Role of Vasoconstriction in Experimental Arteriosclerosis

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The peculiarities of human arterio-atherosclerosis, its morphology, anatomical distribution, and variation with age, sex, diet, and social conditions, have made agreement difficult concerning its etiology. Observations in clinical medicine and in the laboratory suggest a relationship between vasoconstriction and arteriopathies. Essential hypertension, a disease of small arteries and arterioles, begins as a functional vasoconstrictor state which eventuates in structural vascular changes. Not clear in this change from reversible to irreversible phases, however, is the role of resulting hypertension. Elsewhere, the relationship of mean blood pressure to structural changes is less doubtful. In Raynaud's syndrome, for example, a chronic constrictor state of the digital arteries, without known mean blood pressure alterations, is associated with intimal fibrosis of the involved arteries. Indeed, if laminar flow is preserved in these vessels, intravascular pressure would be lowered in accordance with Bernoulli's theorem of hydrodynamics. Implicit in the treatment of human coronary disease with vasodilators is the possibility that pathogenic mechanisms of the arterial disease may be arrested. That arterial injury may occur under the influence of vasoactive agents has also been shown in the laboratory, and the resulting pathological changes may resemble the human disease. A consideration of such well-known observations has stimulated interest in the present investigation concerning the role of vasoconstriction in experimental arteriosclerosis and, more particularly, the part played by neural mechanisms in executing that role.

Little information is available on the effect of neuroexcitation in experimental arteriopathy. In all mammalian species, vessels of the size of the elastic and muscular arteries are well supplied with an autonomic nerve distribution capable of inducing episodic vasomotion. Epinephrine and related substances are present in arterial walls. In addition, stimulation of such nerve fibers results in the release of biochemical transmitter substances at receptor sites and into the closely associated venous return. Such substances, for example catecholamines, when administered parenterally to animals in long-term experiments, can induce arteriopathies and have been given some consideration in the genesis of arteriosclerosis. However, they act diffusely and involve many bodily functions, making it difficult to concentrate on the desired vascular observations. To examine the effects of regional protracted neuroexcitation on in vivo arterial vessels, a remote control radio-frequency method of stimulation of vasomotor nerves was devised. This method was carried out in freely moving unanesthetized animals, and left the subjects completely undisturbed. It soon became apparent that neuroexcitation was always accompanied by local vasoconstriction, and it was not possible in these experiments to determine whether it was the elaboration of neuropathological substances or the physical effects of constriction, or perhaps both, which was effective in influencing the pathological changes.

Methods

Under light pentobarbital sodium anesthesia, 2.5 mg./100 Gm. body weight, laparotomy was per-
formed on 12 albino rats of both sexes, two to three months of age, averaging 150 Gm. in weight. Stimulating electrodes of no. 36 teflon-coated platinum wire carried in an outer polyethylene catheter were sutured to the right diaphragmatic crus over the location of the lesser splanchnic nerve. The electrodes were attached to a small radio receiver, 2.0 cm. in diameter, previously embedded in epoxy resin, and the receiver was implanted in the dorsal subcutaneous space. The receiver consisted of a small tuned circuit in resonance at 3.8 megacycles. Three days after recovery, the animals were placed in nonmetallic lucite cages, around which were wound loops of antenna wire issuing from an adjoining transmitter, which was transmitting at a frequency of 3.8 megacycles. The carrier wave was amplitude-modulated by a 60-cycle sine-wave frequency. Experiments with varying frequency disclosed that there was no essential difference in stimulatory effect within a range of 30 to 60 cycles. Nor did the wave shape, whether round or square, play an important part. Thus, for reasons of convenience, the modulation was accomplished by using the readily available 60-cycle line current to modulate the audio amplifier stage of the transmitter. A repeat cycle timer allowed the radio field in the cages to be generated at intervals of 11.5 minutes, and to last for 30 seconds by supplying plate voltage to the transmitter at these intervals (fig. 1). The cage voltage was maintained between 5 and 8 volts. The animals were subjected to this protracted stimulation for a period of three to four weeks, since in this time, barring nerve refractoriness, a minimum of some 2,500 stimuli was received. They were maintained on ordinary rat pellet diet with water ad libitum, after which they were sacrificed and the abdominal aortas examined histologically with multiple serial sections. Hematoxylin and eosin stains were employed routinely. Special staining reagents, such as phosphotungstic acid hematoxylin (PTAH), periodic acid Schiff (PAS), elastics and van Gieson (EVG), were employed when thought

