

ARTERIAL HYDRATION DURING VASOCONSTRICTION

by



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ABSTRACT

The relationship between vasoconstriction and the hydration of the artery wall was examined using the tail artery of the rat.

Freeze substitution was used to prepare histological sections of arteries fixed in a known state of constriction. Measurements of wall dimensions showed that the more constricted arteries had smaller wall and media cross-sectional areas than the less constricted arteries. The constant length of the constricting artery meant that the wall volume decreased by 14%.

Considering the vascular smooth muscle cell as a double cone enabled formulation of relationships between the cell radius, length, surface area, and volume. Radius and length measurements of the smooth muscle cells of the freeze-substituted arteries demonstrated that the cell radius doubled and the length decreased by half during vasoconstriction. These measurements revealed that the surface area of the double cone model of the cell remained constant, while the volume increased during vasoconstriction. This suggested that water entered the contracting vascular smooth muscle cells.

Water and ion content determination of paired control and constricted in vitro arteries indicated that the artery wall lost 16% of its water. This represented a 13% decrease in the wall volume. The associated decreases in the Na and Cl contents and in the inulin space, as well as the constant K content implied that the water was expelled from the extracellular space of the constricting artery. While this was true for arteries constricted with both norepinephrine and high K solutions, it seemed that water lost from arteries constricted with PLV-2, a synthetic vasopressin, may have

come from inulin-inaccessible phases of the wall. The size of the water loss depended upon the duration of vasoconstriction: the losses were largest 30 seconds after the start of constriction.

Perfused rat tail arteries exhibited pressure-flow characteristics during vasoconstriction which suggested that the permeability of the wall had increased. It was discovered that the changes in permeability induced by vasoconstriction were drastically affected by changes in the intravascular pressure. In a third perfusion experiment, the dilution of Evans blue dye passing through the lumen of a constricting artery also indicated a permeability increase during vasoconstriction.

Arteries were incubated in one of five isosmotic solutions of different ionic composition. Comparison of arterial contents and perfusion pressures showed that the absence of monovalent ions in the bathing media resulted in a decrease in arterial water, an increase in divalent ion content, and higher perfusion pressures. These observations can be explained by changes in the tension of the vascular smooth muscle cells and possibly by an ion exchange process in the paracellular matrix which caused conformational changes in the matrix, in turn causing an altered wall hydration.

Arteries, cooled overnight at 2°C, were rewarmed in one of three solutions of different Na concentration. The arteries were transferred from a solution at 2°C to one at a temperature between 2° and 37°C for 15 minutes. Comparison of the arterial contents showed that a small amount of K was gained while large amounts of water and Na were lost from the artery wall during these short rewarming periods. Postulation of a 1:1 exchange of K for Na for the cell metabolic Na-K pump means that a fast Na component was

extruded, independent of K, from the rewarming artery wall. The extrusion of the wall water may have been related to the extrusion of this extra Na component, because they both had the same temperature and external Na concentration dependencies. The monitoring of the intravascular pressure of perfused rewarmed arteries revealed pressure changes with the same temperature and external Na concentration dependencies as the above water content changes. Calculations indicated that changes in wall volume caused by changes in water content could partially explain the intravascular pressure changes during rewarming.

The wall water loss, the permeability changes, and the cell water increase associated with vasoconstriction, are discussed in terms of an osmotic and hydrostatic pressure balance between the artery wall and its surroundings which is upset by vasoconstriction.

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CHAPTER I

INTRODUCTION

The artery is a living, working tissue, supplied by its own blood vessels and by blood flowing through its lumen. The bulk of the wall of the artery consists of spirally oriented smooth muscle cells immersed in a network of collagen, elastin, and protein-polysaccharides. The inside of the wall is lined with endothelial cells butted against an elastic lamina and the outside is a loose coating of collagen fibers, the adventitia. The volume of the artery lumen can be passively altered by changes in the intraluminal pressure. In addition, very fine nervous and humoral controls can change the degree of tension in the artery wall by changing the activity of the smooth muscle cells, resulting in an active change in lumen volume. This means that the artery wall regulates blood flow by varying the volume it encloses.

