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THE HISTOCHEMISTRY OF HYALURONIC ACID AND RELATED MUCOSACCHARIDES IN THE CEREBRAL AND OTHER ARTERIES OF THE DOG*

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ABSTRACT

In the cerebral and other arteries of dogs of different ages, histochemical studies have been made on hyaluronic acid and related mucosaccharides by means of digestion procedures with Streptomyces and testicular hyaluronidases. In the cerebral arteries, hyaluronic acid is either scanty in amount or absent, but other arteries contain histochemically demonstrable amounts of this mucosaccharide which decreases, however, with advancing age of the animals. The suppressive effects of digestion with testicular hyaluronidase upon the alcianophilia (pH 1.0) of all the arteries examined indicate that varying amounts of isomeric chondroitin sulfates are present in the arterial tissues. The technique of digestion with Streptomyces hyaluronidase established previously[1] has been found to be useful for the histochemical identification of hyaluronic acid in the tissues of dog arteries.

INTRODUCTION

In some higher mammals, the cerebral arteries have been found to exhibit a number of structural and functional peculiarities, as compared with arterial vessels in other regions of the body[2)3)4)5)]. In keeping with such peculiarities, it seems possible that the cerebral arteries can be distinguished in mucosaccharide composition from other arteries. Therefore, histochemical studies on mucosaccharides in cerebral arteries as compared with those in other arteries must be promising for better recognition of the peculiar functions of the cerebral vessels. For the histochemical demonstration of particular acid mucosaccharides, the technique of digestion with testicular hyaluronidase[6)7)] has proved to be useful and the procedure of digestion with Streptomyces hyaluronidase established recently[1)] appears to open a new avenue for the histochemical identification of hyaluronic acid.

In the present study, the procedures of digestion with Streptomyces and testicular hyaluronidases were employed for the tissues of cerebral and other

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arteries from dogs of different ages, in an attempt to gain an insight into the mucosaccharide compositions of the vessels. As a result of this, the cerebral arteries were shown to be nearly devoid of histochemically demonstrable hyaluronic acid, whereas other arteries were found to contain varying amounts of this mucosaccharide. Such histochemical characteristic of the cerebral arteries was discussed in relation to their peculiar functional activities.

MATERIALS AND METHODS

Six mongrel adult dogs and three mongrel puppies of both sexes were used in the present study. As premedication, these animals were subcutaneously injected with morphine chloride (0.8 mg per kg of body weight). Thirty minutes after the premedication, they were anesthetized by intermittent intravenous injection of 2.5% Isozol [Sodium 5-allyl-5-(1-methyl-buthyl)-2-thiobarbiturate] (Yoshitomi Pharm. Co. Ltd., Japan). From the anesthetized animals, basilar, mesenteric and popliteal arteries were dissected out as quickly as possible. All these arteries were cut into small pieces and fixed for 12 to 24 hours at 4°C in two fixing solutions; 2% calcium acetate in 10% formalin[8] and 10% formalin in 95% ethanol[9]. Then, the tissues were dehydrated in ethanol series of ascending concentrations and embedded in paraffin in the usual manner. Sections were cut at 6 or 8 μ in thickness and the following five stainings of histology and histochemistry were performed; (a) hematoxylin and eosin, (b) alcian blue (pH 1.0)[10], (c) alcian blue (pH 2.5)[11], (d) periodic acid-Schiff (PAS) (modification of McManus[12]) and (e) alcian blue (pH 2.5)-PAS[13].

Hyaluronidase digestion experiments

(1) Digestion with Streptomyces hyaluronidase

Prior to staining, some sections were digested with Streptomyces hyaluronidase; incubation for 2 hours at 60°C in 0.1 M phosphate buffer (pH 5.0) containing Streptomyces hyaluronidase (Amano Pharm. Co. Ltd., Japan) at an activity concentration of 100 Turbidity Reducing Units (TRU)/ml[1].

(2) Digestion with testicular hyaluronidase

Prior to staining, other sections were digested with testicular hyaluronidase; immersion for 2 hours at 37°C in 0.1 M phosphate buffer (pH 5.5) containing testicular hyaluronidase (Sigma Chem. Co. Ltd., U.S.A.) at a concentration of 1 mg/ml[1].

