Two-Dimensional in-Vitro Studies of Femoral Arterial Walls of the Dog
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ABSTRACT
Analyses of the vascular system have characteristically been based on assumptions that the vessel wall is isotropic and that its properties and behavior can be linearized. To test the validity of these assumptions, excised arteries of dogs were submitted to stepwise strains tangentially and longitudinally during conditions of control, vasoconstriction, and inhibition of muscle metabolism. These strains were recorded simultaneously with the associated stresses, and the relations between stress and strain were studied during both dynamic and steady-strain states. The vessels were anisotropic within most of the physiological ranges of tangential and longitudinal stress and strain. During control conditions the tangential modulus of elasticity was higher than the longitudinal; during vasoconstriction the situation was reversed for wall tensions corresponding to a blood pressure equal to or smaller than 190 mm Hg; however, above this value isotropic behavior prevailed. Vasoconstriction increased the non-linearity of the stress-strain relation, and decreased the modulus of elasticity of the arterial wall. The viscoelastic properties of the femoral artery were found to be different tangentially and longitudinally, as evidenced by a much larger amount of stress relaxation in the former direction than in the latter.

ADDITIONAL KEY WORDS: distributing arteries, anisotropy, nonlinearity, viscoelasticity, stress relaxation, vasoconstriction, norepinephrine, metabolic inhibitors.
remains linear for physiological changes in tension. The second assumption, that of isotropy of vascular tissue, presupposes identical moduli of elasticity in all directions. If two of the moduli of the material determined in the two perpendicular directions are different, it proves the anisotropy of the material; although its full characterization will require the determination of at least three moduli depending upon the degree of anisotropy.

A few isolated reports of the anisotropy of the vascular tissue can be found in the literature. As early as 1902, MacWilliam (6) published stress-strain curves which show a different mechanical behavior in the circumferential and longitudinal directions, the longitudinal modulus being slightly higher than the circumferential. Similar results were obtained by Roach and Burton (7) on human iliac arteries removed a few hours after death. Fenn’s studies (8) of Poisson’s ratios of vascular tissue pointed to a lower modulus of elasticity in the longitudinal direction than in the circumferential. In this laboratory a series of previous studies (9) confirmed such findings. However, it was felt that more direct evidence of the anisotropic behavior of blood vessels was needed and consequently the present studies were designed to confirm these earlier findings by classical stress-strain analysis, carried out both tangentially and longitudinally, of the wall of one of the main distributing arteries. They also include considerations of an anisotropic effect both within and beyond the physiological ranges of vessel-wall strain and with regard to vasomotion.

Characterization of the blood-vessel wall is complicated because it consists not of a purely elastic, but of a viscoelastic material; a feature which affects its dynamic behavior. Depending upon the arrangements of the viscoelastic elements within the wall, the vascular tension corresponding to a given strain will vary and insight can be gained into the functional and structural characteristics of the wall by observing the time course of the stress in response to strain applied to the vessel wall in two perpendicular directions. These studies therefore include some considerations of the phenomenon of stress relaxation of the arterial wall in both the tangential and longitudinal directions.

Methods

Material

The femoral arteries of six mongrel dogs weighing between 50 and 60 lbs were removed a few seconds after the animals had been killed by a blow on the head.1 The arteries were cleaned of loose adventitia and blood and kept overnight at 0°C in a standard salt solution2 through which a 95% O2-5% CO2 mixture was bubbled. The following morning the longest possible segment (2 to 3 cm) was cut from the specimen between two branches; such a segment can be assumed to have a uniformly distributed structure. From this sample a ring 3 to 4 mm in thickness was selected for the measurement of the tangential modulus, and the remainder for that of the longitudinal modulus.

