOH</td>
<td>15 minutes</td>
</tr>
<tr>
<td>6</td>
<td>95% ETOH</td>
<td>2 x 15 minutes</td>
</tr>
<tr>
<td>7</td>
<td>100% ETOH</td>
<td>3 x 20 minutes</td>
</tr>
</tbody>
</table>

The retia were freeze-fractured in liquid nitrogen. Later, alcohol was substituted by freon TF and critical point drying was followed after the replacement of freon by carbon dioxide. Finally, the specimens were mounted on stubs, sputter coated with palladium-gold for three minutes and were examined with the SEM scope previously described.

Sixteen sheep and four goats were used for histochemical studies. The animals were decapitated and the carotid rete-cavernous sinus complex was expeditiously collected. Trials for detection of alkaline phosphatase, 5'-nucleotidase, and adenosine triphosphatase enzymes were performed (Table 3).
Table 3. Histochemical studies

<table>
<thead>
<tr>
<th>Number of animals used</th>
<th>Species and tissues</th>
<th>Technique used</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>sheep retia</td>
<td>Embedded in paraffin</td>
<td>Alkaline phosphatase enzyme detection</td>
</tr>
<tr>
<td>4</td>
<td>sheep retia</td>
<td>Fixed in polyvinyl pyrrolidone and frozen with spra-freeze</td>
<td>Adenosine triphosphatase enzyme detection</td>
</tr>
<tr>
<td>6</td>
<td>sheep retia</td>
<td>Frozen with either liquid nitrogen or spra-freeze^a</td>
<td>5'-nucleotidase and adenosine triphosphatase enzymes detection</td>
</tr>
<tr>
<td>2</td>
<td>goat retia</td>
<td>Frozen with spra-freeze</td>
<td>Detection of all three enzymes</td>
</tr>
<tr>
<td>1</td>
<td>goat liver</td>
<td>Frozen with spra-freeze</td>
<td>Detection of all three enzymes</td>
</tr>
<tr>
<td>1</td>
<td>goat retia^b</td>
<td>Perfusion of polyvinyl pyrrolidone solution and frozen with spra-freeze</td>
<td>Detection of all three enzymes</td>
</tr>
</tbody>
</table>

^aLaboratory Supplies Company, Inc., 29 Jefry Lane, Hicksville, NY 11801.

^bPerfusion was performed after ligation of one common carotid artery and canulation of the other one and 400 ml of the solution was injected towards the head.
Animals used for alkaline phosphatase detection were processed as follows:

- Cold acetone for 24 hours
- Room temperature acetone for 1 hour with 2 changes
- Benzene for \( \frac{1}{2} \) hour with 2 changes
- Embedded in paraffin

Sections 5-10 \( \mu \text{m} \) thick were cut and the calcium method for alkaline phosphatase enzyme detection was used (Gomori, 1952).

**Preparation of incubation medium**

- Sodium \( \beta \)-glycerophosphate, 2 percent: 2.5 ml
- Sodium veronal (barbitone): 2.5 ml
- Calcium chloride (\( \text{CaCl}_2 \)), 2 percent: 4.5 ml
- Magnesium sulfate (\( \text{MgSO}_4 \)), 2 percent: 0.2 ml
- Distilled water: 0.3 ml

The pH was adjusted to 9.2 and the incubation medium was stored at 4°C. Two controls were used, one by substituting sodium \( \beta \)-glycerophosphate with an equal amount of distilled water and the other one with distilled water only.

In addition, the retia and livers of goats were frozen and sectioned in a range of 10-15 \( \mu \text{m} \) thick at -20°C using the cryostat. Sections were placed on slides directly, left to dry for 15-20 minutes and placed in a prewarmed incubation medium. The controls were treated exactly the same way except for the removal of the sodium \( \beta \)-glycerophosphate from the incubation medium.
Procedure:

Deparaffinize sections and bring to water (for those embedded in paraffin)

Incubate for one to three hours at 37°C (one group was left over night)

Rinse quickly in distilled water

Treat in two percent cobalt nitrate (or acetate) for two minutes

Wash for one minute to remove excess cobalt chloride

Treat with one percent ammonium sulfide for one minute

Wash for two to three minutes

Dehydrate through graded alcohol, clear with xylene and mount.

Results:

Black deposits of cobalt sulfide represented the sites of enzyme activity while control was completely negative.

The method for sodium-potassium activated adenosine triphosphatase enzyme was used after Guth and Albers (1974). Cryostat sections of 10-15 μm thick at -20°C were used on slides after being left for 15-20 minutes to dry.

Preparation of polyvinyl pyrrolidone perfusion solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>polyvinyl pyrrolidone (M.W. 40,000)</td>
<td>12 percent</td>
</tr>
<tr>
<td>calcium chloride (CaCl₂)</td>
<td>1.8 mM</td>
</tr>
<tr>
<td>histidine</td>
<td>100 mM</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.25 with HCl.
Preparation of the incubation medium:

- 2-Amino-2-methyl-1-propanol 70mM
- P-Nitrophenyl phosphate 5mM
- Potassium chloride (KCl) 30mM
- Magnesium chloride (MgCl₂) 5mM
- Dimethyl sulfoxide 25% V/V

The pH was adjusted to 9.0 with HCl. For the control, 1 mM of ouabain (final concentration) was added to the incubation medium. In addition, sections were placed in distilled water only as a control.

Procedure:

- Incubate for one to three hours at 37°C
- Place sections directly into two percent solution of cobalt chloride for five minutes
- Rinse briefly in distilled water
- Wash in three changes of 2-amino-2-methyl-1-propanol buffer (70 mM, pH 9.0) for 30 seconds each
- Place in two percent ammonium sulfide solution for three minutes
- Wash in running tap water.
- Dehydrate through graded alcohols, clear in xylene and mount.

Results:

Sites of Na⁺/K⁺-activated ATP-ase enzyme were yellowish brown to black while the control sections showed no reaction product.

The lead method for 5'-nucleotidase was used after Wachstein and Meisel (1957) except that 10-15 μm thick sections at -20°C were directly placed on slides instead of freely-floated in the incubation medium.
Preparation of the incubation medium:

- adenosine 5-phosphate, 0.125 percent: 4.0 ml
- tris buffer, 0.2 M, pH 7.2: 4.4 ml
- magnesium sulfate, 0.1 M: 1.0 ml
- lead nitrate, 2 percent: 0.6 ml

All solutions were prepared with CO₂-free distilled water, though a small amount of precipitate was formed when the lead nitrate was added. The solution was filtered before use. Control was used by adding distilled water instead of adenosine 5-phosphate.

Procedure:

- Incubate sections at 37°C for 30 to 60 minutes
- Transfer to formal-saline fixative for 30 minutes
- Transfer through two changes of CO₂-free distilled water
- Treat with two percent ammonium sulfide for two minutes
- Rinse in distilled water
- Mount in glycerin jelly

Results:

5'-nucleotidase activity appeared brown to black while control was negative.
RESULTS

Gross

The nomenclature used conforms to the second edition of the Nomina Anatomica Veterinaria (N.A.V.) recommended by the International Committee on Veterinary Anatomical Nomenclature (ICVAN, 1973, 1975) and Latin terms were translated to accepted English equivalents.

