

Histochemical Demonstration of Adrenergic Fibers in the Smooth Muscle Layer of Media of Arteries Supplying Abdominal Organs

by

KIKUO MOHRI, NAOHIRO OHGUSHI, MASANAO IKEDA,
KUNITARO YAMAMOTO and KENGO TSUNEKAWA

From the 2nd Surgical Division, Kyoto University Medical School

(Director : Prof. Dr. CHUJI KIMURA)

MOTOHATSU FUJIWARA and TAKASHI MURYOBAYASHI

Department of Pharmacology, Kyoto University Medical School

Received for Publication Jan. 10, 1969

INTRODUCTION

Our present knowledge of sympathetic innervation and vasomotor activity in the splanchnic vasculature is based predominantly on physiological studies. The splanchnic vascular bed is the largest of the regional circulation, about 30% of the total blood circulation. The change of splanchnic circulation plays a great role in the function of digestive organs. Clinically, intestinal ischemia or arterial occlusive lesions of splanchnic areas have been reported by many surgeons. These lesions are now being repaired with the technique of such revascularizations, as endarterectomy or graft bypass, but the splanchnic vasculature has often remained resistant to surgeons. Therefore, the principles of revascularization learned in the limbs in our clinic are applicable in the splanchnic vascular areas. Thus, sympathectomy is also indicated for intestinal ischemia or arterial occlusive lesions of splanchnic areas, as well as for ischemic change of arterial occlusion in the limbs. It is hoped that the regional sympathectomy may cause vasodilatation of splanchnic vasculature.

Regarding the sympathetic innervation of arteries supplying abdominal organs, celiac artery, superior mesenteric artery and so on, many investigators have reported the existence of vascular efferent fibers in the adventitia and in the media of the arteries, but there is much argument about the exact localization and ramification of autonomic efferent fibers into the arteries. A specific histochemical fluorescence method for the detection of catecholamines and 5-HT in tissues has recently developed. Several tissues have been shown to be capable of taking up and bind catecholamines and it is now generally accepted that this occurs predominantly in the adrenergic nerves.

Thus, in the course of our experiments to investigate the mode of distribution of adrenergic nerve fibers in different vasculatures in the body, we have been able to find a specific fluorescent material representing catecholamines and adrenergic nerve fibers in the smooth muscle layer of the media in the arteries supplying abdominal organs in animals and human.

A group of vessels, which includes the arteries leaving the abdominal aorta, and all branches of these vessels supplying the viscerae, were studied by our experiments. Our experimental studies revealed that these arteries contained adrenergic nerve fiber in the media and adventitia.

METHODS AND MATERIALS

Thirty dogs, weighing 10-20 kg, and five rats and ten guinea pig, and also several clinical materials were used. Small tissue specimens of arteries, which included celiac, hepatic, splenic, gastric, gastroepiploic and gastroduodenal arteries, and also superior mesenteric and inferior mesenteric arteries, were biopsied from animals anaesthetized with sodium pentobarbital (25 mg/kg, i.v.).

Immediately after excision, the specimens were freeze-dried, treated with paraformaldehyde, embedded in paraffin, sectioned (5-7.5 μ thick) and mounted in Entellan for fluorescence microscopy, as described elsewhere.

Sections, adjacent to those which were exposed to paraformaldehyde for fluorescence microscopy, were stained with hematoxylin-eosin. To intensify the fluorescence of the artery, dogs were treated with noradrenaline (50 μ g/kg, i.v.) at various time intervals before excision of specimen. Some animals were given reserpine (0.3 mg/kg, 3 times, i.p.) to deplete catecholamines and test the specificity of the observed fluorescence.

In 4 dogs, chronic resection of the paravertebral sympathetic trunk at the level of Th 3~L 6, including preaortic ganglia, was performed 10 days previously. To differentiate the catecholamine fluorescence from non-specific autofluorescence, sections were treated with sodium borohydride.

RESULTS

Normal distribution of adrenergic nerves to arteries supplying abdominal organs.

Treatment with formaldehyde gas of freeze-dried sections of the arteries supplying abdominal organs of normal animals produced a strong, specific greenish fluorescence.

The fairly rich localization of the fluorescent material to the arteries dominates the fluorescent microscopical picture in arterial wall of blood vessels supplying abdominal organs, which are illustrated in Fig. 1, 2, and 3.

Celiac artery of dog (Fig. 4)

Several bundles of fluorescent adrenergic fibers are present in the media of the celiac artery of dog.

Common hepatic artery and proper hepatic artery of dog (Fig. 5 and 6). Several bundles of fluorescent adrenergic fibers are seen in the media of the common hepatic artery of dog.

Fluorescent adrenergic fibers are observed between the media and the adventitia of the proper hepatic artery of dog.

Gastroepiploic artery, gastric artery and gastroduodenal artery. Several bundles of fluorescent adrenergic fibers are observed in the media of the arteries (Fig. 7).