In these studies the ring samples were tested as intact loops in preference to the usual helical strips for two main reasons. (a) Observation of the pictures of dissected vascular smooth muscle published by Benninghoff (11) and subsequent personal observation of fresh and histological samples revealed that the smooth muscle bundles generally run in such a close helix that they are practically perpendicular to the longitudinal axis of the vessel; therefore, any helical cutting of a sizable strip would necessarily transect smooth muscle bundles and so destroy some of the essential properties of the wall in the circumferential direction. On the other hand, the borders of a ring would essentially be parallel to the direction of the muscle bundles. (b) The attachment of the helical strip to the measuring apparatus necessitates some type of crushing of the tissue to avoid slippage of the sample at high tension. However, the intact ring sample can be held very simply between two smooth platinum U-shaped pins whose arms are screwed to the measuring apparatus.

1The blow is delivered with a Shermer mechanical stunning apparatus (Shermer Humane Killer, model M.S., Alfa International Corp., New York, N. Y.). This method is the one legally accepted for the euthanasia of domestic animals. It kills the animal in a fraction of a second and permits avoidance of changes in vascular properties inherent to any type of general anesthesia.

2The composition of this solution, suggested by Bohr (10), contains: NaH2PO4:1.2 mm; MgSO4:7H2O:1.2 mm; CaCl2:2.5 mm; NaHCO3:22.5 mm; NaCl:121.3 mm; K2SO4:2.25 mm; glucose:5.5 mm.

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Diagram of the apparatus and recording equipment described in the text. The solenoid which delivers the stepwise strains is mounted on a rail; after each step it is moved away from the sample over a distance equivalent to the next step strain. The ring sample is mounted between two platinum pins fixed to the arms of the apparatus. The longitudinal sample is mounted on two hollow, plastic cylinders in which small holes have been drilled to permit circulation of the surrounding liquid.

The longitudinal specimens were used as tubes instead of strips to avoid transecting the smooth muscle bundles. They were mounted in the bath by attaching them to small plastic tubes which had a diameter roughly similar to that of the unstretched sample. Holes were drilled along the side of the mounting pieces so that the fluid bathed all the surfaces of the sample and to ascertain that the transmural pressure was zero throughout the experiment.

The samples were incubated for two hours at 37°C in an oxygenated standard solution; in turn, they were mounted into the bath of the special isometric apparatus described below.

APPARATUS

Figure 1 depicts the isometric system used for the studies. The strain is applied to the sample in a stepwise manner by a solenoid. The time course of the applied strain is measured by a differential transformer mounted as shown in the diagram; the output of the transformer is recorded on magnetic tape. This system is calibrated with a caliper mounted parallel to its axis. The accuracy of the readings is within 0.01 mm.

The stress resulting from the applied strain is recorded by a transducer composed of a rigid arm on either side of which silicone semiconductor strain gauges have been mounted to form two arms of a Wheatstone bridge. The output of this transducer is amplified and recorded on magnetic tape simultaneously with the output of the strainometer. Calibration is performed by hanging a series of weights ranging from 0 to 100 g in 5 g steps.

The length of the samples was measured before each run with a precision divider whose span was determined under a machinist microscope, permitting accurate reading to 0.0025 mm. Small rings were cut immediately adjacent to the tested sample, and the radii and wall thickness of these unstressed samples were measured under the same microscope.

PROCEDURE

After two hours of incubation at 37°C in the standard salt solution, the sample was stretched once to 60 or 70% of its resting length to overcome the constriction due to the prolonged unstretched state (12). The initial length (l0) was defined as being the greatest length at which the tension was still zero, then a series of strains in the form of step functions was applied in the manner described in Figure 2. The magnitude of each step was maintained at between 4 and 8% of the original length in order to correspond to physiological ranges of strain.

After a control run the sample was allowed to return to its original length and norepinephrine was introduced into the bath until a concentration of 3/µg/ml was achieved. After 15 minutes a certain amount of tension had developed; the ring sample was then allowed to contract till the tension dropped to zero. This new length was measured and the new cross section estimated as accurately as possible with precision calipers. These two measurements were later used to correct the strain and stress values for the norepinephrine response. The sample was then returned slowly to its original length and a series of step functions identical to those of the control run was applied to the sample. Preliminary experiments performed by Dr. G. S. Thind had shown that with such a concentration of norepinephrine, a steady state of constriction is reached within ten minutes and persists for over one hour thus providing grounds to assume that during the time required for the tests (half an hour), the smooth muscle was in a steady activated state.