The carotid rete (ICVAN Rete mirabile epidurale rostrale, 1973)

The main blood supply to the brain of sheep and goat was via rostral and caudal rete branches\(^1\) of the maxillary artery (Figs. 1, 2, 3 and 4). These branches arborized into a network of small arteries to form the carotid rete. In both sheep and goat, the caudal epidural carotid rete was absent.

The carotid rete of the sheep (Fig. 1)

In adult sheep, the carotid rete was composed of intertwined arteries which anastomosed freely with each other forming a meshwork of vessels. The rete branches of the maxillary artery consisted of rostral branches (two to four) and one caudal branch. The rostral branches (G, H, I and J) were variable in their origins: two branches (J and H) were consistently present in all specimens. A branch (J) arose from the maxillary artery usually opposite to the origin of the external

\(^1\) In the text, arteries and rete branches were used synonymously.
Figure 1. The carotid rete of sheep and its connection (schematic drawing)

A. Maxillary artery
B. Lingual artery
C. Inferior alveolar artery
D. Maxillary artery
E. Caudal rete branch
F. Carotid rete (Rete mirabile epidurale rostrale)
G.-J. Rostral rete branches
K. External ophthalmic artery
L. Anastomotic branch between F and N
M. Anastomotic branch between J and N
N. Ophthalmic rete (Rete mirabile ophthalmicum)
O. Supraorbital artery
ophthalmic artery (K). This branch had an anastomotic connection (M) with the ophthalmic rete mirabile (N). It was distributed in the rostral portion of the carotid rete. The branch (I) departed very close to the origin of the external ophthalmic artery or sometimes it arose by a common trunk with the latter. The largest branch (H) arose from the maxillary artery (D) few millimeters caudal to the origin of the external ophthalmic artery. This branch usually divided before entering the carotid rete. Frequently, it arose by a common trunk with another branch (G) in animals having two or three rostral rete branches only. The fourth branch (G) was usually small and arose caudal to the origin of the preceding branch (H) and was distributed in the middle of the ventral aspect of the rete.

Connections or anastomoses of the carotid rete

A small direct anastomotic branch between the carotid rete and the ophthalmic rete was evident in four sheep (Figs. 2 and 3). From the ventral aspect of the caudal rete branch arose two arteries: one close to its origin and the second from its middle. These arteries were distributed in the pharyngeal region. At the caudal region of the rete, there were two anastomotic branches between left and right retia. The larger dorsal one arose at the level of the origins of the caudal cerebral arteries. The smaller ventral anastomotic branch originated at the level of the termination of the caudal rete branch. The above two ventral anastomotic branches, one from each side, joined together forming a single artery which ran caudally paralleling the course of the basilar artery.
Plate 2

Figures 2 and 3. Batson's #17 cast of the carotid rete of an adult sheep. Lateral view. Line scale = 2.9 mm.

A. Maxillary artery
B. Caudal rete branch
C. Rostral rete branches
D. External ophthalmic artery
E. Anastomotic branch between D and H
F. Anastomotic branch between H and the ophthalmic rete
G. Cerebral arterial circle
H. Carotid rete

Figure 4. Decopour cast of the carotid rete of an adult sheep. Lateral view. Line scale = 2.6 mm.

A. Maxillary artery
B. Caudal rete branch
C. Rostral rete branches
D. Carotid rete
E. Broken end of the cerebral arterial circle
Innervation of the carotid rete

The carotid rete was supplied by a branch of the trigeminal nerve which originated from the latter before its division. The branch entered the rete after piercing through the dura mater caudal to the hypophysis cerebri and extended rostrally. It became closely related to the carotid rete branches as well as the intracranial portion of the internal carotid artery. Grossly, so far this branch was the only one observed in all specimens examined.

The internal carotid artery

The extracranial portion of the internal carotid artery was involuted post-natally. The intracranial portion of the internal carotid artery was well-developed and emerged from the mid-portion of the rete. It extended rostrally lying over the surface of the rete for a short distance then joined the cerebral arterial circle (Circulum arteriosus cerebri) or circle of Willis a few millimeters caudal to the rostral end of the rete.

The carotid rete of the goat

The morphology of the carotid rete of the goat was basically similar to that of sheep with a few differences. The rete consisted of two to three rostral rete branches. One arose close to the origin, while the second one arose a few millimeters rostral to the origin of the external ophthalmic artery. The former usually divided into several branches before entering the rete. An inconsistent very small third
branch arose rostral to the origin of the second branch. It had anastomotic connections with the ophthalmic rete and in one instance with the second rostral branch. The caudal branch arose similar to that of sheep. A small anastomotic branch between the rostral portion of the carotid rete and the ophthalmic rete was observed in one specimen.

The carotid rete of the sheep fetus

The carotid rete was well-developed in near term sheep feti. Postnatally the extracranial portion of the internal carotid artery was involuted except in one specimen where a small artery persisted. There was one caudal rete branch and usually two to three rostral rete branches. The largest of the rostral branches arose next to the origin of the external ophthalmic artery. The most caudal of the rostral branches split into several small branches before entering the rete. The two retia were connected caudally by two small transverse branches (dorsal and ventral), while rostrally there were two anastomotic branches which formed a loop around the rostral border of the hypophysis cerebri (pituitary gland). A bilateral or a unilateral connection between the rostral portion of the carotid rete and the ophthalmic rete was usually present.

Light Microscopy

The carotid rete of adult sheep

The carotid rete branches were medium-sized, muscular arteries and their thickness varied in different segments of the rete. They possessed a well-developed internal elastic lamina which showed a number of changes, such as splitting, fenestration, doubling, and thickenings which varied
between sections. The endothelial lining of these branches was bulging into the lumen in most sections. The tunica media consisted of three to five smooth muscle layers, coursing irregularly in all directions depending on the incidence of sectioning, with a few intervening collagen and elastic fibers (Fig. 5). Usually, these smooth muscle cells were typically fusiform, but sometimes stellate-shaped. The tunica adventitia was composed mainly of collagen fibers and a few elastic fibers. A distinct external elastic lamina was lacking and it consisted of only a few elastic fibers towards the periphery. The rete branches shared a common tunica adventitia with the satellite veins of the cavernous sinus. Seemingly, there were no arteriovenous anastomoses in the carotid rete-cavernous sinus complex, but large numbers of epithelioid cells in the wall of several small arterioles were observed (Fig. 6). In addition, intrarterial bolsters simulating intimal thickenings or pads were noticed in several sections (Fig. 7). These intimal projections appeared either with smooth muscle cells or collagen fibers which were surrounded by the split internal elastic lamina.