Vasoconstriction is a decrease in the size of the arterial lumen due to an increase in active tension: both radii of the artery wall decrease while the wall thickness increases. By possessing sufficient elasticity, elastin acts in cooperation with the smooth muscle to allow the total wall tension to automatically change as the artery changes its size (1). This means that the artery is capable of very fine gradations in its degree of constriction (1). Consequently, there are a multitude of physical states of the wall during the transition from relaxed to fully constricted states of the artery. The wall can thus undergo very slight or very drastic physical changes between these different states (see 2). These changes

occur quickly and are thus much more difficult to study than the very slow changes that occur with age (3) and disease (4,5).

An understanding of the physical changes in the artery wall associated with constriction could lead to an understanding of how the components of the artery wall work together. Isolated effects of vasoconstriction have been considered: morphological changes (2,6), ion movements with respect to the artery wall (7,8) and the smooth muscle cells (9,10), membrane potential changes (11), visco-elastic changes (12,13), and hemodynamic changes (14). In addition, the causes of constriction have been examined in terms of the contractile proteins of the smooth muscle cells (15,16), the initiation of constriction (17,18), and the actions of vasoconstrictive agents (19). However, a synthesis of these isolated findings is not yet possible. One of the problems is that few of the studies attempt to relate the observed effects of constriction to the action of the whole wall. The dynamic nature of the artery wall has really only been visualized in studies on the propagation of pulse pressures down the arterial tree (20), in some of the discussions on the question of hyper-reactivity of the hypertensive artery wall (21,22,23), and in various models depicting the interactions of the different wall components (1,24,25).

One component which has received little attention in regard to vasoconstriction is the water in the artery wall. For a muscular artery, about 75% of its weight is water (26): water fills the smooth muscle cells and the extracellular spaces between them. It seems unlikely that this water simply remains a static solvent during the profound changes in the structure of the constricting artery wall.

There have been observations of losses of water from the artery wall during vasoconstriction. From electronmicrographs, Rhodin (27), found smaller intercellular spacings and extracellular space in contracted mouse intestinal smooth muscle than in the relaxed muscle. An increase in the percentage dry weight of the rat aorta exposed to norepinephrine was observed by Rorive et al. (28). Daniel (29) found that extracellular fluid was squeezed out of contracted uterine muscle. Türker et al. (30) observed that angiotensin-induced constriction of both carotid artery and uterus strips resulted in a large decrease in the inulin space. Small insignificant losses of water from the constricting artery wall have also been reported (7,31,32). However, the only discussion in these 7 papers of the implications of this finding was Rorive's comment that the water loss may have been due to the distribution of ions between the different tissue compartments (28).

The reluctance to consider the implications of fluid leaving the constricting artery wall may arise from the assumption that the wall has a constant volume during vasoconstriction (13,33). However, this assumption was carried over from Lawton's observation on the incompressibility of the artery wall subjected to small strains (34) which has nothing to do with vasoconstriction. There are in fact two distinct processes: stretch, to which the artery wall responds passively without the expenditure of energy (1), and vasoconstriction, which is an active process arising within the wall itself, requiring energy, and involving large strains. [This distinction, of course, is for stretch without a myogenic response (see 35).]

Examination of the effect of constriction on arterial water can lead to a better understanding of how the artery constricts. Models of the artery wall consider it as a network of smooth muscle cells and extracellular

solids which act as a unit to alter the degree of constriction (1,24,25). There may be an analogy between the artery wall and a gel or ion exchange resin: the degree of swelling, i.e. the water content, is affected by the elastic forces within the network of the gel (36,37). Alterations in the elastic forces alter the hydration of the gel. Similarly, changes in arterial water content may imply alterations in the elastic forces within the artery wall. This point is discussed more fully in Chapter VI.

The increased water content of hypertensive arteries (21,26) may be related to the increased Na and mucopolysaccharide contents (26,38). Knowledge of the factors affecting normal arterial hydration may help in understanding some of the physical changes associated with hypertension.

In this study, the relationship between vasoconstriction and arterial hydration was examined from several different experimental angles. Some of the aspects considered were: the constancy of the volume of the constricting artery wall (Chapter II), changes in size and shape of the contracting vascular smooth muscle cells (Chapter II), the effect of constriction on arterial hydration (Chapter III), the relationship between the constricting artery wall and its pressure-flow characteristics (Chapter IV), and the role of the paracellular matrix in arterial hydration during ion exchange and cooling-rewarming procedures (Chapter V). The initial experiments compared the extremes: relaxed and constricted arteries, while the later experiments considered the process of vasoconstriction.