Model 7DCDT-100 Hewlett-Packard Company, Sanborn Division, Waltham, Massachusetts.

Model N01-16-350 Micro Systems Incorporated, Pasadena, California.
At the end of this run the standard salt solution was replaced by a solution containing a mixture of metabolic inhibitors, and after two hours of equilibration the series of step functions was again repeated.

The procedure described above was applied to the ring sample to determine the tangential mechanical properties of the artery under conditions of control, smooth muscle contraction, and smooth muscle metabolic inhibition. The longitudinal sample was then tested under similar conditions.

Results

For each step function the relation between stress and strain was determined twice. The first, corresponding to point A in Figure 2, was made at maximal tension resulting from the stepwise strain. The second measurement of tension, corresponding to point B in Figure 2, was obtained after a steady state had been reached.

Strain values were computed as the ratio of the incremental change in length to the initial maximum length at which tension was zero. For the longitudinal samples this length was measured between the two ties which attached the vessel to the mounting pieces (Fig. 1, bottom). The initial length of the ring samples was taken as being one half the circumference of the unstressed rings. This is justified by the fact that our samples assumed a flattened shape once mounted in the bath before any tension could be detected on the monitoring oscilloscope. The latter was calibrated to a sensitivity of 0.03 v/g. It should be pointed out that our samples were narrow rings, 3 to 4 mm in width, cut from the femoral arteries of large dogs. These vessels have an average diameter of 4.5 mm. Such rings have a tendency to collapse under tension so small that our recording apparatus could not detect it. This, however, is not the case for smaller vessels which retain their circular shape at zero tension and flatten more gradually when submitted to stretch. Figure 3 shows a typical side view of a ring sample at zero tension and initial length where the longitudinal axis of the blood vessel is perpendicular to the plane of the picture. Since the arm of the stress transducer is not infinite-
ly stiff the necessary correction of the recorded elongation was made to represent the actual strain of the vessel specimen. This effect, however, is a linear function of tension. Since tension is a stress it has to be expressed as force/unit area; this area being the surface of the tested material which is perpendicular to the direction of the force. The initial cross sections of the ring samples were computed as the surface area of a rectangle whose two adjacent sides were respectively the width of the ring and twice the wall thickness. The initial cross section of the longitudinal samples was taken as \( \pi (r_0^2 - r_i^2) \), where \( r_0 \) and \( r_i \) are respectively the outer and inner radii of the unstretched tube.

The volumes of the samples were computed from their initial cross sections and initial lengths. It was assumed that the initial volume did not change during subsequent application of increasing strains, so that it was possible to compute new cross-sectional areas for each step.

The measured tensile forces resulting from each stepwise strain were divided respectively by the appropriate cross-sectional area of the specimens and are reported as stress (force/unit area in g/cm²). Since the ring samples already assumed a flattened shape at zero tension and initial length (Fig. 3) one is justified in assuming that, even at low stress, the strains were applied in a direction parallel to the majority of the smooth muscle bundles (excepting the few adjacent to the pins) and that the moduli thus obtained are comparable to the tangential or circumferential moduli.

Figure 4 represents the control stress-strain relations obtained at points A and B in both the tangential and longitudinal directions. Each point represents the mean of six experiments. At low strain, the longitudinal modulus of elasticity is slightly higher than the

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Throughout the text the term modulus of elasticity refers to the modulus determined by the tangent to the stress-strain curve at any point.
tangential modulus, but due to the marked nonlinearity of the relationship, there is a cross-over at a tension of about 500 g/cm². While the longitudinal modulus changes very little, the tangential modulus rises sharply with increasing strain. From this diagram it is evident that the total stress relaxation, as expressed by the difference between the A and B curves for each run, is very small in the longitudinal direction but larger in the tangential direction. Furthermore, in higher tension ranges the stress relaxation increases both tangentially and longitudinally.