The internal carotid artery of adult sheep

The extracranial portion of the internal carotid artery was absent in the specimens examined, which is in agreement with the findings of Balankura (1954), Baldwin (1964), Daniel et al. (1953), Getty (1975) and McGrath (1977).

The intracranial portion of the internal carotid artery had a distinct internal elastic lamina. The tunica media consisted of five to six
smooth muscle layers which ran uniformly in a circular fashion, contrasting the smooth muscle of the carotid rete branches which coursed irregularly in all directions (Fig. 8). The well-developed tunica adventitia was almost twice the thickness of the corresponding tunica media. The outer surface of the tunica adventitia of the internal carotid artery was surrounded by endothelial cells (simple squamous epithelium). Underneath the epithelium were a large number of blood vessels (vasa vasorum) and nerve fibers (nervi vasorum). In addition, there were islets or clusters of epithelioid cells in the tunica adventitia of the artery at the branching site. They were of two types: small, rounded, darkly-stained and larger, irregular (oval to oblong), lightly stained cells (Figs. 9 and 10). Peripherally these cells were surrounded by fibroblasts, separating them from the surrounding adventitial collagen fibers of the artery, thus forming a sheath or mantle around them.

In some carotid rete branches as well as in the tunica adventitia of the internal carotid artery, there were similar structures referred to above except that these structures contained large numbers of both myelinated and unmyelinated nerve fibers only. Moreover, large myelinated nerve trunk of the trigeminal coursed in close or intimate relationship through the carotid rete from caudal to rostral regions (Figs. 11 and 12). No valves were observed in arteries of the carotid rete in sheep.

**The cavernous sinus of adult sheep**

The structure of the cavernous sinus of adult sheep differed from region to region. It consisted of endothelial lining surrounded by a
Plate 3

Figure 5. Micrograph of an artery of the carotid rete surrounded by a vein of the cavernous sinus. Gomori one-step trichrome stain. Line scale = 263 μm.

A. Tunica media of an artery.
B. Tunica adventitia (common to both the artery and the vein surrounding it)
C. Tunica media of a vein
D. Lumen of the cavernous sinus

Figure 6. Epithelioid cells in the wall of an arteriole in the carotid rete-cavernous sinus complex of a sheep. Line scale = 30.5 μm.

Figure 7. Intimal proliferation or intraarterial bolster (pedunculated). Weigert's stain. Line scale = 98 μm.
Plate 4

Figure 8. Cross section of an artery of the carotid rete. Van Gieson's stain. Line scale = 100 μm.

a. Tunica media of an artery
b. Tunica adventitia (common to both the artery and the vein)
c. Tunica media of a vein of the cavernous sinus
d. Endothelial lining of the cavernous sinus

Figure 9. Micrograph of the wall of the intracranial portion of the internal carotid artery. Van Gieson's stain. Line scale = 15.6 μm.

A. Tunica media of the internal carotid artery
B. Tunica adventitia
C. Carotid body
D. Tunica media of an adjacent carotid rete branch

Figure 10. Magnified carotid body. Van Gieson's stain. Line scale = 8 μm.

a. Small cells
b. Large cells
Plate 5

Figure 11. Paraffin section of the carotid rete-cavernous sinus complex. Myelinated nerve bundles appeared interposed between the arteries. Weigert's stain. Line scale = 91 μm.

A. Blood filled lumen of the cavernous sinus
B. Carotid rete arteries
C. Myelinated nerve bundles

Figure 12. Magnified area of the carotid rete of sheep showing the close relationship between the artery (A) and the myelinated nerve (B). Weigert's stain. Line scale = 11.4 μm.

Figure 13. Intimal proliferation or intraarterial bolsters in an artery of the carotid rete of the sheep fetus. Weigert's stain. Line scale = 131 μm.
thin layer of connective tissue (mostly collagen and some elastic fibers). However, at the emergence site of the intracranial portion of the internal carotid artery, in addition to the endothelial lining with its connective tissue the cavernous sinus had one to several layers of smooth muscle depicting a common tunica adventitia with the carotid rete branches (Figs. 8 and 9). These longitudinally or circularly arranged smooth muscle cells were heavily infiltrated with elastic fibers. In the region where smooth muscle cells were few or altogether absent, a corresponding thickening of the tunica adventitia was evident. The smooth muscle cells of the cavernous sinus always ran in a different direction than those of the adjacent carotid rete branches.

Throughout the length of the cavernous sinus, valve-like structures in the form of extensions of the lining endothelium were consistently observed. These valve-like structures were composed of a collagenous core surrounded by elastic fibers. In cross sections, these areas appeared as a solid mass of collagen and elastic fibers, and the elastic fibers tended to appear larger in quantity towards the center than the periphery. Frequently, a thickening of the tunica intima with the presence of a few smooth muscle cells was discernible at the branching site or at the site of anastomoses between two or more compartments of the cavernous sinuses.

The carotid rete-cavernous sinus complex of the sheep fetus

In one sheep fetus, the flexuous rete branches were well-developed having relatively thinner walls with well-developed internal elastic laminae. In the absence of the external elastic lamina a few elastic
fibers were scattered at their periphery. The tunica media consisted of two to three smooth muscle layers with a few intervening elastic fibers, especially seen towards the periphery. From the wall of the rete branches, a number of intimal projections into the lumen were observed. These projections were covered by the internal elastic lamina and endothelium having a muscular or a fibrous core (Fig. 13). The wall of the cavernous sinus was devoid of smooth muscle cells and was only represented by endothelium and connective tissue, which was mainly of collagenous fibers and few sub-endothelial elastic fibers.

The carotid rete-cavernous sinus complex of the goat

The histomorphology of the carotid rete and the cavernous sinus of the goat was almost identical to that of sheep with a few species differences. The tunica intima had several thickenings and splitting of the internal elastic lamina which was more pronounced. The tunica media of the carotid rete branches was relatively thicker than that of sheep, consisting of five to eight smooth muscle layers; a few collagen fibers were seen in between the smooth muscle cells. In the wall of the cavernous sinus, no smooth muscle cells were evident. Epithelioid cells were present in the wall of some arterioles, but no arteriovenous anastomoses or valves were observed in carotid rete branches.

Scanning Electron Microscopy

The carotid rete of sheep was obtained as a complete cast with some areas showing extravasation due to over injection (Fig. 14). The sites
of entrance of the rostral and caudal rete branches were visible. In addition, the rostral part of the carotid rete had an anastomotic branch with the ophthalmic rete. The carotid rete (Rete mirabile epidurale rostrale, ICVAN, 1973) of sheep was composed of medium-sized, muscular arteries bathing in a venous lake - the cavernous sinus. The main blood supply to the carotid rete was via branches off the maxillary artery.