(3) Controls

Two types of control experiments for the above digestion procedures were conducted. (a) Some control sections were incubated in buffer solutions without enzymes under the incubation conditions of the same duration and temperature. (b) Other control sections were immersed in buffer solutions with heat inactivated enzymes under the same incubation conditions as control (a).

RESULTS

1. The staining reactions of adult dog arteries

(a) Reactions of the basilar arteries

Microscopic observations of hematoxylin and eosin stained sections indicate that the basilar arteries of the dog consist of three layers; the intima, media and adventitia. The alcian blue (pH 1.0), alcian blue (pH 2.5), PAS and alcian blue-PAS reactions of the three layers in the basilar arteries of the control, Streptomyces hyaluronidase digested and testicular hyaluronidase digested specimens are summarized in Table 1.

As is illustrated in Table 1, digestion with Streptomyces hyaluronidase fails to alter both the intensity and shade of the four staining reactions in the three layers (Figs. 1 and 2). In contrast to this, digestion with testicular hyaluronidase suppresses apparently or abolishes the alcianophilia (pH 1.0 and pH 2.5) of all the three layers (Fig. 3). Furthermore, this enzyme digestion tends to increase the PAS staining intensity of the three layers, while such effects being most distinguished in the intima. The digestion with the testicular enzyme suppresses or abolishes the alcianophilia of the three layers that reacted to alcian blue-PAS.

(b) Reactions of the mesenteric arteries

There are three layers in the mesenteric arteries of the dog, as in the basilar vessels. The four staining reactions of the three layers of the mesenteric arteries in the control and experimental specimens are presented in Table 2.

Digestion with Streptomyces hyaluronidase does not induce any appreciable changes in the intensity and shade of the alcian blue (pH 1.0) and PAS stainings in the three layers of the arteries. However, this digestion procedure results in an apparent diminution in intensity of the alcianophilia (pH 2.5) of the three layers (Figs. 4 and 5). The enzyme digestion tends to suppress the alcianophilia of the three layers stained by the alcian blue-PAS method. The alcian blue (pH 1.0 and pH 2.5) reactions of the three layers, above all those of the media and adventitia are strikingly diminished in intensity following digestion with testicular hyaluronidase (Fig. 6). The digestion with this enzyme characteristically increases the PAS staining intensity of the media, while it suppresses or abolishes the alcianophilia of the three layers stained with the alcian blue-PAS procedure.

(c) Reactions of the popliteal arteries

The three layers, intima, media and adventitia are the constituents of the popliteal arteries as in the above mentioned vessels. In Table 3, the four staining reactions of the three layers of the popliteal arteries in the control and experimental specimens are illustrated. As in the case with the mesenteric arteries, digestion with Streptomyces hyaluronidase fails to alter the alcian blue (pH 1.0) and PAS reactions of the three layers in the popliteal arteries,

TABLE 1. Mucosaccharide staining reactions of the basilar arteries in the adult dog

		AB (pH 1.0)	AB (pH 2.5)	PAS	AB-PAS
Control tissues	Intima	1~2 B	1~2 B	3 M	3 BM
	Media	1 B	1~2 B	1~2 M	1~2 BM
	Adventitia	1 B	1 B	±~1 M	1 MB
Streptomyces hyaluronidase digested tissues	Intima	1~2 B	1~2 B	3 M	3 BM
	Media	1 B	1~2 B	1~2 M	1~2 BM
	Adventitia	1~2 B	1 B	±~1 M	1 MB
Testicular hyaluronidase digested tissues	Intima	±~1 B	±~1 B	3~4 M	3~4 M
	Media	−~±B	±B	2 M	1~2 M
	Adventitia	±B	±~1 B	±~1 M	1 BM

AB=Alcian blue, PAS=Periodic acid-Schiff, B=Blue, M=Magenta, BM=Bluish magenta, MB=Magenta blue, PM=Purplish magenta, −=A negative reaction, ±=A feeble or doubtful reaction, 1~n=The number being proportional to the intensity of reaction. The abbreviations in Table 1 apply to all the following Tables.