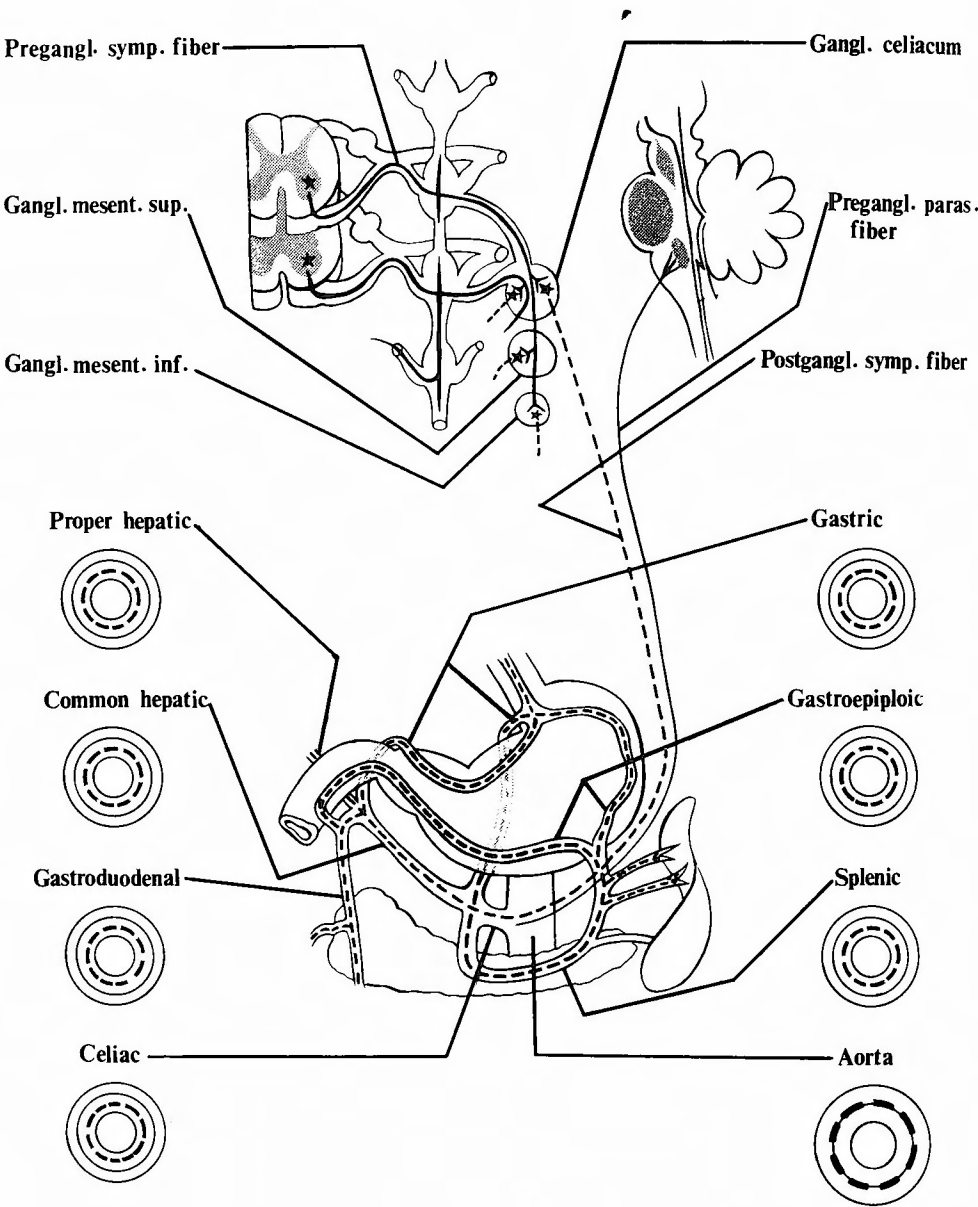


Fig. 1

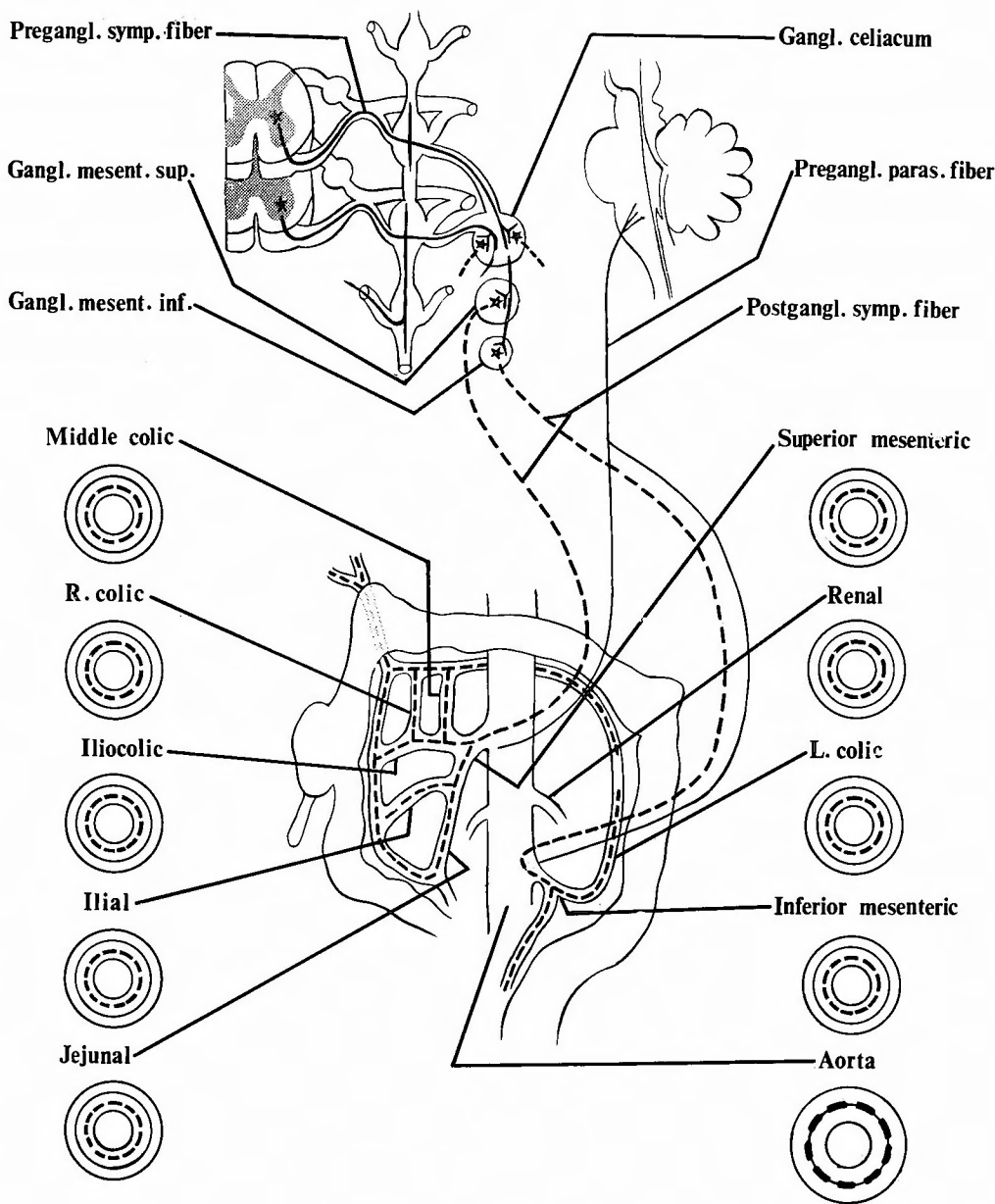


Fig. 2

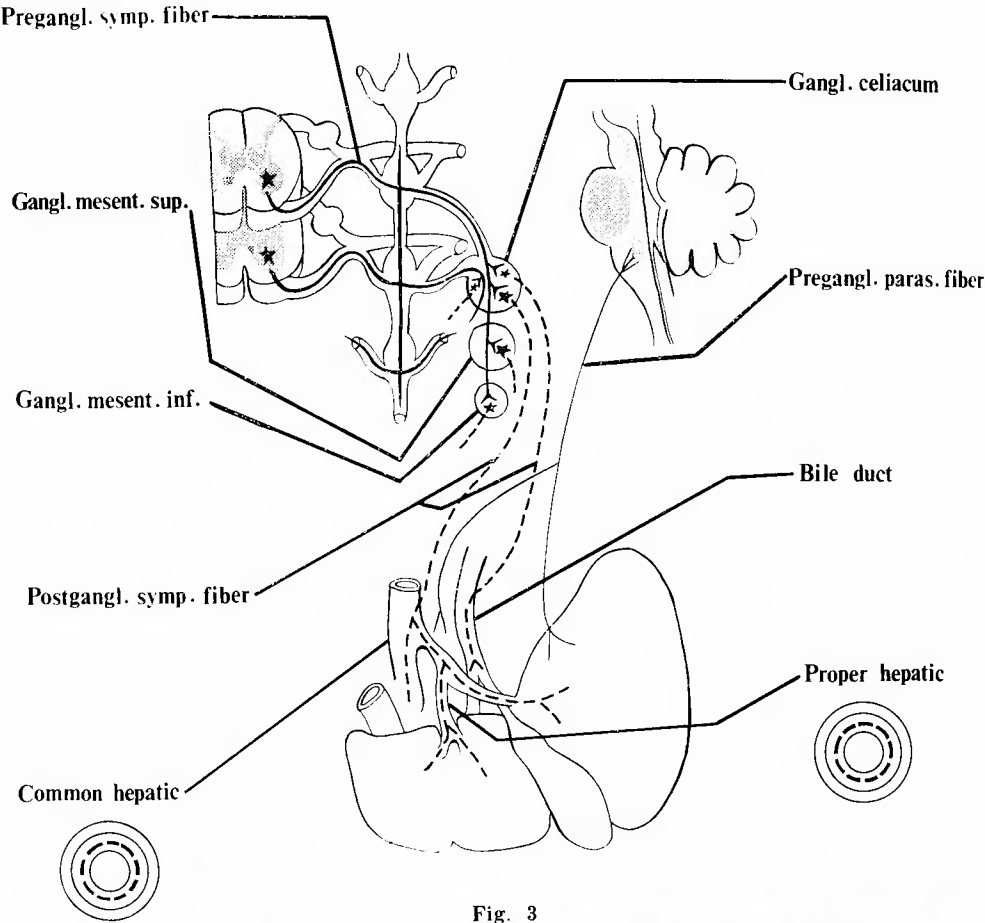


Fig. 3

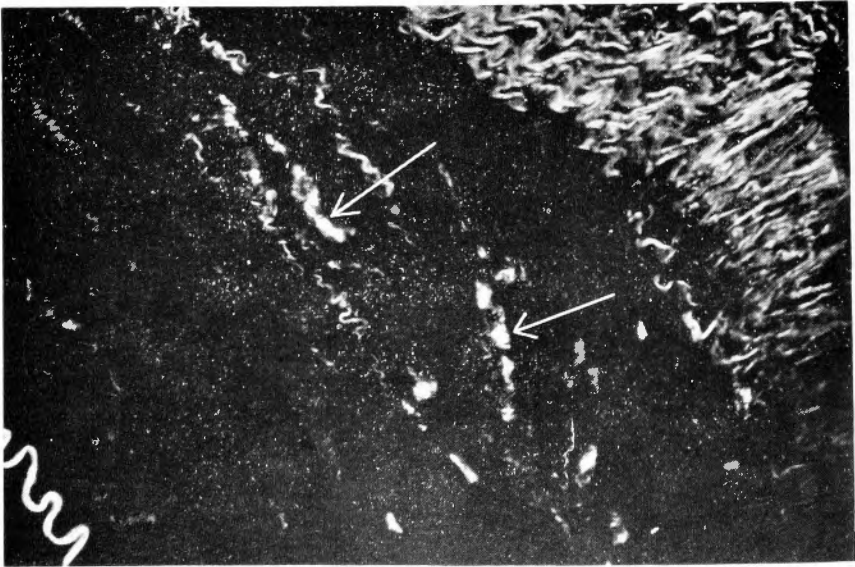


Fig. 4 Celiac artery of dog.
Cross section.