The carotid rete specimens prepared by freeze-fractured technique showed distinct tunics characteristic of normal muscular arteries in other regions of the body. A well-developed internal elastic lamina was present in all specimens, and it was apparently folded because of the procedure used to kill the animals (Fig. 15). The differences were obvious between the razor-blade sectioned rete artery, obscuring all details and the freeze-fractured one showing clearly all arterial wall layers (Fig. 16). A scanty amount of connective tissue was seen separating the internal elastic lamina from the endothelial cells (Fig. 17). This connective tissue was mainly collagen fibers entangled with a large number of red blood, as well as a few white blood cells smeared from the lumen of the artery during preparation of the specimen. The endothelial cells were longitudinally oriented with their long axes in the direction of blood flow (Fig. 18). On the other hand, the cavernous sinus was lined by rounded to ovoid endothelial cells oriented differently than those of the arteries as the blood flowing in the opposite direction (Fig. 19). Commonly, the vein's endothelial cells run circularly while those of the arteries run in a longitudinal direction. The central part of some of these endothelial cells protruded toward the lumen due to the presence of the underlying
Figure 14. Scanning electron micrograph of the carotid rete of sheep (Batson's #17 cast). Ventrolateral view. Line scale = 0.8 μm.

A. Rostral rete branch

B. Caudal rete branch

C. Overinjection resulted in partially filling the cavernous sinus
Plate 7

Figure 15. Freeze-fractured artery showing the tunics of the arterial wall. Line scale = 36 μm.

A. Endothelial lining
B. Internal elastic lamina
C. Tunica media
D. Tunica adventitia

Figure 16. Razor-blade section of an artery of the carotid rete. Tunics are not clearly delineated. Line scale = 22 μm.

A. Endothelial lining of an artery
B. Endothelial lining of a vein
Figure 17. Magnified tunica intima of an artery from the carotid rete of sheep. Line scale = 11 μm.

a. Lumen
b. Internal elastic lamina
c. Connective tissue
d. Red blood cells
e. White blood cell

Figure 18. Tilted tangential section of an artery. Line scale = 110 μm.

a. Internal elastic lamina
b. Endothelial lining of an artery
c. Tunica media
d. Tunica adventitia of both the artery and the cavernous sinus
e. Endothelial lining of the cavernous sinus
nuclei. In some arteries a few craters were also observed at the edge of the endothelial cells (Fig. 20).

The tunica media, which consisted of circularly arranged smooth muscle cells was found to be well-developed and was thicker than the tunica adventitia in most arteries. The latter lacked a distinct external elastic lamina, although in most sections a few scattered elastic fibers were observed, which appeared shiny and white in color.

The intravascular injection technique used in this investigation showed that Batson's #17 yielded better results than Decopour. However, higher magnification of a vessel wall (injected with Batson's #17) revealed numerous depressions or holes of unknown origin (Fig. 21). The Batson was used as it is without any modification because the size of the arteries of the carotid rete was large enough to be filled with its viscosity. This procedure facilitated studying of the afferent and efferent branches to the carotid rete. On the other hand, Decopour had resulted also in good filling of the carotid rete branches but was not tough and pliable enough, so when the specimen was dry it broke easily posing some difficulties in identifying the continuity of vessels.

Histochemistry

Alkaline phosphatase enzyme (A.P.)

The localization of the alkaline phosphatase enzyme (A.P.), with a few exceptions, was found to be circumscribed in and around areas where capillaries were present. The endothelial cells of some arteries showed weak reaction to A.P. and this reaction was not continuous all around the lumen
Plate 9

Figure 19. Cross section of a thick-wall muscular artery. Line scale = 26 μm.

a. Tunica media
b. Tunica adventitia
c. Endothelial lining of the cavernous sinus

Figure 20. Tilted tangential section of an artery showing the craters in the endothelial lining (arrows). Line scale = 17 μm.

Figure 21. Magnified arterial wall (Batson's cast) showing depressions or holes of varying sizes. Line scale = 83 μm.
of the artery. In some sections, the area of the folded internal elastic laminae of the rete branches was lightly stained, which indicated the presence of a small amount of enzymic activity on the endothelial surface (Fig. 22). However, the endothelial cells of the cavernous sinus positively reacted and the intensity of the reaction was several times stronger than that of carotid rete branches. In addition, in areas where the cavernous sinuses anastomosed with each other or came in contact with adjacent sinus, the enzymic activity fairly increased.

In all specimens, the tunica media and the tunica adventitia of all carotid rete branches, in general, showed no reaction and were colorless. But, the outer layer of the tunica adventitia of rete branches, which was in contact with the dura mater, showed positive reaction. Also, the osteoblasts of the internal periosteum of the skull bone and the liver specimens were intensely stained (Figs. 24, 25 and 26).

The endothelial cells, as well as the pericytes of capillaries were darkly stained, especially in peripheral areas of the tunica adventitia. Some of these capillaries showed obliterated lumen because of their small luminal diameters and the intensity of the stain (Fig. 27).

As a trial, one specimen was kept overnight in the incubation medium to detect the presence of a very small amount of the enzymic activity which was otherwise not possible by the usual three hours incubation. This trial revealed an increased enzymic activity in surrounding tissues of the carotid rete-cavernous sinus complex, but revealed no enzymic activity on the rete structure (Fig. 28).
Figure 22. A cross section of an artery of the carotid rete of sheep showing black deposition at the site of alkaline phosphatase enzyme. Line scale = 23 μm.

a. Tunica intima
b. Tunica media
c. Tunica adventitia
d. Blood capillary

Figure 23. An artery of the carotid rete of sheep showing positive reaction to alkaline phosphatase enzyme. Line scale = 125 μm.

a. Intraarterial bolsters
b. Split internal elastic lamina
c. Tunica media
d. Tunica adventitia
e. Blood capillaries

Figures 24 and 25. Cross sections of small pieces of skull bone. Notice the osteoblasts stained dark-black in Fig. 24, while no staining in the control section, Fig. 25. Line scale = 143 μm.
Figure 26. Liver lobe used as control showing the concentration of alkaline phosphatase enzyme around the central vein. Line scale = 33 μm.

Figure 27. Enlarged wall of an artery showing positive reaction to alkaline phosphatase enzyme. Line scale = 23 μm.
   a. Intraarterial bolster
   b. Tunica intima
   c. Tunica media
   d. Tunica adventitia
   e. Blood capillaries

Figure 28. A cross section through an artery showing the intense reaction at the periphery due to prolonged incubation period (overnight). Line scale = 18 μm.
   a. Lumen of an artery
   b. Tunica intima
   c. Tunica media
   d. Tunica adventitia
   e. Endothelial lining of the cavernous sinus
   f. Lumen of the cavernous sinus
Adenosine triphosphatase enzyme (Na\(^+\)-K\(^+\), ATP-ase)

The reaction sites for the enzymic activity appeared as black clusters of small granules and not a uniformly stained field. In different trials, the result of this study was inconsistent on different sections. These black clusters of small granules were variably distributed along the entire thickness of carotid rete branches (Fig. 29). Seemingly, they were more concentrated at the luminal surfaces of rete branches as well as the cavernous sinus, which indicated that endothelial surfaces had a higher enzymic activity in comparison to the rest of the wall. However, the activity of the enzyme consistently appeared to be much higher on the endothelial lining of carotid rete branches than that of the cavernous sinus. In addition, the endothelial cells showed intense staining reaction forming a ring inside the lumen of the artery.