TABLE 2. Mucosaccharide staining reactions of the mesenteric arteries in the adult dog

		AB (pH 1.0)	AB (pH 2.5)	PAS	AB-PAS
Control tissues	Intima	1~2 B	2 B	1~2 M	1~2 BM~MB
	Media	1~2 B	1~2 B	1~2 M	1~2 BM~MB
	Adventitia	1~2 B	1~2 B	1~2 M	1~2 BM~MB
Streptomyces hyaluronidase digested tissues	Intima	1~2 B	1~2 B	1~2 M	1~2 BM
	Media	1~2 B	± B	1~2 M	1~2 BM
	Adventitia	1~2 B	±~1 B	1~2 M	1~2 BM
Testicular hyaluronidase digested tissues	Intima	±~1 B	±~1 B	1~2 M	1~2 BM
	Media	−~±B	−~±B	2~3 M	1~2 M
	Adventitia	−~±B	± B	1~2 M	1~2 M

TABLE 3. Mucosaccharide staining reactions of the popliteal arteries in the adult dog

		AB (pH 1.0)	AB (pH 2.5)	PAS	AB-PAS
Control tissues	Intima	2 B	1~2 B	1~2 M	1~2 MB
	Media	1~2 B	1 B	1~2 M	1~2 MB
	Adventitia	1~2 B	1 B	1~2 M	1~2 MB
Streptomyces hyaluronidase digested tissues	Intima	2 B	1~2 B	1~2 M	1~2 BM
	Media	1~2 B	±~1 B	1~2 M	1~2 BM
	Adventitia	1~2 B	1 B	1~2 M	1~2 BM
Testicular hyaluronidase digested tissues	Intima	1~2 B	±~1 B	1~2 M	1~2 BM
	Media	−~±B	−~±B	1~2 M	2 M
	Adventitia	±B	±B	1~2 M	1~2 M

but the enzyme digestion tends to suppress the alcianophilia (pH 2.5) of the media (Figs. 7 and 8) and to diminish the blue stainability of all the three layers that reacted to alcian blue-PAS. The effects of digestion with testicular hyaluronidase upon the alcian blue (pH 1.0 and pH 2.5) and alcian blue-PAS reactions of the three layers in the popliteal arteries are nearly comparable to those of the mesenteric arteries (Fig. 9).

2. The staining reactions of puppy arteries

(a) Reactions of the basilar arteries

The three layers, intima, media and adventitia, are well differentiated in the basilar arteries of the puppy, as in the adult dog. The four staining reactions of these three layers of the basilar arteries in the control and experimental specimens are summarized in Table 4. As is shown in Table 4, the effects of digestions with Streptomyces and testicular hyaluronidases upon the mucosaccharide staining reactions of the basilar arteries in the puppy are nearly comparable to those of the same arterial tissues of the adult dog (Figs. 10, 11 and 12).

(b) Reactions of the mesenteric arteries

The three layers are differentiated in the mesenteric arteries of the puppy as in the basilar arteries. In Table 5, the staining reactions of the three layers of the mesenteric arteries in the control and experimental specimens are illustrated. Except that all the four staining reactions of the three layers in control tissues tend to be vivid, the reactions of the tissues are found to undergo changes which are similar to those in adult animals following digestions with the two hyaluronidases.

(c) Reactions of the popliteal arteries

As in the above mentioned two arteries, the popliteal arteries of the puppy consist of three layers. The staining reactions of the arteries in the control and experimental tissues are shown in Table 6. The results obtained here

TABLE 4. Mucosaccharide staining reactions of the basilar arteries in the puppy

		AB (pH 1.0)	AB (pH 2.5)	PAS	AB-PAS
Control tissues	Intima	1~2 B	2~3 B	3 M	3 BM~MB
	Media	1~2 B	2 B	2 M	2 MB
	Adventitia	1~2 B	1 B	1 M	1 MB
Streptomyces hyaluronidase digested tissues	Intima	2 B	2 B	3 M	3 BM~MB
	Media	2 B	2 B	2 M	1~2 MB
	Adventitia	2 B	1~2 B	1 M	1 MB
Testicular hyaluronidase digested tissues	Intima	1 B	−~±B	3~4 M	3 PM~M
	Media	1 B	1 B	2~3 M	2 PM~M
	Adventitia	−~±B	−	1~2 M	1~2 PM~M

TABLE 5. Mucosaccharide staining reactions of the mesenteric arteries in the puppy