Specific fluorescence of the adrenergic fibers are found in the smooth muscle layer of the media of the artery. Fluorescence micrograph $\times 128$.

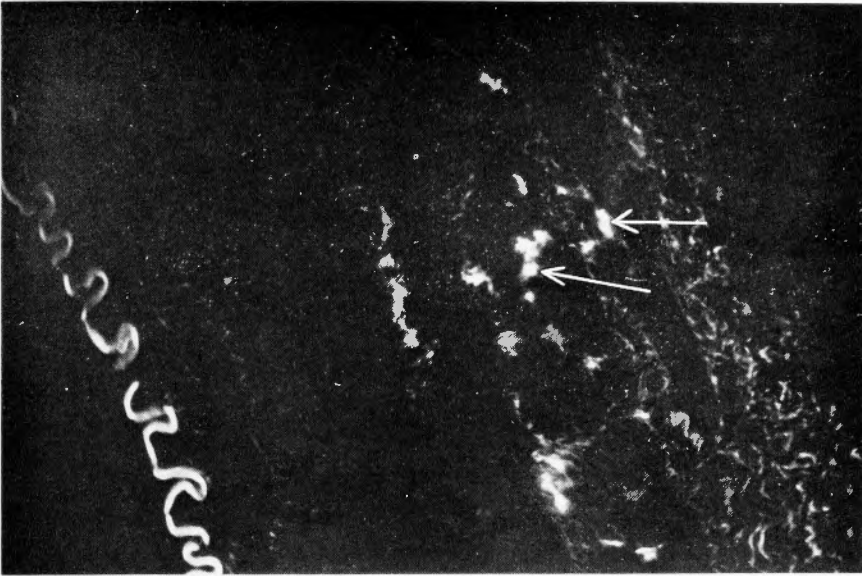


Fig. 5 Hepatic artery of dog.