The intensity of the enzymic activity present in the tunica media gradually decreased towards its outer layer adjacent to the tunica adventitia. The tunica adventitia, on the other hand, showed very little or sometimes no activity, while the endothelial cells of the cavernous sinus showed moderate activity. In some sections, the smooth muscle cells of the tunica media, closer to the internal elastic lamina, showed moderate activity all around the artery leading to a ring-like formation around the cross sectional area (Fig. 30). The intensity of the staining increased in areas where two anastomosing rete branches came close together because of the reaction of the overlying endothelial cells of the cavernous sinus. Also, the staining intensity increased in the caveolae of the endothelial cells, especially in those of the carotid rete branches.
Plate 12

Figure 29. An artery of the carotid rete of the goat showing black granules at the site of adenosine triphosphatase enzyme. Line scale = 46 μm.

AL. Arterial lumen
VL. Venous lumen

Figure 30. Enlarged wall of an artery of the carotid rete of the goat showing black staining at the site of adenosine triphosphatase enzyme. Line scale = 12 μm.

a. Lumen of an artery
b. Tunica intima
c. Tunica media
d. Tunica adventitia

Figure 31. Concentration of staining at both ends indicating the presence of adenosine triphosphatase enzyme in larger quantities in the endothelial walls of both the artery and the vein. Line scale = 46 μm.

AL. Arterial lumen
VL. Venous lumen

Figure 32. Enlarged portion of the wall of an artery of the carotid rete of the goat. Line scale = 12 μm.

a. Lumen of the artery
b. Tunica intima
c. Tunica media
In some sections, the reaction products were limited only to endothelial cells of both rete branches and the cavernous sinus with very little staining of the tunica media of rete branches (Fig. 31). In addition, a relatively small reaction zone was observed between the tunica media and the tunica adventitia. These observations were, however, evident in branches separated from the carotid rete or branches at the edge of it and were not surrounded by other arteries.

In areas where smooth muscle cells were present in the wall of the cavernous sinus, small, lightly stained sites were observed which indicated the presence of a small quantity of the enzyme. Besides, the enzymic activity tended to decrease in areas where some deformities of the internal elastic laminae and resultant change in the shape of endothelial cells were encountered (Fig. 32).

5'-Nucleotidase Enzyme (5'-N)

The endothelial cells as well as the internal elastic laminae of all rete branches were intensely stained. The tunica media, however, showed no reaction at all which indicated the absence of this enzyme (Figs. 33 and 34). The tunica adventitia was darkly stained in all specimens and the staining was continuous all around branches of the carotid rete. This region was common to the tunica adventitia of the adjacent cavernous sinus and both of them showed intense staining (Figs. 35 and 36). In addition, the endothelial cells of the cavernous sinus were darkly stained which, in turn, became continuous with its tunica adventitia.
Also, the capillaries were darkly stained and they were difficult to
discern from the surrounding tunica adventitia because of the intensity
of the staining which sometimes masked the surface or completely obscured
the lumen of the capillaries.
Plate 13

Figure 33. Dark staining in the walls of the carotid rete-cavernous sinus complex indicating the presence of 5'-nucleotidase enzyme. Line scale = 125 μm.

a. Lumen of the artery
b. Tunica media (unstained)

c. Lumen of the cavernous sinus

d. Common tunica adventitia of both an artery and a vein

Figure 34. Enlarged arterial wall of the carotid rete of sheep. Line scale = 53 μm.

a. Lumen of the artery
b. Tunica intima
c. Tunica media (unstained)
d. Common tunica adventitia of both an artery and a vein

Figure 35. Micrograph of positively reacted carotid rete arteries of sheep to 5'-nucleotidase enzyme. Line scale = 125 μm.

a. Lumen of an artery
b. Tunica media (unstained)
c. Lumen of the cavernous sinus

d. Common tunica adventitia of both an artery and a vein

Figure 36. Enlarged portion of Fig. 35. Line scale = 56 μm.

a. Tunica intima of an artery (lumen is not clear)
b. Tunica media
c. Common tunica adventitia of both an artery and a vein
d. Lumen of the cavernous sinus
DISCUSSION

Gross

In the elk, deer and pronghorn the veins drain relatively cool blood from the nasal area to the cavernous sinus. The anatomical differences among these species were contributed to different capacities for heat exchange which may correlate with their physiological needs affected by internal and external temperature changes (Carlton and McKean, 1977). Taylor (1974) developed an equation which predicts heat load during exercise and running. The internal heat generated during running is very large in quantity and it depends on the body mass of the animal and running speed.

The carotid rete of sheep and goat were supplied by one caudal rete branch and two rostral rete branches (ICVAN, 1973). In this study, the variations observed in the number of rostral rete branches could be due to differential growth between the rete and the specific branch during development. In some specimens, a rete branch divided shortly before it entered the rete, but the division was incorporated completely into the rete as the rete itself grew faster than that of the specific branch. In another instance, the rete branch divided at its origin from the maxillary artery and continued to arborize with the growth of the carotid rete, while its proximal portion got absorbed into the maxillary artery resulting in either two separate branches or a common trunk depending on the degree of fusion with the maxillary artery.
The complexity of the carotid rete and its close relationship with the cavernous sinus suggested that these structures could be a site for conditioning blood temperature before reaching the brain. This has been proven to be true in several mammalian species (Baker and Hayward, 1968a; Krabill, 1979; Magilton and Swift, 1968). In the calf, Uehara et al. (1978) found that close relationship between the structure of the carotid rete and the cavernous sinus existed and the main source of blood draining toward the cavernous sinus was relatively cool nasal venous blood. Similar findings were reported in sheep (Baker and Hayward, 1968b; Khamas and Ghoshal, 1982a). Baker and Chapman (1977) reported that the blood circulating through the brain of the dog, during heavy exercise in a warm environment, was 1-3°C cooler than the temperature of the blood leaving the heart. Therefore, maximum cooling of the brain occurs during exercise in carotid rete species. In contrast, during heat stress the temperature of the blood supplying the brain is the same as the core temperature blood. Jessen and Pongratz (1979) stated that during cold stress (i.e., hypothermia) the hypothalamic temperature in the goat was uncoupled from the temperature of the upper respiratory surfaces and presented an undistorted body core temperature due to shunting of blood away from the cavernous sinus to the jugular vein via the facial, i.e., reversing its usual flow pattern during normothermia and hyperthermia. The opposite is true in heavy exercise. Cabanac and Caputa (1979) found the above change in the pattern of circulation to be true in man.

In addition, Kluger and D'Alecy (1975) showed that the upper respiratory passages cool the brain even in noncarotid rete species such as the rabbit. They hypothesized that the venous blood draining the nasal
cavity, cooled by evaporation and convection, has two possible mechanisms for the cooling of the brain viz., countercurrent heat exchange between the carotid rete and the veins in the cavernous sinus in carotid rete species (the internal countercurrent heat exchange mechanism of Magilton and Swift, 1968) and by conduction from the cavernous and ventral petrosal sinuses to the brain. The last one accounted for the change in temperature in non-carotid rete species.