		AB (pH 1.0)	AB (pH 2.5)	PAS	AB-PAS
Control tissues	Intima	2~3 B	2~3 B	3 M	2 MB
	Media	2 B	2 B	2 M	2 MB
	Adventitia	2 B	2 B	2 M	2 MB
Streptomyces hyaluronidase digested tissues	Intima	3 B	1 B	3 M	3 BM~M
	Media	2 B	1 B	2 M	2 BM~M
	Adventitia	2 B	−~±B	2 M	2 BM~MB
Testicular hyaluronidase digested tissues	Intima	1 B	1 B	3 M	3 M~PM
	Media	1 B	±~1 B	2~3 M	2 M
	Adventitia	−	−~±B	3 M	2 M

TABLE 6. Mucosaccharide staining reactions of the popliteal arteries in the puppy

		AB (pH 1.0)	AB (pH 2.5)	PAS	AB-PAS
Control tissues	Intima	3 B	3 B	2 M	2~3 MB
	Media	2 B	2 B	2 M	2 MB
	Adventitia	2 B	2~3 B	2 M	2 MB
Streptomyces hyaluronidase digested tissues	Intima	3 B	1 B	2 M	1~2 BM
	Media	2 B	±B	1~2 M	1 BM
	Adventitia	2 B	±~1 B	2 M	1~2 MB
Testicular hyaluronidase digested tissues	Intima	±B	±B	2 M	2 PM~M
	Media	−	−~±B	2 M	2 PM~M
	Adventitia	−~±B	−	2 M	2 PM~M

are essentially similar to those observed in the mesenteric arteries.

DISCUSSION

It is generally accepted that the alcian blue (pH 1.0) method is a specific histochemical means of demonstrating sulfated mucosaccharides[10) 11) 14) 15) 16)]. In the present study, the three layers of all the control arteries examined have been found to stain with the alcian blue (pH 1.0) procedure. This finding indicates that in these arteries sulfated mucosaccharides are apparently present. The alcian blue (pH 2.5) method is believed to give rise to alcianophilia in the tissues which contain weakly acidic mucosaccharides such as hyaluronic acid, sialomucins and weakly acidic sulfated mucosaccharides[11) 14)]. In all the control arteries studied here the three layers have been found to exhibit alcianophilia (pH 2.5), when they are stained by the alcian blue (pH 2.5) or alcian blue (pH 2.5)-PAS procedure. Therefore, it is apparent that the three

layers contain weakly acidic mucosaccharides such as hyaluronic acid, sialomucins and weakly acidic sulfated mucosaccharides.

Streptomyces hyalurenidase first introduced into the histochemistry of acid mucosaccharides by Yamada[1], is known to exhibit an absolute substrate specificity; hyaluronic acid is the only mucosaccharide which can be degraded by this enzyme and various other acid mucosaccharides are never affected by it. Thus, the alcianophilia (pH 2.5) of any histologic structures abolished by digestion with the Streptomyces enzyme is conceived to be due to the presence of hyaluronic acid in tissues[1]. In the present study, digestion with Streptomyces hyaluronidase fails to alter the alcian blue (pH 2.5) reaction of the three layers of the basilar arteries in both the adult and young dogs, indicating that hyaluronic acid is either negligible in amount or absent in the tissues. In contrast, digestion with Streptomyces hyaluronidase has been found to diminish, to a variable extent, the alcianophilia (pH 2.5) of the three layers of the mesenteric and popliteal arteries in both the adult and young dogs. Such susceptibility of the alcian blue reaction to the enzyme is taken to indicate that hyaluronic acid is present in the tissues. In the present observations, the Streptomyces hyaluronidase labile alcianophilia (pH 2.5) of the arterial tissues from puppies is found to be more distinguished in intensity than that of the tissues from adult dogs. Therefore, the hyaluronic acid content is significantly higher in the tissues of puppies than in those of adult dogs. Such diminution in hyaluronic acid content of the arterial tissues with advancing age was previously reported by Zugibe[17], who examined the alcian blue stained human arteries digested with streptococcal hyaluronidase. The substrate specificity of testicular hyaluronidase is histochemically known to be lower than that of Streptomyces hyaluronidase; a series of acid mucosaccharides such as hyaluronic acid, chondroitin, chondroitin sulfates A and C are degraded by the testicular enzyme[1,7]. In the present study, digestion with the testicular enzyme has been shown to reduce differently the alcianophilia (pH 1.0 and pH 2.5) of the three layers in all the arteries examined. In addition, the effect of digestion with the enzyme upon the alcianophilia (pH 2.5) of the tissues is more marked than that of digestion with the Streptomyces enzyme. Hence, it is obvious that chondroitin, chondroitin sulfates A and/or C are present in the tissues. It has been reported that in human arteries there is no great increase in total acid mucosaccharides with aging[17,18]. However, the nature of acid mucopolysaccharides does alter in the senescent artery; isomeric chondroitin sulfates involved undergo qualitative and quantitative changes with aging[18,19,20]. In the present study, however, it seems uncertain whether the amount of histochemically demonstrable acid mucosaccharides other than hyaluronic acid does or does not vary with age in all the arteries examined.