Cross section.

Several bundles of fluorescent adrenergic fibers are present in the media of the artery. Fluorescence micrograph $\times 128$.

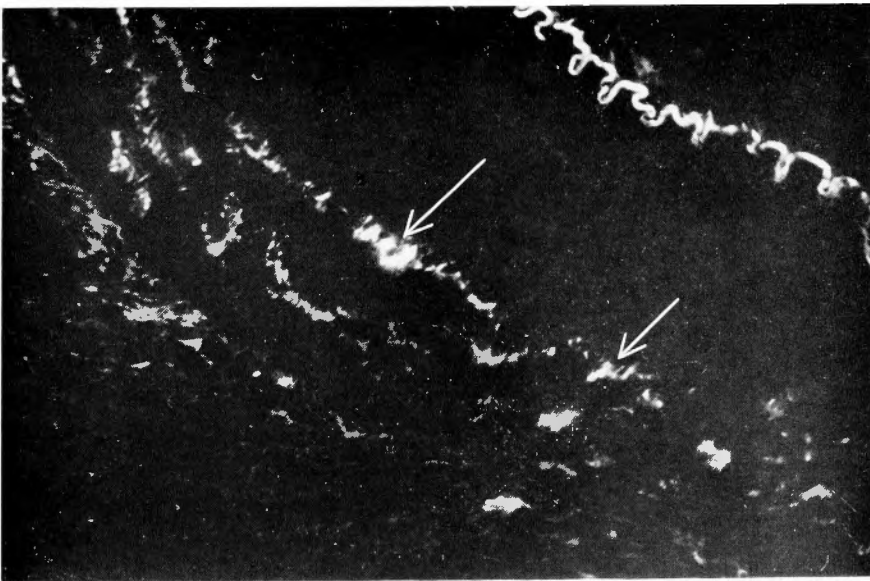


Fig. 6 Proper hepatic artery of dog.
Cross section.