Since the whole vascular system, perfused with blood, was much too complex for this investigation, isolated arteries perfused with a physiological solution were used. All the information was gathered from in vitro and in situ preparations of the rat tail artery. Vasoconstriction was

induced by different agents which apparently have different mechanisms of action (for example, 19). Since this study was concerned with the effects, not the causes of constriction, the differences between the agents were only briefly considered. Adult male albino rats of a specific pathogen-free Wistar strain (SPF, Woodlyn Farms) were used. The rats were anesthetized with 3.33 mg sodium pentobarbital per 100 g body weight administered intraperitoneally, and 6 mg sodium phenobarbitone per 100 g administered subcutaneously.

The methods and literature for each of the experiments are given in the separate chapters. The final chapter contains a discussion which relates the individual findings to the overall problem of vasoconstriction and arterial hydration. The references in the Bibliography are arranged according to the chapters.

CHAPTER II

CHANGES IN VASCULAR DIMENSIONS DURING CONSTRICTION

Arterial dimensions have been measured in situ (1-4) and from histological sections (5,6,7). By providing information on the appearance of the artery in a given physiological state, these methods are a good complement to the usual biochemical and physiological approaches. Baez has examined relaxed and constricted microvessels in vivo (2) but there is very little data on dimensional changes of constricting muscular arteries. The present study attempts to provide some of this information.

Several approaches were taken to this problem of changing dimensions of a constricting artery: (a) direct measurements of the arterial radii using a TV scan technique (1) (see Appendix I), (b) measurements of the spiral angle of vascular smooth muscle cells from histological sections (see Appendix II), (c) histological procedures to fix the artery in a known functional state. It was found that the best method for determining vascular dimensions was to use histological sections of freeze-substituted arteries.

A. CHANGES IN DIMENSIONS OF ARTERY WALL DURING CONSTRICTION

It is usually assumed that the cross-sectional area of a constricting artery is constant (6,7). However, there are indications that this may not be so (8,9). Using freeze-substituted arterial sections, the present study examined: (a) the wall cross-sectional area and other arterial dimensions during constriction, and (b) the effects of different vasoconstrictive agents on these dimensions.

1. Methods

The ventral tail artery of the rat was exposed at the base of the tail for cannulation. To remove neurogenic influences, the whole tail was removed from the rat. The tail artery was then perfused with Krebs solution (see Chapter III for composition) at 37°C using a constant infusion pump. The intravascular pressure was monitored with a Statham transducer proximal to the cannula. A segment of the artery was exposed, the collaterals tied, and a strip of foil placed under the segment. Through a microscope, the artery was photographed and its outer diameter measured using a micrometer eyepiece. The artery was then frozen in less than 1.5 seconds by directly applying a silver probe which had been cooled to -180°C in liquid nitrogen. The artery was cut free and plunged with the attached foil and probe into absolute alcohol at -80°C. Fixation was by freeze substitution (5,10). The artery was transferred to a solution of 1% osmic acid and absolute alcohol at -80°C for 7 days, then allowed to warm up to room temperature over 48 hours. During this rewarming period, the arteries were washed with absolute alcohol at the same temperature as the rewarming artery. In this manner the problem of refreezing any water remaining in the artery was at least partially solved. The arteries were embedded in paraffin, cut in cross-section, and stained with Mallory trichrome. The above procedure was done in cooperation with the late Dr. G. Scott.

Colour photographs of the artery cross-sections were projected onto a table top. From these projections, the dimensions of the cross-sections were measured with a planimeter. The measurements were calibrated using the projection of a micrometer scale photograph. The total magnification was 458 x for most sections and 284 x for the larger sections. The photographs

of the arteries in situ were used to determine the outer radii for comparison with those measured in situ through the microscope and with those calculated from the histological sections.

Three arterial segments, each about 1.5 cm long, were taken serially from each rat tail artery at 8, 5, and 2 cm from the base of the tail respectively. The three segments were treated in the manner described above. The middle artery segments were perfused with Krebs solution to which vasoconstrictive drugs had been added. A constant infusion pump (B. Braun) was used for the perfusion. These middle segments were photographed before and after the drug application. Two spots of India ink placed on these segments enabled the photographs to be used for determining any changes in the lengths of the segments. The following 7 groups of 6 rats, differing only in the treatment of the middle artery segment, were used:

1. untreated
2. norepinephrine (levarterenol bitartrate, Winthrop), maximal pressor dose (2 μ g)
3. PLV-2 (phenylalanine² - lysine - vasopressin, Sandoz), non-pressor dose (10 mU)
4. PLV-2, pressor dose (80 mU)
5. angiotensin (angiotensin II amide, Hypertensin, Ciba), non-pressor dose (20 μ g)
6. angiotensin, pressor dose (150 μ g)
7. low Na solution (Krebs solution with all but 1.5 meq Na/liter replaced by lactose).