Relatively little information is available on the physiological functions of acid mucosaccharides in animal tissues. However, the natures of the physio-

logical activity of hyaluronic acid have been rather well elucidated; hyaluronic acid is an essential component of the interfibrillar ground or cement substances in some connective tissues[21], has molecules with a very large hydrodynamic specific volume which is essential for the preservation of water in tissues[22], is a negatively charged macromolecule which can act like a cation exchange resin, regulating the behavior and concentration of cations in intercellular spaces[22] and performs many different functions such as the regulation of diffusion of substances in tissues, lubrication and shock absorption in joint and holding cells together in jelly like matrix[21][22]. It remains to be settled how the scantiness or absence of hyaluronic acid in the tissues of the dog basilar arteries is to be related to the structural and functional peculiarities of the cerebral arteries in general, even though the possible physiological functions of this mucosaccharide are taken into consideration. It has been reported that the hyaluronic acid level declines in rabbit aorta with not only increasing age of the animals but with progressing stages of experimental arteriosclerosis in them[23]. According to the statistical data of previous studies on different arteries in human beings[24][25], the cerebral arteries are highly liable to undergo arteriosclerotic changes[18]. Based upon such sets of facts, the scantiness or absence of hyaluronic acid in the dog basilar arteries may interestingly be comprehended; in view of the possible physiological functions of hyaluronic acid mentioned, the scantiness or absence of this acid mucosaccharide appears to result in a disturbance of metabolic processes in the basilar arteries, which should lead easily to arteriosclerotic changes in the arterial tissues. Such concept is consistent with the results of previous works by Bollet and coworkers[26] and Gerö and coworkers[27], who reported that a changed composition of mucopolysaccharides produced experimentally in the arteries of animals is associated with the deposition of lipids and other substances in the arterial tissues.

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REFERENCES

1) Yamada, K., Effects of novel (Streptomyces) hyaluronidase digestion upon some mucosaccharide stainings of the cartilages and aortas in the rabbit and rat, *Histochemie*,