Fluorescent adrenergic fibers are obscured between the media and the adventitia of the artery. Fluorescence micrograph $\times 128$.

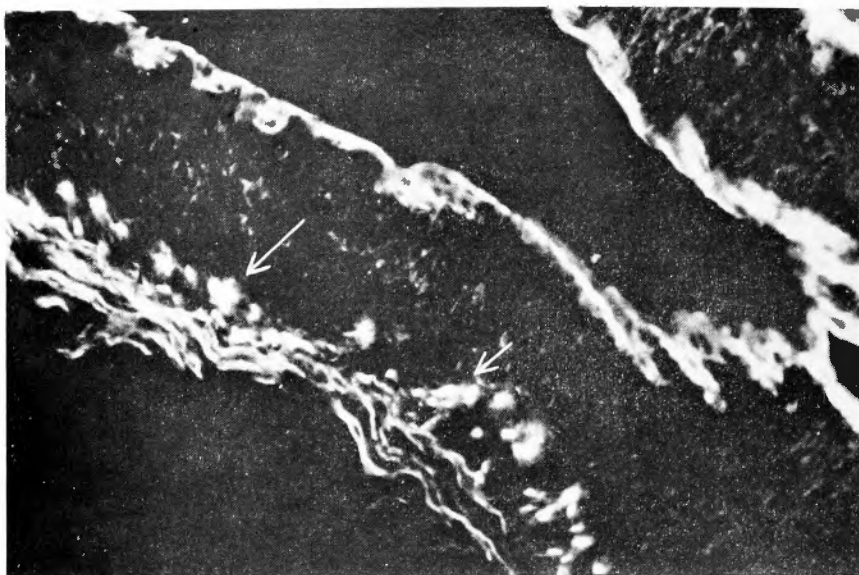


Fig. 7 Gastroepiploic artery of dog.

Cross section.

Several bundles of fluorescent adrenergic fibers are seen, on the leftside, in the media of the artery. Fluorescence micrograph $\times 256$.

Splenic artery of dog (Fig. 8)

Several bundles of fluorescent adrenergic fibers are also present in the media of the artery.

Superior mesenteric artery and its branches (Fig. 9).

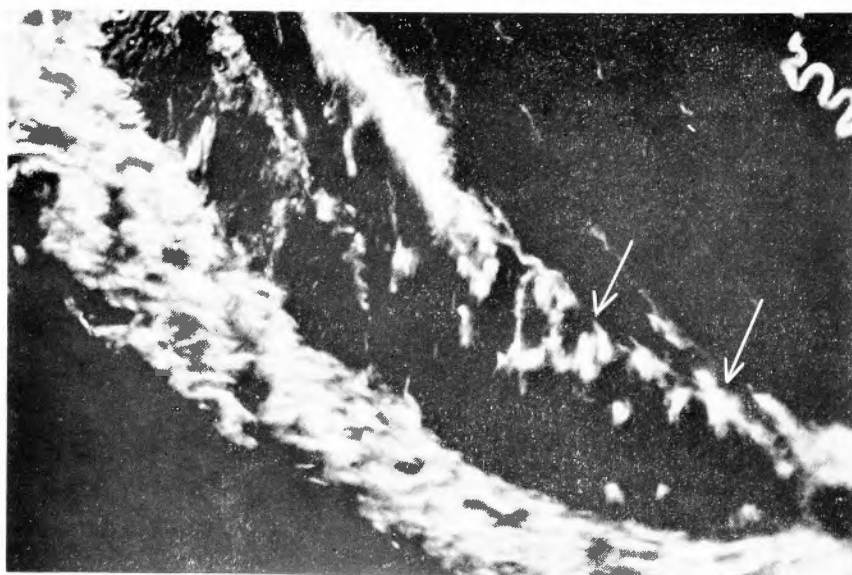


Fig. 8 Splenic artery of dog.

Cross section.

Several bundles of fluorescent adrenergic fibers are present in the media of the artery. Fluorescence micrograph $\times 128$.

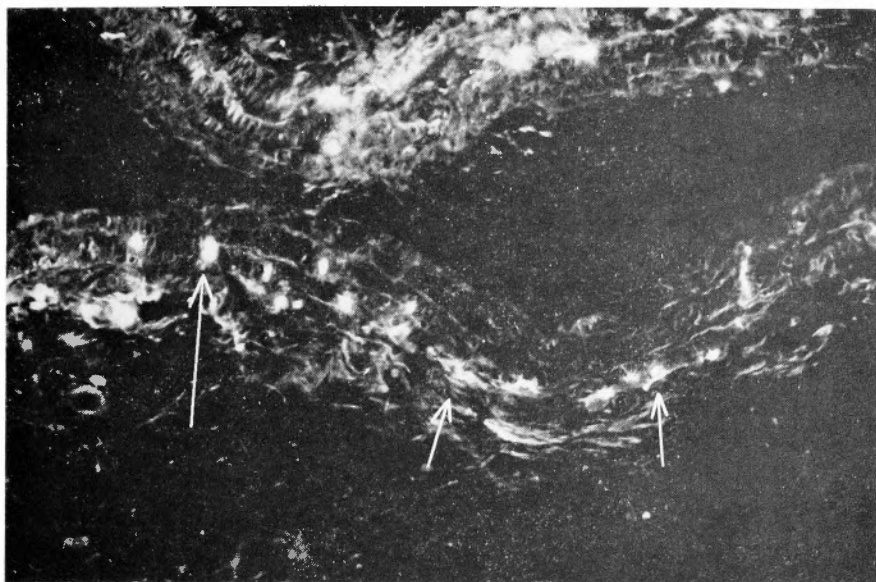


Fig. 9 Artery of mesentium in human being.