There was no increase in the intravascular pressure of artery segments treated with non-pressor doses of PLV-2 or angiotensin. Altogether, 126 artery segments were processed.

2. Results

The arterial dimensions measured from the histological sections were:

A_o = cross-sectional area of lumen + wall

A_e = cross-sectional area of lumen + media

A_i = cross-sectional area of lumen

There was some difficulty measuring A_o because the outside of the adventitia was very irregular and occasionally torn or broken off. Quite accurate measurements of A_e were made because of the sharp colour contrast between the media (red) and the adventitia (blue). Except for the very constricted sections, A_i was easily measured. From these 3 cross-sectional areas, the following calculations were made:

A_w = cross-sectional area of the artery wall = $A_o - A_i$

A_m = cross-sectional area of the artery media = $A_e - A_i$ (includes intima)

A_{ad} = cross-sectional area of the adventitia = $A_o - A_e$

r_o = outer radius of the wall = $(A_o/\pi)^{1/2}$

r_i = inner radius of the wall = $(A_i/\pi)^{1/2}$

r_e = outer radius of the media = $(A_e/\pi)^{1/2}$

R = wall to lumen ratio = $\delta/2r_i$

δ = thickness of the wall = $r_o - r_i$

d = thickness of the media = $r_e - r_i$

Δ = thickness of adventitia = $r_o - r_e$

The films of the arterial cross-sections were observed, without identifying the frames, and the sections were ranked as 1,2,3, or 4 according to the degree of constriction. The ranking was based upon the appearance of the artery cross-sections (see 5): (1) relaxed arteries: thin wall with a

smooth intimal surface and long thin smooth muscle cells and nuclei, (2) slightly constricted arteries: slightly thickened wall with occasional slight wrinklins in the intima, (3) moderately constricted arteries: thicker wall with a wrinkled intima, (4) fully constricted arteries: small lumen and very thick wall with a very convoluted intima and shortened smooth muscle cells and nuclei. An example of each of these four states of constriction is shown in Fig. 1.

The errors in the planimeter measurements and calculations were quite small. To estimate the error, an arterial section was photographed at the high and low magnifications--for maximum differences in the measured and calculated values. The largest difference for the areas, radii, and thicknesses was 1.6%.

The lengths of the arterial segments remained constant during constriction. Before and after the application of the vasoconstrictive agent, the separation of the 2 India ink spots on the middle segment was measured. The differences between the final and initial separations were zero for 13/33 measurements, positive for 8/33 (maximum 1.5%), and negative for 12/33 (maximum -2.2%). The average separation before the drug was 1.87 ± 0.006 mm (19.9 cm on the projection). The average length change associated with the application of the drug was essentially zero: -0.0006 ± 0.0004 mm. This value was smaller than the error in the measurements which was about ± 0.1 cm on the projections, or ± 0.01 mm on the arterial segments. Of these 33 middle artery segments, 3 were classed as relaxed, 9 slightly constricted, 9 moderately constricted, and 12 were fully constricted. These static measurements indicate that the length of these arteries did not change.