27, 277, 1971.
2) Forbes, H. S. and Cobb, S. S., Vasomotor control of cerebral vessels, *Brain*, **61**, 211, 1938.
3) Shenkin, H. A., Harmel, M. H. and Kety, S. S., Dynamic anatomy of the cerebral circulation, *Arch. neurol. psychiat.*, **60**, 240, 1948.
4) Lassen, N. A., Cerebral blood flow and oxygen consumption in man, *Physiol. Rev.*, **39**, 183, 1959.
5) Nelson, E. and Rennels, M., Innervation of intracranial arteries, *Brain*, **93**, 475, 1970.
6) Zugibe, F. T., The demonstration of the individual acid mucopolysaccharides in human aortas, coronary arteries and cerebral arteries. 1. The methods, *J. Histochem. Cytochem.*, **10**, 441, 1962 a.
7) Leppi, T. J. and Stoward, P. J., On the use of testicular hyaluronidase for identifying acid mucins in tissue sections, *J. Histochem. Cytochem.*, **13**, 406, 1965.
8) Leppi, T. J., Morphochemical analysis of mucous cells in the skin and slime glands of hagfishes, *Histochemie*, **15**, 68, 1968.
9) McManus, J. F. A. and Mowry, R. W., Effects of fixation on carbohydrate histochemistry, *J. Histochem. Cytochem.*, **6**, 306, 1958.
10) Lev, R. and Spicer, S. S., Specific staining of sulphate groups with alcian blue at low pH, *J. Histochem. Cytochem.*, **12**, 309, 1964.
11) Spicer, S. S., Horn, R. G. and Leppi, T. J., Histochemistry of connective tissue mucopolysaccharides. The connective tissue, Edited by DE Smith, BM Wagner, The Williams and Wilkins Company, Baltimore, 1967, p. 251.
12) McManus, J. F. A., Histological and histochemical uses of periodic acid, *Stain Technol*, **23**, 99, 1948.
13) Mowry, R. W., The special value of methods that color with both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcian blue 8GX and their combinations with the periodic acid Schiff reaction, *Ann. N.Y. Acad. Sci.*, **106**, 402, 1963.
14) Pearse, A. G. E., Histochemistry, Theoretical and Applied. Vol. 1, J. and A. Churchill, Ltd., London, 1968, p. 346.
15) Sorvari, T. E. and Sorvari, R. M., The specificity of alcian blue pH 1.0 alcian yellow pH 2.5 staining in the histochemical differentiation of acidic groups in mucosubstances, *J. Histochem. Cytochem.*, **17**, 291, 1969.
16) Yamada, K., Dual staining of some sulfated mucopolysaccharides with alcian blue (pH 1.0) and ruthenium red (pH 2.5), *Histochemie*, **23**, 13, 1970.
17) Zugibe, F. T., The demonstration of the individual acid mucopolysaccharides in human aortas, coronary arteries and cerebral arteries. II. Identification and significance with aging, *J. Histochem. Cytochem.*, **10**, 448, 1962 b.
18) Adams, C. W. M., Vascular histochemistry. In relation to the chemical and structural pathology of cardiovascular disease, LLOYD-LUKE (Medical books), Ltd., London, 1967, p. 35 and p. 176.
19) Kaplan, D. and Meyer, K., Mucopolysaccharides of aorta at various ages, *Proc. Soc. exp. Biol. Med. (N.Y.)*, **105**, 78, 1960.
20) Zugibe, F. T. and Brown, K. D., Histochemical studies in atherogenesis: Human cerebral arteries, *Circ. Res.*, **9**, 897, 1961.
21) Meyer, K., The biological significance of hyaluronic acid and hyaluronidase, *Physiol. Rev.*, **27**, 335, 1947.
22) Kuwano, S., Study of acid mucopolysaccharides in the pathological synovial fluid, *Igaku Kenkyu*, **39**, 119, 1969.
23) Gurskaya, O. A., Histochemical characteristics of aortic wall mucopolysaccharides in animals of various ages with experimental atherosclerosis, *Tr. Vses. Konf. Gerontol.*

Geriat., **2**, 215, 1969.
24) Wolkoff, K., Normal structure and age-changes of the cerebral arteries, *Beitr. Path. Anat.*, **91**, 515, 1933.
25) Manley, G. and Hawksworth, J., Distribution of mucopolysaccharides in the human vascular tree, *Nature* (*Lond.*), **206**, 1152, 1965.
26) Bollet, A. J., Wang, C. and Adlersberg, D., Experimental atheromatosis: Acid mucopolysaccharide content of the aorta, *Circ. Res.*, **8**, 88, 1960.
27) Gerö, S., Gergely, J., Farkas, K., Dévényi, T., Kocsár, L. Jakab, L. Székely, J. and Virág, S., Role of intimal mucoid substances in the pathogenesis of atherosclerosis, II. Changes of tissue and blood mucopolysaccharides in cholesterol-fed animals, *J. Atheroscler. Res.*, **2**, 276, 1962.

LEGENDS TO FIGURES

FIG. 1. Basilar artery of an adult dog. The three layers, intima, media and adventitia of the arterial tissues are stained in a blue shade of different intensities. Alcian blue (pH 2.5) stained. ×200.

FIG. 2. Basilar artery of an adult dog. Digestion with Streptomyces hyaluronidase fails to alter both the intensity and shade of the alcian blue (pH 2.5) stain in the three layers. Streptomyces hyaluronidase digested and alcian blue (pH 2.5) stained. ×200.

FIG. 3. Basilar artery of an adult dog. Digestion with testicular hyaluronidase suppresses the alcianophilia (pH 2.5) of all the three layers. Testicular hyaluronidase digested and alcian blue (pH 2.5) stained. ×200.