(Branch of superior mesenteric artery)

Many bundles of fluorescent adrenergic fibers are present in the media of the artery.

Fluorescence micrograph $\times 256$.

Fluorescent adrenergic fibers are present in the media of the artery and its branches.

Inferior mesenteric artery and its branches. Fluorescent adrenergic fibers are also present in the media of the artery and branches. These fluorescent materials surrounding the arteries were not only directly superimposed on the smooth muscle layer of the media, but also penetrated from adventitia to the smooth muscle layer of the media.

No fluorescence was observed in the abdominal aorta of dog and human.

On the other hand, the fluorescent materials surrounding the abdominal aorta of guine pig were directly superimposed on the smooth muscle layer of the media, but they were seen to penetrate this layer only seldom (Fig. 10). The elastic tissues of the adventitia showed an intense greenish auto-fluorescence, and a bright specific fluorescent material was found near the border to the media. The specific fluorescence in the media was increased in number and intensity after the injection of noradrenaline. Such an increase lasted for at least 30 min.

Injection of reserpine or chronic resection of sympathetic trunk resulted in a complete disappearance of specific fluorescence in the smooth muscle layer of the media (Fig. 11). Concomitantly, fluorescence of the adventitia also somewhat faded in the greenish tone, as compared with that of untreated animal.

Treatment with sodium borohydride affected the fluorescence of the media and adventitia, in a similar way to reserpine treatment or sympathectomy.

DISCUSSION

Distribution of catecholamine fluorescent adrenergic nerve fibers in the arteries supplying abdominal organs in dog. Two types of fluorescent adrenergic innervation of the arteries were detected by our observation (Fig. 12).

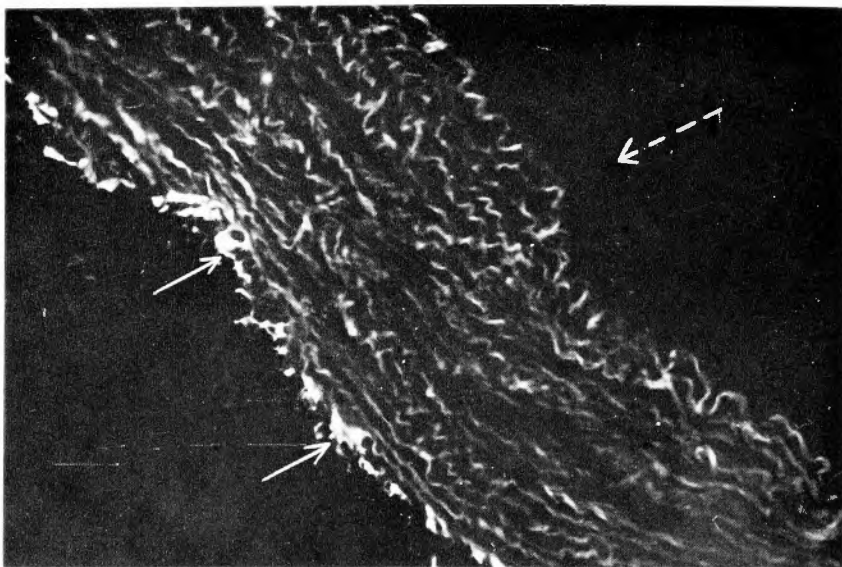


Fig. 10 Abdominal aorta of guinea pig

Cross section.

Several bundles of fluorescent adrenergic fibers are directly superimposed on the smooth muscle layer of the media. Fluorescence micrograph $\times 128$. ($\rightarrow$ Intima)