**FIGURE 1**
(Upper) Schematic diagram of transmitting apparatus. (Lower) Circuit diagram of implanted receiver; circuit elements consist of miniature coil, two miniature capacitors, and small diode.

**FIGURE 2**
to be useful. Control observations were made on the thoracic aorta and femoral arteries of the same animals, as well as on the abdominal aorta of a comparable group of rats in which operation was carried out and electrode wires sutured in place as before, but without the addition of the tuned receiver. Hence, in these animals, no exciting stimulus was passed to the distribution of the splanchnic nerve other than that possibly caused by mechanical irritation of the fibers. A third group of 12 animals was examined for spontaneous lesions. These were similar in age and weight to the experimental subjects.

Since the lesser splanchnic nerve in the rat supplies the lower one-third of the abdominal aorta and only occasionally innervates the iliacs and femorals, the thoracic aortas in the experimental animals were essentially unstimulated.

Vasoconstriction following neurostimulation was tested in the following way: Normal blood volume changes in the limbs were observed through their effect on light absorption by using a sensitive photoconductive cell coupled to a single channel recorder.* Alternate increases and decreases of volume with each pulse affects light absorption which is converted into electrical energy, amplified, and recorded. Obliteration of the cyclical written record indicated disappearance of the plethysmographic changes mediated through the intravascular pressure difference of each pulse. That vasoconstriction rather than vasodilatation occurs is demonstrated by the direction in which the baseline moves. Recordings of blood flow from the hind footpad and tail of the animal in this way disclosed obliteration of the pulse, indicating that stimulation of the lesser splanchnic nerve is followed by vasoconstriction of the aorta (fig. 2). No obliteration of pulses in the anterior footpad was demonstrated, indicating that only the lower aorta is activated.

Only a few of the subjects gave evidence of involvement of pain fibers in excitation of the splanchnic nerve, but within a few days, all were comfortable upon stimulation and behaved in a normal fashion. Thus there was no "awareness" on the part of any of the animals that they were participating in the experiment.

**Results**

The histological results will be discussed under the following categories: changes in endothelial morphology, changes in subendothelial matrix, changes in elastica, subendothelial cell proliferation, calcinosis, and coagulation phenomena.

One or more of the changes described was present in 80 per cent of the animals, although some were more advanced than others. These changes were distributed as follows: endothelial morphology, 6 animals (50 per cent); changes in elastica, 7 animals (58.3 per cent); subendothelial cell proliferation, 2 animals (16.6 per cent); calcinosis, 2 animals (16.6 per cent); coagulation phenomena, 10 animals (83.3 per cent).

The reasons for differences in severity will be discussed below. Vascular segments from the control areas did not disclose any of the features to be described. The aortas of animals in which electrodes only were placed in contact with the lesser splanchnic nerve were

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* Grass model 5 polygraph. Grass Instrument Company, Quincy, Massachusetts.
examined in the same manner. These failed to disclose any of the features described indicating that mechanical irritation of the nerve fibers was not effective in generating vasoconstrictor phenomena. Nor did these animals demonstrate any abnormality of the "pulse record" when examined periodically. In the second group of control animals, no spontaneous lesions of the abdominal aorta were found.

In addition, a similar series of six unanesthetized rats was subjected to a daily intermittent stimulating process for several hours a day with the use of a direct external stimulator instead of by the remote-control radio frequency method and showed similar morphological alterations of the abdominal aorta. This method, however, places the animal under restraints and is therefore less desirable.