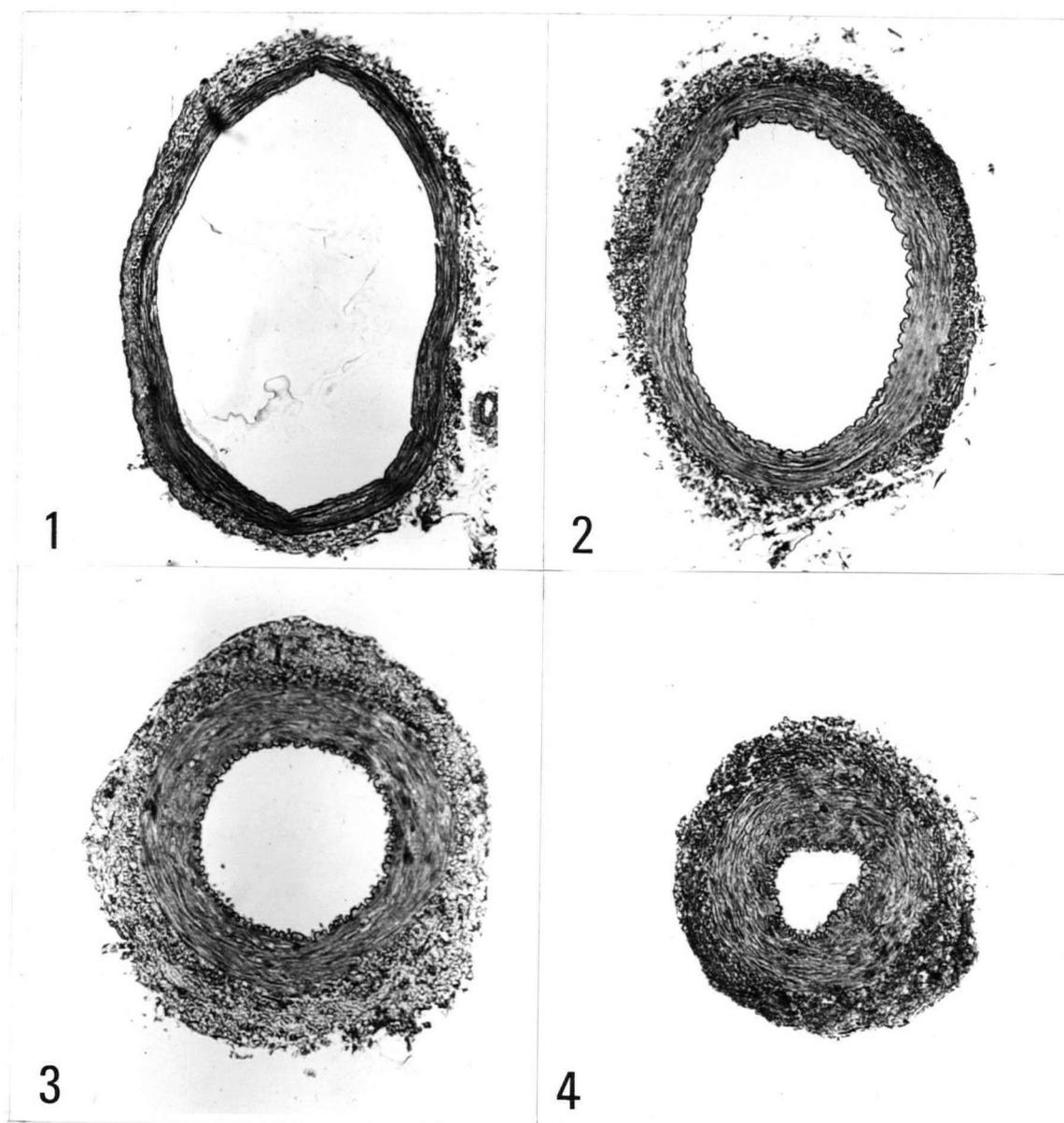


Fig. 1 Four degrees of constriction of the rat tail artery:
1. relaxed, 2. slightly constricted, 3. moderately
constricted, 4. fully constricted.

Since the artery segments were tethered at either end, this result was not unexpected (see 11). Constant length means that the cross-sectional area of the artery wall will reflect any changes in the volume of the wall.

The dimensions of the arterial cross-sections are given in Table I for the three artery segments and the four states of constriction. The distal and proximal segments were not treated with the vasoconstrictive agents while the middle segments were. However, the middle segments were not more constricted than the other two. There was simply a spread of the various degrees of constriction for all three segments. In fact, most of the distal segments were fully constricted and many of the proximal segments were relaxed. The states of these segments before the experimental treatment were not determined, but the "relaxed" proximal segments may have been distended in reaction to the treatment of the middle segment. The constricted appearance of the distal segments suggests that they may have been hyper-reactive or that the rat tail artery is usually fairly constricted. In any case, for the primary analysis, the 126 sections were grouped together, regardless of the cause of constriction.

The relationships between the ranked degree of constriction, 1,2,3,4, and the radii, r_0 and r_i , and the wall to lumen ratio, R , of all 126 sections are shown in Fig. 2. The 3 curves indicate that the wall to lumen ratio is a good index of vasoconstriction (see 5,12,13). Fig. 2 also shows that the greatest increase in constriction was between states 3 and 4. Fig. 3 illustrates the relation between the wall to lumen ratio and the cross-sectional areas of the wall and media, using the average values for the 4 states of constriction rather than all 126 pairs of values. Fig. 3 also shows that

TABLE I. Dimensions (\pm S.E.) of wall of rat tail artery in various states of constriction, calculated from histological cross-sections. The number of sections is given in parenthesis.