FIG. 4. Mesenteric artery of an adult dog. The three layers, intima, media and adventitia of the arterial tissues are stained in a blue shade of different intensities. Alcian blue (pH 2.5) stained. ×200.

FIG. 5. Mesenteric artery of an adult dog. Digestion with Streptomyces hyaluronidase apparently diminishes the alcian blue (pH 2.5) stainability of the three layers. Streptomyces hyaluronidase digested and alcian blue (pH 2.5) stained. ×200.

FIG. 6. Mesenteric artery of an adult dog. Digestion with testicular hyaluronidase markedly suppresses or abolishes the alcianophilia (pH 2.5) of the arterial tissues. Testicular hyaluronidase digested and alcian blue (pH 2.5) stained. ×200.

FIG. 7. Popliteal artery of an adult dog. The three layers of the arterial tissues are stained in a blue shade of different intensities. Alcian blue (pH 2.5) stained. ×200.

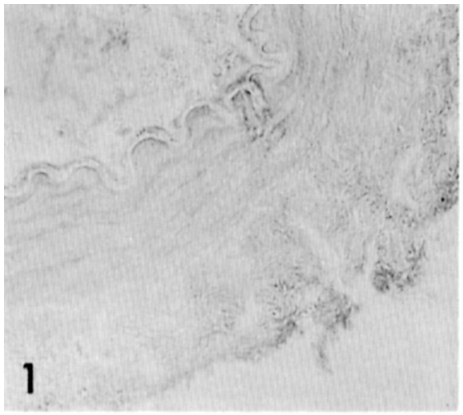
FIG. 8. Popliteal artery of an adult dog. Digestion with Streptomyces hyaluronidase tends to suppress the alcianophilia (pH 2.5) of the arterial tissues. Streptomyces hyaluronidase digested and alcian blue (pH 2.5) stained. ×200.

Fig. 9. Popliteal artery of an adult dog. Digestion with testicular hyaluronidase conspicuously diminishes or abolishes the alcian blue (pH 2.5) reaction of the three layers. Testicular hyaluronidase digested and alcian blue (pH 2.5) stained. ×200.

FIG. 10. Basilar artery of a puppy. The three layers of the arterial tissues are stained in a bluish magenta or magenta blue shade of different intensities. Alcian blue (pH 2.5)-PAS stained. ×400.

FIG. 11. Basilar artery of a puppy. Digestion with Streptomyces hyaluronidase fails to alter both the intensity and shade of the alcian blue (pH 2.5)-PAS stain in the three layers. Streptomyces hyaluronidase digested and alcian blue (pH 2.5)-PAS stained. ×400.