In this study, the carotid retia were connected to each other by several anastomotic branches, but these connections were nonfunctional under normal physiological conditions, and they presumably operate only when changes in the pressure between the two sides take place (Gillilan, 1974). The same consideration might be true in the case of the cavernous sinuses. In both sheep and ox the blood destined for the cerebral arterial circle at first passes through the well-developed intracranial carotid rete (Baldwin, 1964).

The blood flow in the basilar artery in ruminants is from rostral to caudal, i.e., away from the cerebral arterial circle, as evidenced by its decreasing caliber caudally and the branching arising from it at an acute angle in the direction of the flow. In the goat, the brain receives no contribution from the vertebral arteries (Anderson and Jewell, 1956). Further, the anastomoses between basilar and vertebral arteries are small and extremely sparse. Edelman et al. (1972) showed that the flow in the basilar artery of the goat was negligible, which was supported by the findings of Godynicki and Frackowiak (1979) in sheep. They stated that the blood flow to the carotid rete through the afferent arteries was relatively
greater than that carried by the internal cartoid artery, which was transporting the blood away from the rete to the cerebral arterial circle. In this investigation, there were two ventral anastomotic branches between the left and right retia, which gave rise to a branch from the middle of their anastomoses, and descended caudally paralleling the course of the basilar artery. Therefore, these ventral anastomotic branches represented an auxiliary vascular pathway carrying some of the extra blood away from the carotid rete in addition to the portion carried by the basilar artery.

The variations in the connection of the carotid rete with other structures like the ophthalmic rete have been reported and they may play a role in decreasing temperature gradients between the two structures. Moreover, cyclic alternation of airflow through both nostrils at a certain time interval has been reported in the man, rat and rabbit (Moller and Fahrenkrug, 1971; Principato and Ozenberger, 1970; Stoksted, 1952). It is conceivable that cyclic breathing might result in cooling of one side of the cavernous sinus and consequently the connections between two cavernous sinuses or the two carotid retia might enhance or decrease its effect on certain regions of the brain. In addition, alternation in brain function in man was reported, and this may be attributed to a difference in the production of heat between two hemispheres.

This rather important function (heat exchange) should not lead someone to overlook the other possible functions of the carotid rete. These functions include: (1) the extensive side-to-side communications within the rete insure maintenance of adequate cerebral blood flow in case of interruption of flow in one carotid artery (Daniel et al., 1953;
Edelman et al., 1972); (2) to protect the brain from unordinarily high perfusion pressure while allowing augmentation of cerebral blood flow in physiological circumstances (Edelman et al., 1972); (3) to act as a capacitor to lower high systolic pressure with a minimum effect on mean perfusion pressure, an ideal situation to the brain, which is very sensitive to edema formation, but requires relatively high blood flow for proper function (Edelman et al., 1972); (4) regulation of blood flow under normal circumstances (Ask-Upmark, 1935; Daniel et al., 1953); and (5) to assist in venous return from the cranium due to the absence of valves in the venous system of the cranial cavity (Barnett and Marsden, 1961).

Light Microscopy (L.M.)

The carotid rete is enmeshed by the cavernous sinus in sheep and the ox (Baldwin, 1964), which Gillilan (1974) described as a venous plexus in the cat. The veins in the cavernous sinus were thin-walled and resembled thin strands of connective tissue in un.injected specimens. In some regions, smooth muscle cells were visible in the wall of the cavernous sinus. These smooth muscle cells were apparently not that of an emissary vein passing through, because they completely surrounded the rete branches without any interruption. Therefore, the cavernous venous plexus would probably be the appropriate designation for the cavernous sinus in sheep. No smooth muscle cells were, however, noticed in the cavernous sinus of sheep fetus or the goat, and that could be due to the limited number of specimens used in this experiment.
In this experiment, no smooth muscle cells were seen in the subendothelial region. Structural differences of the carotid rete-cavernous sinus complex, such as frequent loss of smooth muscle cells in the wall of the cavernous sinus and sharing a common tunica adventitia with the rete branches, possibly augment the efficiency of the internal counter-current heat exchange system, which several investigators have described in other species. In addition, other factors, such as the temperature gradients, blood flow velocity in both the rete and the sinus, conductivity of the intervening vessel walls, etc., play a role in enhancing the heat exchange mechanism.

Intimal cushions or intraarterial bolsters were present in the arteries of the carotid rete of sheep, the goat and sheep fetus. Dahl (1976) stated that the intimal cushions and the folded internal elastic laminae may represent developmental defects. Stehbens (1960) considered the intimal proliferation of arteries in infants as normal structural components. Recent evidence suggests that turbulence may occur at stagnation points, such as bifurcations and branching sites of cerebral arteries. Several functions for the intimal cushions or intraarterial bolsters were reported: for instance, cell skimming (Fourman and Moffat, 1961); to influence the blood flow to a side branch and to increase the peripheral resistance (Hassler, 1961, 1962; Serban and Ovula, 1961); and finally to occlude or narrow the lumen of certain arteries (Elias and Pauly, 1966). In addition, the presence of a large number of epithelioid cells in the tunica media of small arterioles as well as in the intimal thickening was presumably for regulating the blood flow and for regulating the pressure in response
to sudden changes in general circulation.

The carotid body cells were observed in aberrant positions because of the postnatal degeneration of the extracranial portion of the internal carotid artery. These cells monitor changes in pH reflected by partial pressure of CO₂ or O₂ in the blood destined to supply the brain. De Castro (1926, 1928) suggested that the carotid body might be a receptor and concluded that the epithelioid cells were receptor cells with afferent fibers innervating them. Biscoe (1971) mentioned that sensory cells are small nonmyelinated nerve terminals enclosed in type II cells. The level of excitability of the receptor is set forth by a balance between the excitatory sympathetic efferents and depressant sinus nerve efferents.

The myelinated and unmyelinated axons in the adventitia of carotid rete branches and the internal carotid artery were easily recognizable because the myelin sheath was removed by alcohol during preparation of the tissue. Pease and Paule (1960) indicated that the lack of innervation of tunica media in the rat thoracic aorta was suggestive of its functional activity, which must depend upon intrinsic activation rather than upon nervous stimulation. Further, Krupp (1969) reported that the local blood flow in the cortex, as in many other organs, showed spontaneous rhythmical fluctuations. The rhythmical fluctuations were independent of the arterial pressure and of the respiratory cycle. In addition, the cerebral blood flow is regulated not only by the metabolic rate of the brain, but also by vasoactive substances released by nervous structures. On the other hand, Owman et al. (1974) proposed that the autonomic innervation of
cerebral arteries participates in the control not only of the cerebral circulation but also of associated intracranial pressure phenomena.