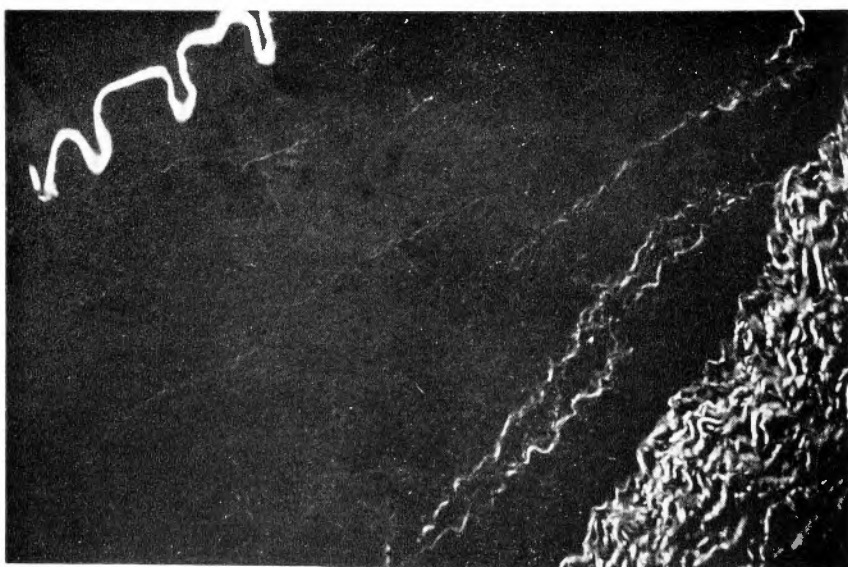
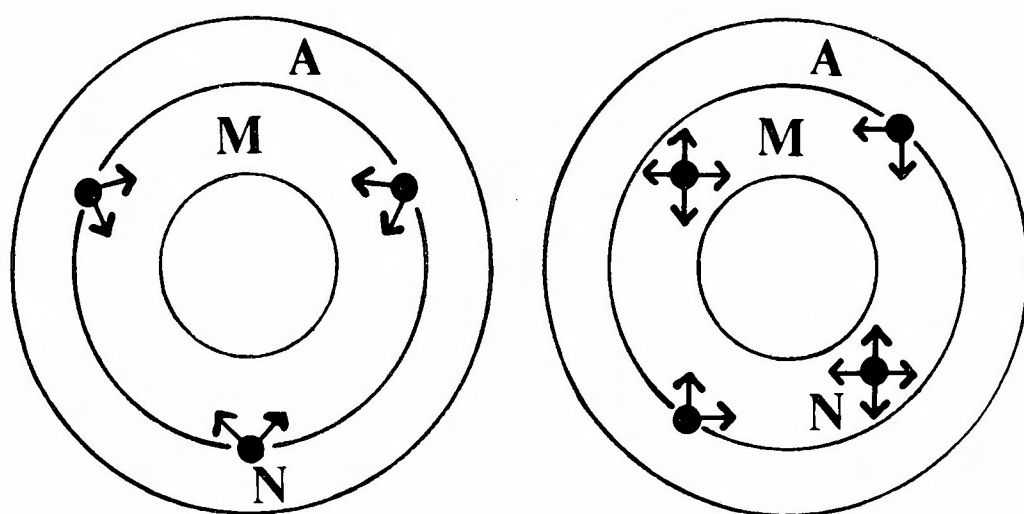


Fig. 11 Celiac artery of dog. Reserpine administration.

Cross section.

No specific fluorescence of the adrenergic fibers are found in the smooth muscle layer of the media of the artery, after reserpine administration. Fluorescence micrograph $\times 128$.



M : Media A : Adventitia N : Catecholamine fluorescent nerve ending

Fig. 12

Only aorta of guine pig and rat had fluorescent adrenergic nerve fibers between the media and the adventitia, except arterioles. Fluorescent adrenergic nerve fibers were not observed in aortas of human being and dog with our researches. This type of vessels had fluorescent adrenergic nerves fibers only in the adventitia or between the adventitia and the media, but it had none of them in the media. Other type of vessels such as celiac artery, superior mesenteric artery, inferior mesenteric artery and their branches had fluorescent adrenergic nerve fibers in the media of vessels. Vessels differ in their architectural structure and their behavior according to their varied task, from the stand point of classical histology. There are, in general, four different vessel types. On the arterial side are elastic arteries and muscular arteries, but it is hard to say where the one ends and the other begins, since the structural changes are gradual. Usually the aorta, the subclavian artery, and the common iliac artery are regarded as elastic artery. Arteries more peripheral than the above, down to the arterioles, are classed as muscular arteries. After these are the capillaries. Then there are veins. Therefore it seems that visco-elastic and plastic behavior of elastic arteries depends mostly on elastic tissue, collagen tissue, and ground substance, but only to a small degree on smooth muscles. These elastic arteries with above mentioned physiological properties, such as aorta, had smaller need for sympathetic innervation resulting vasoconstriction. The muscular arteries with the physiological function which controls the circulating blood volume, had need for sympathetic innervation to make vasoconstriction.

Silver Impregnation method and Histochemical fluorescence method of catecholamine. With BILSCHOWSKY's silver impregnation method, the vascular efferent fibers are carried in paravascular filaments which can sometimes traced alongside arteries for considerable distances before they break up into finer fascicles. These fascicles unite in an open perivascular plexus from which small groups of fibers penetrate into the vessel walls, sometimes alongside the vasa vasorum, to ramify and form much finer networks in the adventitia

and in the media. Some fibers reach the junction and between the media and intima, but it is doubtful if they enter the latter. These appearance led KIMURA, CAJAL and DOGIEL to describe superimposed and inter-connected networks; the first and best defined in the adventitia a second, very delicate, in the media; and a third often difficult to distinguish, in the zone between the media and intima.

But our experimental studies with a recently developed specific histochemical fluorescent method for the detection of catecholamine, showed the above mentioned results, including that two types of adrenergic innervation existed in the arteries, an that adrenergic nerve fibers were not yet found in the inner layer of the media and also between the media and intima.

Physiological studies and histochemical fluorescent method for adrenergic nerve.

With physiological studies of many investigators, there is general agreement that splanchnic stimulation increases the resistance to blood flow through the mesenteric circuit. Vagal stimulation probably has little if any significant influence on the mesenteric blood flow, except insofar as flow is changed secondary to an increase in motility in the stomach and gut. This fact suggests a sympathetic innervation. Therefore this physiological significance was supported by our histochemical findings, which meant the rich existence of adrenergic innervation on the arteries supplying abdominal organs.