Figure 5 shows the comparison between the tangential and longitudinal stress-strain relationship during the response of the vascular smooth muscle to norepinephrine. The tangential relation is represented twice, the upper curve (broken line) representing the relation after the development of a steady isometric contraction, i.e., at the beginning of the norepinephrine run the length of the samples was identical to that of the control condition. The lower curve (dotted line) represents the same relation corrected for the decrease in initial length due to the shortening of the samples produced by the action of norepinephrine. As an example of how this correction was made, one can assume that a ring sample had an initial length of 3.5 mm at zero tension during control conditions. While being maintained at this length, it was submitted to the action of norepinephrine and was found to have developed a tension of 300 g/cm². If the sample were then allowed to shorten until the tension dropped to zero and if the new length were found to be 2.7 mm, this value would then become the new \( l_0 \) for the norepinephrine run and the corrected strain corresponding to 300 mm Hg would become \( \frac{3.5 - 2.7}{2.7} = 0.29 \). Similarly, all subsequent corrected strains would be expressed with 2.7 mm as the initial length instead of 3.5 mm. Corrections were unnecessary for the longitudinal relation (solid line) since norepinephrine did not produce any initial increase in tension in that direction. The correction applied to the tangential relation affects the curve markedly; the uncorrected points all lie well above the longitudinal curve but the corrected values remain significantly below.

Figure 6 shows the stress-strain relations in the tangential direction during three conditions; control, constriction of the smooth muscle by norepinephrine, and after inhibition of the metabolism necessary for muscle contraction. Again the norepinephrine curve appears twice: uncorrected and corrected for the new \( l_0 \). The standard errors were omitted on this graph; however, they were identical to those shown in Figures 4 and 5. The curve of the samples whose muscle metabolism had been inhibited is, curiously enough, not very different from the control. The two relations overlap in the low ranges of tension and
above 300 g/cm² the relation for the poisoned samples lies just below the control; the A values of the former being practically identical to the B values of the latter. The uncorrected norepinephrine curve lies much above that of the control until the 1,500 g/cm² range of tension is reached; above this value the curves overlap. On the contrary, the corrected norepinephrine curve overlaps the control relation until tension is about 300 g/cm²; thereafter, it runs well below the control over the remaining range of the experimental data.

Another outstanding feature of the tangential norepinephrine-induced stress-strain relationship is the marked increase in stress relaxation as compared to control. The poisoned samples, on the other hand, exhibited a small decrease in the amounts of stress relaxation.

Figure 7 represents relations similar to those of Figure 6 but in the longitudinal direction. The overlap of the three curves control, norepinephrine, and after metabolic inhibition is evident.

Discussion

Information concerning certain mechanical characteristics of the blood vessel wall can be
FIGURE 6
Tension-strain relations in the tangential directions during control conditions, after norepinephrine, and after inhibition of the smooth muscle metabolism. As in Figure 4, the norepinephrine curve appears twice, uncorrected and corrected for decreased initial length.

Gained from the results presented here. In particular, the validity of the two basic assumptions usually made in the analyses of vascular system characteristics (isotropy and linearity) may be evaluated.

ANISOTROPY
The arteries of all animals studied were found to be anisotropic over most of the ranges of tensions studied. This is evident from Figure 4 which illustrates a marked difference between the tangential and the longitudinal stress-strain relationships with, however, a region of isotropy within a range of tension varying from 300 to 450 g/cm².

Below this range the longitudinal modulus is slightly higher than the tangential but beyond, the situation is reversed. It is important to determine whether the narrow region of isotropy of the blood vessel wall lies within the physiological operating ranges of tension and strain in both tangential and longitudinal directions.