FIG. 12. Basilar artery of a puppy. Digestion with testicular hyaluronidase markedly suppresses or abolishes the alcianophilia (pH 2.5) of all the three layers. Testicular hyaluronidase digested and alcian blue (pH 2.5)-PAS stained. ×400.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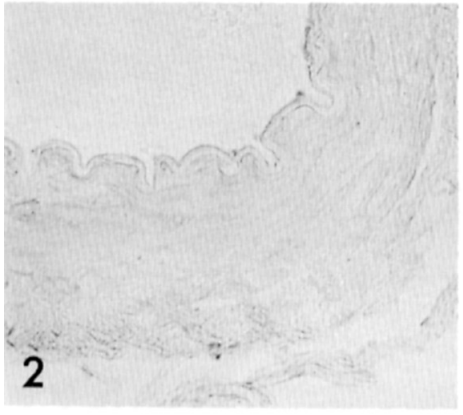
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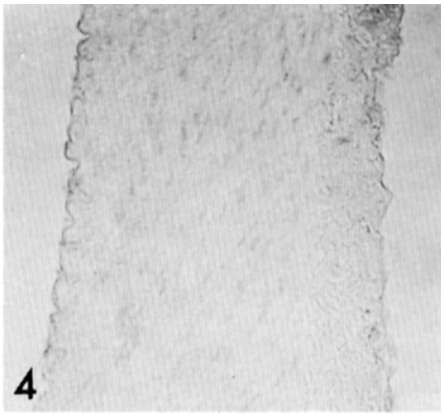
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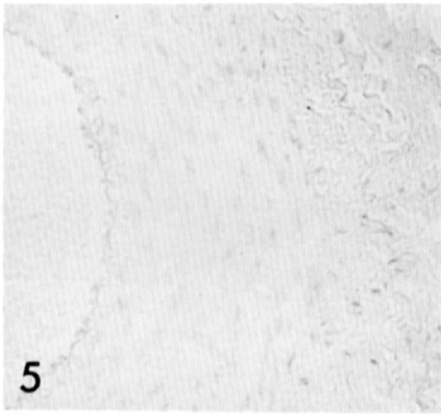
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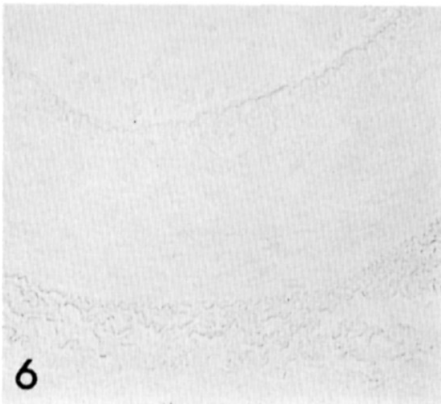
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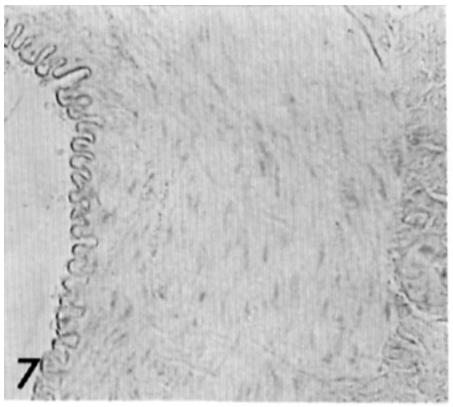
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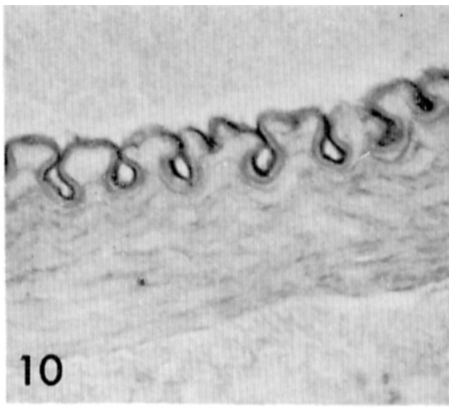
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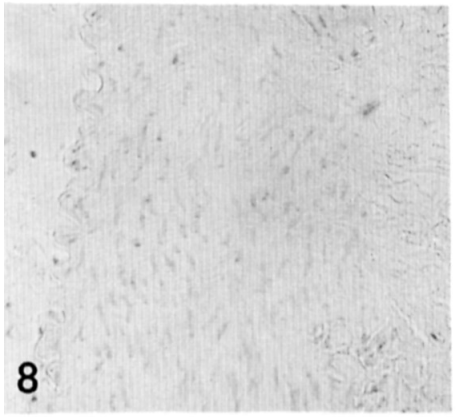
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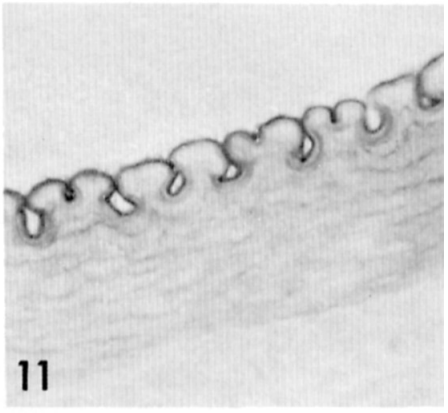
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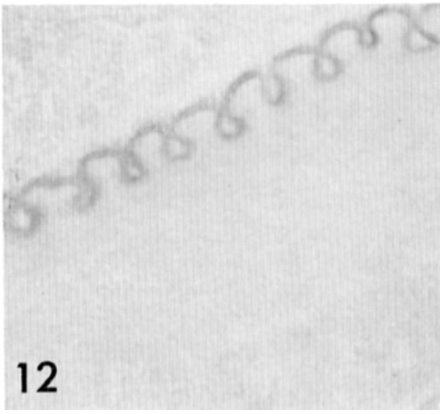
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