**Scanning Electron Microscopy (SEM)**

The vessels contributing to the formation of the carotid rete in sheep were similar to the observations of other investigators (Baldwin, 1964; Godynicki et al., 1981). However, scanning electron microscopic (SEM) study of this vascular complex was seemingly lacking in the literature. The intravascular injection technique used in this investigation showed that Batson's #17 yielded better results than Decopour under the SEM. Nopanitaya et al. (1979) suggested that Batson's should be diluted with Sevriton (a monomeric methyl methacrylate) for exceptional details and reproducible vascular casts. Further, they recommended this mixture for obtaining casts from highly vascularized organs as the eye, brain, tongue and kidney. In this experiment, however, Batson's was used without any dilution because of the size of the carotid rete branches.

The arterial branches of the carotid rete shared a common tunica adventitia with the cavernous sinus which might be a structural modification facilitating its probable role in the thermal regulation of the brain. The shape of the endothelial cells was generally influenced by fixation procedure (Buss and Hollweg, 1977), but their irregularities in this experiment could also be due to the intertwining of vessels of this complex. Buss and Hollweg (1977) stated that veins frequently showed a very regular arrangement of large, flat endothelial cells with slightly protruding nuclei.
The internal elastic lamina was folded in specimens examined which could be due to several factors, like the muscle tone resulting from bleeding of animals to death and the contraction of the internal elastic lamina resulting in folding of the endothelium (Edanaga, 1974).

Craters in the endothelial cells have been described in ischemic conditions as a consequence of temporary clamping or prolonged washing of the specimen prior to fixation (Fonkalsrud et al., 1976; Kawamura et al., 1974). Although a direct relationship between the formation of craters and balloons has not been proven, it seemed that craters usually represent ruptured balloons. In rabbits fixed by perfusion, Edanaga (1974) observed craters in the brachiocephalic trunk and at branching sites of dorsal intercostal arteries. Although he did not link the appearance of craters to any physiological condition, he considered them as possible result from any damage to the endothelial cells, especially at the branching and bending sites of vessels. Further, Nelson (1973) hypothesized that crater formation is a nonspecific reaction of endothelial cells to injury. These craters could alter the permeability of the blood vessels and might contribute to the formation of thrombi. In this study, a few craters were observed in some branches of the carotid rete but not in the wall of the cavernous sinus. Their occurrence might be attributed to the fixation procedure since the glutaraldehyde was directly injected into the arteries resulting in maximum effect on their walls. Buss and Hollweg (1977) attributed to the presence of craters to collapsed or emptied blebs or vacuoles of altered endothelial cells created by multiple causes during preparation of the specimens. In addition, Clark and Flagov (1976),
after using perfusion of fixatives at a physiological pressure, concluded that vascular surface artifacts could be minimized but are not entirely avoidable with currently available techniques.

Histochemistry

Alkaline phosphatase enzyme

Three types of alkaline phosphatase (A.P.) enzymes have been described by Moss (1969). Their high concentrations are usually associated with absorptive cells and calcification sites. In this study, the presence of A.P. enzyme was usually detected in and around areas where blood capillaries were present. This is indicative of active transport of materials through the capillary membrane, concordant with the findings of Gomori (1941) in dogs, gophers, guinea pigs, cats, mice and ground hogs, and Doty (1980) in the mice, rats, dogs, chicks, cell culture and human biopsy. Further, Jancso et al. (1975) demonstrated the presence of this enzyme in the cytoplasm of endothelial cells of the capillaries of brain smears of albino rats. There was also a very small amount of A.P. enzyme on the endothelial surface of arteries which indicated some activities in that region.

One of the specimens investigated was kept overnight in the incubation medium at 37°C to ensure the detection of even very scanty amount of enzyme. No difference was, however, observed in the carotid rete-cavernous sinus complex because of the absence of the enzyme in the wall of this structure.

Gomori (1941) indicated that acetone is a good preservative for A.P.
enzyme on both human and animal tissues under normal and pathological conditions. The procedure used for the detection of the enzyme in the present study is adapted from Gomori (1952). It was selected because it gives good results in both human and animal tissues. Further, fresh frozen retia, without fixation, were used to assure the detection of any A.P. enzyme in conjunction with a fresh frozen liver specimen as a control. Several previous studies reported the availability of this enzyme in the liver of man, mouse, dogs, cats, guinea pigs and goats (Gomori, 1941; Nanda et al., 1979).

Because of the wide distribution and presence of this enzyme in different organs in the body, the specific function or functions cannot be attributed to A.P. (Low and Finean, 1977). Danielli (1953) suggested that the enzyme acts in the transport of materials across the plasma membrane, thus adjusting the fluid and electrolyte concentration. This might help in adjusting the blood perfusion pressure directly or indirectly. In the carotid rete-cavernous sinus complex, this enzyme might have an insignificant role to play because of its apparent scarcity, although it might help in hydrolyzing residual phosphates, such as monophosphates, pyrophosphates or adenosine triphosphates (Cox and Griffin, 1965; Moss and Walli, 1969).

Adenosine triphosphatase enzyme

Several types of adenosine triphosphatase (ATP-ase) enzymes have been described in the literature. These included mitochondrial, myosine—mainly in striated muscle (Balo et al., 1948), cytoplasmic (Hori and Chang,
1963) and finally the (Na\(^+-\)K\(^+\))-activated ATP-ase (Tormey, 1966; Guth and Albers, 1974; Firth, 1980). In this study, attempts were made to discern the presence of the (Na\(^+-\)K\(^+\))-activated ATP-ase. The presence of this enzyme has been linked to active transport of materials across the plasma membrane and it has usually been associated with the (Na\(^+-\)K\(^+\)) pump on the cell membrane (Novikoff and Essner, 1960; Tormey, 1966; Kus, 1967). The pump activity is dependent on the presence of ATP and thus it may regulate or control cell volume due to the removal of Na\(^+\) from the intracellular space resulting in water withdrawal to the extracellular space. In this experiment, the function of ATP-ase enzyme on the endothelial surfaces of both carotid rete branches and the cavernous sinus was probably to regulate the cell volume. Therefore, it might regulate the diameter of the human vessel (Ernst, 1972; Guth and Albers, 1974). Moreover, it has been experimentally demonstrated that the countercurrent heat exchange system operates between the core temperature blood in carotid rete branches and the relatively cool venous blood in the cavernous sinus. Therefore, increased activity of the endothelial ATP-ase of the cavernous sinus could result in an increase of intercellular fluid circulation around carotid rete branches. Thus, this mechanism could augment the countercurrent heat exchange system.