Within the wall of a cylinder the mean tension in the tangential direction is related to the radial pressure by a geometrical factor which is the ratio of the radius to the wall thickness. Peterson (13, 14) has discussed in detail and justified experimentally the importance of this relation for the estimation of the...
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TENSION

A control
B control
A norepinephrine
B norepinephrine
A poison
B poison

FIGURE 7

Tension-strain relations in the longitudinal direction during control conditions, after norepinephrine, and after inhibition of muscle metabolism.

mechanical properties of the vascular wall. From these considerations it is possible to compute that, within a femoral arterial wall, the mean tangential stress resulting from an intra-arterial blood pressure of 100 mm Hg corresponds approximately to 680 g/cm² (13). The physiological range of blood pressures can be considered to be between 60 and 250 mm Hg, corresponding to a range of vessel-wall stresses extending from 400 to 1,700 g/cm². Therefore, only the low values of the physiological range of wall tension lie within the isotropic region. There are no experimental data available from which one could calculate the in vivo longitudinal stresses of the femoral arteries, but from in vivo longitudinal recoil studies previously made in this laboratory (9) the physiological longitudinal strains were calculated for two positions of the dog's hind limb. When the latter was at a right angle to the vertebral column, the strain was 0.11; and when the leg was extended parallel to the vertebral column, the strain was in the vicinity of 0.7. (This last position of the legs with respect to the trunk is the one usually assumed by the animal while lying on the experimental table.) It is legitimate to assume that within the range of quiet, daily, normal activity of the animal the longitudinal strain will vary around 0.1, and lie, therefore, within the low portion of the stress-strain curve and most likely below the cross over.

On the other hand, the ranges of longitudinal strains for the animal in the experimental position are all well beyond the isotropic region; therefore, in both normally active and experimental conditions the longitudinal modulus is lower than the tangential modulus in the physiological range. Only certain positions of the hind limb (most likely corresponding to certain positions of the running sequence) will bring the longitudinal modulus within the isotropic region, assuming that at this instant the blood pressure remains low.

The results of the present studies confirm previous findings obtained with completely different methods (9) that the femoral arteries are anisotropic and also that within the physiological operating range the modulus of elasticity was lower in the longitudinal than in the tangential direction. These results also confirm earlier work by Fenn (8) who obtained, from arterial samples, Poisson's ratios incompatible with the usual assumption of isotropy.

The question of how vasoconstriction affects the anisotropic condition of the arterial wall led to the norepinephrine experiments. The longitudinal stress-strain relation is not altered by the constriction of the vascular smooth muscle (Fig. 7). The tangential orientation of the muscle bundles is well known; however it is remarkable that the activity of the latter exclusively influences the tangential characteristics of the vessel wall without any significant alteration longitudinally. The manner by which the constricted smooth muscle affects the tangential mechanical proper-
ties of the artery is not clear. As indicated by the uncorrected and corrected norepinephrine curves of the tangential relation in Figures 5 and 6, much depends upon the definition of the initial length \((l_0)\) of the sample reacting to a vasoconstrictor agent. When strain is computed relative to initial unstressed length, the data related to the constricted sample presents a paradox, i.e., the constricted vessel exhibits a lower modulus of elasticity than during control conditions (Fig. 6). This phenomenon has already been reported; as early as 1913, Kesson (15) observed it on cat intestine, and more recently Landgren (16) described a similar condition in the wall of the carotid sinus, as did Alexander (12) in mesenteric veins of dogs and intestines of rabbits. Burton (5) explained the phenomenon by postulating that when the vessel's diameter decreases due to smooth muscle constriction, the elastic and collagen fibers are under lower tension, and thus the stiffness of the vessel's wall is decreased. Converse behavior was observed (9) on dogs' femoral arteries in vivo submitted to a vasodilator agent; the modulus of elasticity was higher during vasodilation than during control conditions. These phenomena are, however, not as paradoxical as they may first appear. Peterson (13, 14) has repeatedly pointed out the importance of geometrical factors in the evaluation of vascular tension. As an example, consider a hypothetical thick-walled blood vessel subjected to a blood pressure of 100 mm Hg having, under control conditions, an outside radius of 2.3 mm and an inside radius of 1.9 mm: the distribution of tensions within the wall can then be represented by the solid line in Figure 8. (Tension distribution can be computed from the following formula derived by Timoshenko (17) \(T = \frac{P}{\frac{b^2-a^2}{2a}}\), where \(a\) is the inside radius, \(b\) the outside radius, and \(P\) the internal pressure.) Assume a new outside radius, after vasoconstriction, of 1.8 mm and an inside radius of 1.3 mm: if the blood pressure remains at 100 mm Hg, the change in geometry lowers the tension within the wall as evidenced by the dotted line. Therefore, it may be concluded that once the smooth muscle has actively altered the geometry of the blood vessel, maintenance of the existing geometry may be achieved at tensions lower than in the relaxed state. The new geometry enables the vascular muscle to maintain a prolonged increase in resistance to the flow of blood with great economy of energy.