CHANGES IN ENDOTHELUM

The normal endothelial cell is flattened, elongated, has a deep staining nucleus, and is closely adherent to the internal elastica. In the young rat, the endothelium lies directly on the internal elastica (fig. 3).

In vessels subjected to stimulation, the endothelial cells were often seen to be greatly enlarged, occasionally contained cytoplasmic vacuoles (resembling in some details Lazzarini-Robertson's "cyto I" cells grown in organ culture), phagocytic in appearance and frequently were lifted away from the underlying elastica (fig. 4). In some instances, there was a local increase in the number of these hypertrophied cells.

CHANGES IN SUBENDOTHELIAL MATRIX

Frequently the endothelium and underlying elastica were thrown into highly convoluted folds. The overlying cells were sometimes rounded and the subelastica appeared less dense, possibly as the result of "insudated" substances from the blood stream.

CHANGES IN ELASTICA

A number of vessels disclosed areas in which the inner surface protruded into the lumen slightly. More detailed examination of such regions revealed a reduplication of the internal elastica, frequently with trapped erythrocytes between the duplicated fibers (fig. 5). In one advanced lesion where the internal elastica had undergone almost complete disappearance, and mononuclear cell proliferation and fibrosis appeared, substances resembling degenerated red blood cells could still be seen.

SUBENDOTHELIAL CELL PROLIFERATION

In addition to trapped red blood cells, mononucleated cells of varying size were often encountered between duplicated elastic fibers. The number of such cells varied from animal to animal, ranging from 2 or 3, to 20 or 25 per high powered field (fig. 6). In some instances, the cells were very large, and except...
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for a deeply staining nucleus resembled the hypertrophied endothelial cells encountered elsewhere. The areas of most active proliferation demonstrated little PAS positive material and were blue with the aniline blue Trichrome preparation, although they did not stain as intensely as the collagen of the outer adventitia. This suggested the appearance of newly formed connective tissue. In the most advanced lesion, a progression of changes could be seen in which this "fibrous" area formed a definite plaque (fig. 7) and could be traced to a normal endothelium in the adjacent circumference of the vessel, thus establishing its eccentric nature.

CALCINOSIS

In a few of the vessels, calcification of the elastic fibers was observed. In the most advanced lesion, the calcified elastic fibers formed almost plate-like deposits, and were degenerated, duplicated, and fragmented (fig. 6). The zone of heaviest calcification in this vessel correlated with the area of greatest subendothelial cell proliferation, although individual elastic fibers elsewhere in the wall were also seen to be encrusted with calcium.

COAGULATION PHENOMENA

Perhaps the most interesting finding present in many of the aortas, in view of the reawakened interest in the thrombogenic theory of atherosclerosis, was the presence of intraluminal as well as intramural thrombi. These were for the most part early, although some were in definite stages of organization. Some of the thrombi were "hyaline" in appearance, while others contained fibrin. Some of the thrombi contained trapped erythrocytes and were undergoing incorporation into the wall of the vessel merging with the vascular endothelium and ill-defined elastica (figs. 8, 9, and 10).

Discussion

Alterations in surface endothelial cells with phagocytic properties have been observed in experimental atherosclerosis as well as in human disease. These have been correlated with hyperlipemia and changes in lipoprotein metabolism in vitro. In the present study, morphological alterations of endothelial cells, although not resulting in typical "foam cells" or lipophages, were seen without reference to excessive exogenous fat.

In this study, lipid stains were not prepared routinely. Thus it was not possible to investigate the nature of the cytoplasmic vacuoles described above. This will be done in a subsequent series of experiments and will be correlated with evaluation of the serum lipids.

Among the earliest lesions observed in human arteriosclerosis is the "insudation" of serum and fibrin with a corresponding lysis of formed elements and swelling of the in-
"Hyaline" thrombus (T), PAS positive, attached to wall of vessel. Periodic acid Schiff, X 150.