Because of its presence in the cell membrane, several functions have been given to the (Na\(^+-\)K\(^+\))-activated ATP-ase enzyme. These functions include the generation of a membrane potential because of its ability to extrude Na\(^+\) which, in turn, results in an increase of the intracellular K\(^+\) (Firth, 1974; Guth and Albers, 1974). Further, the enzyme regulates the
electrolyte concentration within the cell and within the interstitial tissue (Guth and Albers, 1974). In the carotid rete-cavernous sinus complex, the ATP-ase could play all of the above functions. It might be assumed that there were some kind of receptors on the endothelial cells which respond to temperature changes. Their activation because of temperature changes could result in an inhibition of membrane potential due to decrease of Na$^+$ extrusion. Eventually, Na$^+$ and K$^+$ either stimulate or inhibit the smooth muscle cells in the tunica media influencing action potential and its subsequent contraction or relaxation. Therefore, the blood flow and blood pressure could be regulated in the carotid rete-cavernous sinus complex according to physiological needs.

Rosenblum (1970) stated that an increase in plasma viscosity is more effective in prolonging the transit time through the microcirculation than an increase in hematocrit. In small vessels of the carotid rete-cavernous sinus complex, prolonging the transit time could help in augmenting the countercurrent heat exchange mechanism. As Na$^+$ pumped out into the interstitial space, water will follow resulting in an increase of plasma viscosity. This could lead to prolonging the transit time of blood from the rete to the cerebral arterial circle.

Moreover, Eisenberg and Suddith (1979) found that the activity of (Na$^+\text{-K}$)$^+$-activated ATP-ase enzyme and the uptake of rubidium, a potassium analog, were similar in brain microvessels and the choroid plexus. They concluded that the brain microvessels have cation transport systems like those of the choroid plexus. Secretion of cerebrospinal fluid (CSF) by the choroid plexus has been shown to be directly related to the
activity of (Na\(^+-\)K\(^+\))-activated ATP-ase in that structure (Vates et al., 1964). It has also been shown that the (Na\(^+-\)K\(^+\))-activated ATP-ase enzyme was directly involved in the secretion of aqueous humor in cat and man (Simon and Bonting, 1962; Simon et al., 1962).

In view of the distribution of the histochemically demonstrated ATP-ase enzyme in epithelia noted for active transport, one could speculate that the presence of this enzyme is related to active transport. However, Ellis et al. (1963) stated that there is no direct correlation between histochemically demonstrated ATP-ase activity and transcellular transport because of the absence of this enzyme in the secretory epithelium of the avian salt gland, although such glands are notably engaged in electrolyte transport and contain high concentration of sodium pump (Ernst, 1972; Hokin, 1963).

This enzyme was demonstrated in capillaries, tunica intima and tunica media of arteries in the dog kidney (Freiman and Kaplan, 1960; Hori and Chang, 1963) and in smooth muscles and endothelium of large coronary vessels of albino rats (Padykula and Herman, 1955). The endothelial cells of the blood vessels, in general, possess ATP-ase activity mainly on their surface caveolae (Hoff and Graf, 1966; Marchesi and Barnett, 1963). Jancso et al. (1975) found that ATP-ase was localized in the basement membrane of blood capillaries. In this study, the positive reaction of the endothelial surface of carotid rete branches indicated the presence of this enzyme, which might function as previously described.
5'-nucleotidase enzyme

The procedure for the detection of 5'-nucleotidase (5'-N) enzyme was adapted after Wachstein and Meisel (1957), except that the sections were placed directly on slides due to the difficulty in transferring the carotid rete sections between solutions. This was so because the carotid rete branches were interconnected by delicate connective tissue strands which break up easily when placed in solutions.

In this experiment, the enzyme was found to be present in the tunica adventitia of both rete branches and the cavernous sinus, as well as their endothelial cells, which showed intense reaction. As the tunica media of the rete branches did not show any reaction, it has been concluded that this enzyme was absent in that region. Species variations have been reported in the distribution of (5'-N) enzyme (Wachstein and Meisel, 1957). They reported the presence of (5'-N) in the wall of arteries and veins of the rat and guinea pig liver, whereas only the endothelium of vessels positively reacted in the mouse, dog and man.

In addition to blood vessels, the (5'-N) enzyme has been shown to be present in several other structures in the body as the spinal cord in the mouse and cat (Suran, 1974); the brain of the mouse (Scott, 1965) and the liver of the rat, mouse, guinea pig, goat and man (Nanda et al., 1979; Wachstein and Meisel, 1957).

Catabolic functions were attributed to this enzyme by Hardonk and Kondstaal (1968), while Goldberg (1973) mentioned a function of degrading residual nucleotidases. Finally, a function similar to those reported for ATP-ase was mentioned by Novikoff (1958). In this study, the (5'-N) enzyme
perform all functions enumerated above. But, the results indicated that the enzyme was primarily present on the endothelial surfaces and the tunica adventitia of both carotid rete branches and the cavernous sinus. This suggests that the enzyme is related to the surface endothelial cell and function in transporting materials across the plasma membrane. Catabolic function in this case is also possible because of the relatively rapid turn-over of the endothelial cells and their faster regeneration in comparison to that of smooth muscle cells of the tunica media. This might explain the absence of (5'-N) enzyme from the tunica media of rete branches. Other functions of the enzyme previously mentioned should not be overlooked, however.
SUMMARY AND CONCLUSIONS

A total of 56 sheep and goats were used to study the morphology of the carotid rete-cavernous sinus complex. Different intravascular plastic injectable materials were used to depict its detailed structure. The extracranial portion of the internal carotid artery in full-term fetus as well as in adult sheep and goat was usually absent, as is the case with with the caudal carotid rete (Rete mirabile epidurale caudale) in both species. The rostral carotid rete (Rete mirabile epidurale rostrale) was supplied by one caudal and two to four rostral rete branches. Possible genesis of variations in the number of rostral rete branches was discussed reminiscing the embryological development of vessels in the head region.

For light microscopic study, different stains were employed to describe the histomorphology of this structure. The carotid rete consisted of medium-size, muscular arteries with well-developed internal elastic laminae. Thickness of the cavernous sinus wall considerably varied between regions from a thin-walled structure to a venous plexus with smooth muscle cells in its wall. Because of regression or involution of the extracranial portion of the internal carotid artery early in life in both species, the carotid body cells were observed in an aberrant location along the intracranial portion of the internal carotid artery within the cavernous sinus.

Further, cryofracture-freeze substituting technique and plastic casts were used for scanning electron microscopic study. They revealed different shapes of endothelial cells of the carotid rete vis-a-vis the
cavernous sinus. Also, craters were discernible in the wall of the carotid rete but not in the wall of the cavernous sinus.

In addition, attempts were made to detect the presence of alkaline phosphatase, Na\(^+\)-K\(^+\) activated adenosine triphosphatase, and 5'-nucleotidase enzymes in this vascular complex. To a varying extent, these enzymes or their reaction products were distributed in different parts of this complex and depending on their sites of localization, various possible functions have been suggested.

In general, the basic organization of the carotid rete-cavernous sinus complex of sheep and goat was very similar. The possible role of this vascular structure in the thermal regulation of the brain (in hypothermia, normothermia, and hyperthermia) as well as the direct or indirect role it might play in influencing the general behavior and various functions of other systems of the body has been discussed.


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