Returning to the problem of anisotropy, it is evident from Figure 5 that during vasoconstriction the tangential modulus is consistently lower than the longitudinal and there is not an isotropic region within the physiological range of tension. It should be noted that due to the change in geometry of the constricted artery this physiological range now extends from about 245 g/cm² to the vicinity of 1,025 g/cm² and that 410 g/cm² represents the mean tension produced in the thickened wall by a blood pressure of 100 mm Hg. In this region the elastic modulus in the tangential direction is lower than the one observed within the physiological range of longitudinal moduli; therefore, the anisotropic properties of the wall are reversed as compared to control conditions. Above 800 g/cm² the slope of the
tangential relation becomes stiffer and is almost parallel to the longitudinal curve. Therefore, if the blood pressure within a constricted vessel is equal to or larger than 190 mm Hg, the vascular wall has almost identical moduli of elasticity in the two perpendicular directions and approaches isotropic conditions. At present, it is difficult to assess how this reversal in anisotropic behavior of the constricted arterial wall and the passage from an anisotropic to an isotropic condition in the high regions of blood pressure affects the normal operation of the cardiovascular system, specially because the results presented here are limited to one type of dog's arteries; a necessary step before generalization can be made would be to study a large array of dog's blood vessels and then to assess possible differences between the vascular mechanical properties of different mammalian species.

LINEARITY

The linearity of the elastic properties of the blood-vessel wall is often debated. Although it has long been recognized that the complete stress-strain curve characterizing the vascular tissue in the tangential direction has an S shape, linear behavior of the blood-vessel wall is usually assumed in most analyses of the cardiovascular system, not only within the range of the pulse pressure but also sometimes for the whole range of physiological wall tensions. The first assumption is more appropriate than the second; however, the results of these studies indicate that before assuming either linearity or nonlinearity the conditions of the vascular tissues must be considered. Figure 6 indicates that in the so-called control conditions the tangential stress-strain relation may be considered linear only above 1,200 g/cm²; before this level of tension the slope of the curve changes continuously even between 550 and 820 g/cm² which are the tensions corresponding to a normal pulsatile pressure of 120/80 mm Hg. Therefore, the assumption of linear behavior in a nonconstricted, femoral artery of a dog is valid only if the pressures under consideration range above 175 mm Hg. On the other hand, constricted vessels have a continuously chang-
among the elastic and viscous elements of the wall in the tangential direction. Furthermore, the dramatic increase in stress relaxation exhibited by the norepinephrine curve and its marked diminution after poisoning indicates that the vascular smooth muscle plays a dominant role in the viscous behavior of the wall. The present analysis of the results does not permit the decision whether, by constricting, the vascular smooth muscle only increases its own viscosity and consequently the overall viscosity of the wall or if, in addition, it alters the coupling between itself and the more purely elastic elements of the wall, thereby carrying more load than during control conditions.

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