The origin and height of sympathetic innervation to arteries supplying abdominal organs. There was much arguments about origin and heights of sympathetic nerve fibers to the arteries. From the basis of classical anatomy, a large number of preganglionic fibers does not terminate in the sympathetic trunk but in a group of preaortic ganglia. Fibers from T5-T9 fuse to form the greater splanchnic nerve, which terminates mainly in the coeliac ganglion. Fibers from T10-T11 fuse to form the lesser splanchnic nerve, which goes primarily to the superior mesenteric ganglion. Fibers from T12 forms the least splanchnic nerve, which goes mainly to the inferior mesenteric ganglion. The ganglia are located on the surface of the aorta, around the origin of the vessels which give them name. The postganglionic fibers go from here, forming periarterial networks, to innervate the vessels to abdominal organs and also the abdominal viscerae themselves. The exact localization, height and ramification, from which the sympathetic nerve fibers to the vessels supplying abdominal organs were originated, are now investigated in our laboratory-group, but the above stated opinions were in general accepted by many investigators. Therefore, the elimination of sympathetic tone on the vessels of abdominal organs was, in our animal experiments, performed from the height T3 to L6, but technical difficulties were occurred to resect trunk, ganglia and nerves of the heights from last thoracic ganglion to first lumbar ganglion (L1).

From the basis of our studies, regional sympathectomy could cause vasodilatation of splanchnic vasculature. Therefore, sympathectomy is one of useful methods for treatment of intestinal ischemia or arterial occlusive lesions of splanchnic areas, as well as for ischemic change of arterial occlusion in the limbs.

CONCLUSION AND SUMMARY

It is very important that catecholamine-fluorescent nerve fibers are detected to penetrate through the muscle layer of the media in the arteries supplying abdominal organs, as

muscular arteries ; celiac artery, gastric arteries, gastroepiploic arteries, splenic artery, gastroduodenal artery, common and proper hepatic arteries, superior mesenteric artery, inferior mesenteric artery and their branches.

Catecholamine-fluorescent nerve fibers are detected very rarely in aorta of some species, such as guine pig or rat, as a elastic artery, between the adventitia and the media of aorta. It is noteworthy, that histological study of artery show two types of arteries, such as elastic artery and muscular artery in their architectural structure and their behavior to their varied task, and also that our histochemical fluorescence method detected two types of arteries with modes of sympathetic innervation, which are described above. These facts might explain the mutual connections and interactions between physiological and histological natures of the arteries.

ACKNOWLEDGEMENT

We wish to express our deepest gratitude to Prof. Dr. Chuji Kimura for his helpful advice and kind guidance throughout this study.

NOTE

This work was presented by Dr. K. Mohri at Symposion of International Society for Neurovegetative Research on Neurohormones and Neurohumores, in Amsterdam, July 24, 1967.

REFERENCE

- 1) T. Kitahaba : A Histological Study on the Innervation of the Large Blood Vessels of the Abdomen. Arch. Jap. Chir. **28**, 1960, 1959
- 2) Ch. Kimura : Vasucular Sensitivity. Acta Neurovegetativa, **14**, 170, 1956
- 3) M. Fujiwara, et al., Nor-adrenaline, Monoamine Oxidase and Acetylcholinesterase in Salivary Glands of Dog. J. Histochem. Cytochem. **14**, 483, 1966
- 4) P. Dow, : Handbook of Physiology, Section 2, Circulation, Publisher Williams & Wilkins Co.
- 5) K. Tsunekawa et al. : Histochemical Demonstration of Adrenergic Fibers in the Smooth Muscle Layer of Media of Dorsal-pedal Artery in Dog. Experientia, **23**, 842, 1967
- 6) K. Mohri : Histochemistry of Catecholamines Arch. Jap. Chir. **31**, 280, 1962
- 7) N. Ohgushi : Adrenergic Fibers to the Brain and Spinal Cord Vessels in the Dog. Arch. Jap. Chir. **37**, 294, 1968

和文抄録

腹部血管のアドレナリン作動性神経の分布に関する研究

京都大学医学部外科学教室第2講座（指導木村忠司教授）

毛利喜久男・大串直太・池田正尚
山本国太郎・恒川謙吾

京都大学医学部薬理学教室

藤原元始・無量林 堯

血管に於ける交感神経の発効器は中膜平滑筋であるが、その神経支配については未解決の問題が多く残されている。

本研究は、蛍光組織化学的方法により、腹部内臓血管のアドレナリン作動性神経支配について検討を加えた。

筋性血管、又は拮抗血管として総括される腹腔動脈、胃動脈、胃大網動脈、脾動脈、胃十二指腸、総肝及び固有肝動脈、上及び下腸間膜動脈及び、上記動脈の枝にはアドレナリン作動性神経が血管外膜と中膜との境界部に分布すると共に、中膜筋層にまで達してい

る。

弾性血管の1つと考えられている大動脈には、ハムスター、ラットにのみ血管外膜と中膜との間にアドレナリン作動神経を認めるが、犬及び人間には証明出来なかった。

以上の成績から、弾性血管と筋性血管の神経支配について、組織化学的証明法により、アドレナリン作動神経分布の差異が認められることは、生理学的実験成績によつても同様に、2種類に分類出来る事実と考え合わせると興味深い。