The young rat possesses no real intima, but in regions where vasoconstriction appears to have existed, there was a rarefaction of the matrix together with dissolution of formed elements and occasional threads of fibrin. It cannot be altogether certain, however, that this change is not artifactual in the present investigation.

Reduplication of the elastica has long been observed in association with human arteriosclerosis and was found frequently in animals of this series. Often such areas contained trapped erythrocytes and appeared as small plaques, since the internal elastica was the layer most often affected. Whether such reduplication was due to mechanical stresses associated with vasospastic phenomena, or mediated chemically through the elaboration of neuropharmacologic substances could not be determined.

Proliferation of intimal cells with attendant fibrosis is of course the sine qua non of arteriosclerosis. In the most advanced lesion of this series, marked proliferative activity of endothelial or subendothelial cells with fibrosis was observed. Some of the larger cells suggested a connection with the hypertrophied surface cells, bringing to mind the concept of inward migration of the endothelium which is considered a possibility by some observers.

Calcification, observed in only a few animals in this series, although very severe in one case, was confined to the elastic fibers and is probably related to the release of epinephrine or norepinephrine. Very rarely is it encountered "spontaneously" in older animals.

Many of the vessels examined revealed small thrombi, some encrusting on the endothelium and showing partial incorporation into the wall. In this respect, it is interesting to note the experiments of Shimamoto, who produced thrombotic deposits and plaques in rabbits' aortas following daily injections of epinephrine. Neuroexcitation of the animals in this series was undoubtedly associated with the release of epinephrine-like substances locally.

Variations in severity or in the number of histological features present in individual vessels may be associated with differences in effective stimulation from animal to animal. Varying thresholds and refractoriness undoubtedly played their part in limiting excitation of the nerve distribution to the aortas. Moreover, it was not always possible to place the electrodes over the lesser splanchnic nerve. At times, the least splanchnic nerve received more of the stimulation than the lesser, as evidenced by urinary and fecal incontinence in two of the animals.

The effect of lipids in diet and blood was not evaluated as it was the purpose to study arterial changes without increase of exoge-
nous fat. The lipid investigation will be undertaken separately. Senility was excluded as a factor in the genesis of the lesions by selecting young rats although the lack of intima in such animals may in its turn limit proliferative activity.

Although neuroexcitation and vasoconstriction has previously not been given much attention in the genesis of arteriopathies, the converse situation has been dealt with. Thus, sympathectomy and injuries of the peripheral and autonomic innervating nerves have been shown to aid the development of arteriosclerosis. The mechanisms involved apparently relate to increased lateral pressure and increased endothelial permeability with resulting "insudation" of vascular components into the blood vessel wall. The high frequency of arterial diseases in man, however, without associated known injuries of the vegetative nervous system, suggests that neuroexcitation rather than neuroinjury may be involved. In the present study thrombus formation was seen more commonly than "insudation" and seems to have played a role in the arteriopathy developed.

A number of changes seen in experimental arteriosclerosis may therefore be induced by neuroexcitation. Whether these alterations are related to physical factors mediated through vasoconstriction or are biochemical in origin, is not known. Possible physical stimuli are shock waves, or "coup de bélier" effects, setting up mechanical stresses on the endothelial surface because of the incompressibility of the blood. Hemodynamic effects, such as turbulence in the boundary layer of flow, may also play a part. Since arteriosclerosis is rapidly showing itself to be a multifaceted problem with respect to its etiology, it is felt that more attention should be given to the role of neuroexcitation and vasoconstriction in experimental and spontaneous animal arteriosclerosis, and possibly in the human form of the disease as well.

**Summary**

A method is given for the study of local neuroexcitation with subsequent vasoconstriction and its relationship to experimental arteriosclerosis in unanesthetized animals under normal laboratory conditions. Histological observations of vasoactive areas of stimulated blood vessels in the laboratory rat disclose some of the features observed elsewhere in experimental arteriosclerosis. Possible pathogenic mechanisms relating neurostimulation of autonomic arterial nerves with in situ arteriopathies are discussed.

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