	Overall	1 relaxed	2 slightly constricted	3 moderately constricted	4 fully constricted
<u>r_o (μ) outer radius</u>					
all 3 segments	(126) 244 \pm 5	(19) 321 \pm 8	(30) 292 \pm 7	(22) 242 \pm 7	(55) 194 \pm 4
distal	(42) 194 \pm 5	(0)	(3) 254 \pm 22	(3) 236 \pm 11	(36) 186 \pm 4
middle	(42) 247 \pm 8	(4) 335 \pm 19	(13) 285 \pm 10	(10) 230 \pm 11	(15) 203 \pm 6
proximal	(42) 291 \pm 7	(15) 318 \pm 9	(14) 300 \pm 8	(9) 258 \pm 11	(4) 237 \pm 25
<u>r_i (μ) inner radius</u>					
all 3 segments	(126) 135 \pm 8	(19) 255 \pm 8	(30) 211 \pm 6	(22) 131 \pm 9	(55) 52.7 \pm 4.1
distal	(42) 54.1 \pm 7	(0)	(3) 180 \pm 24	(3) 107 \pm 2	(36) 39.2 \pm 3.2
middle	(42) 146 \pm 12	(4) 276 \pm 21	(13) 211 \pm 10	(10) 121 \pm 14	(15) 71.8 \pm 5.3
proximal	(42) 204 \pm 9	(15) 250 \pm 8	(14) 218 \pm 8	(9) 149 \pm 15	(4) 103 \pm 23
<u>δ (μ) wall thickness</u>					
all 3 segments	(126) 110 \pm 3	(19) 66.3 \pm 2.5	(30) 77.3 \pm 1.8	(22) 111 \pm 4	(5) 142 \pm 2
distal	(42) 140 \pm 4	(0)	(3) 73.3 \pm 5.9	(3) 130 \pm 9	(36) 147 \pm 3
middle	(42) 101 \pm 5	(4) 58.5 \pm 4.0	(13) 73.5 \pm 2.4	(10) 108 \pm 6	(15) 132 \pm 3
proximal	(42) 87.6 \pm 4	(15) 68.4 \pm 2.8	(14) 81.7 \pm 2.4	(9) 109 \pm 6	(4) 133 \pm 8
<u>d (μ) media thickness</u>					
all 3 segments	(126) 67.4 \pm 2.2	(19) 36.8 \pm 1.4	(30) 43.8 \pm 1.3	(22) 68.9 \pm 2.8	(55) 90.3 \pm 1.8
distal	(42) 90.4 \pm 2.9	(0)	(3) 40.3 \pm 0.7	(3) 82.0 \pm 7.5	(36) 95.3 \pm 2.0
middle	(42) 61.2 \pm 3.2	(4) 31.3 \pm 2.2	(13) 42.1 \pm 1.2	(10) 67.2 \pm 3.4	(15) 81.8 \pm 2.8
proximal	(42) 50.6 \pm 2.5	(15) 38.3 \pm 1.4	(14) 46.1 \pm 2.3	(9) 66.4 \pm 4.3	(4) 77.3 \pm 3.6

TABLE I. (Continued)

	Overall	1 relaxed	2 slightly constricted	3 moderately constricted	4 fully constricted
<u>A_w ($10^3 \mu^2$) wall area</u>					
all 3 segments	(126) 118 ± 2	(19) 121 ± 6	(30) 122 ± 4	(22) 128 ± 5	(55) 110 ± 4
distal	(42) 107 ± 4	(0)	(3) 99.1 ± 12	(3) 141 ± 15	(36) 106 ± 4
middle	(42) 115 ± 3	(4) 111 ± 8	(13) 115 ± 6	(10) 117 ± 7	(15) 114 ± 5
proximal	(42) 131 ± 4	(15) 123 ± 7	(14) 133 ± 6	(9) 136 ± 7	(4) 143 ± 25
<u>A_m ($10^3 \mu^2$) media area</u>					
all 3 segments	(126) 61.4 ± 1.6	(19) 63.8 ± 3.6	(30) 64.9 ± 3.3	(22) 70.3 ± 3.5	(55) 55.1 ± 2.1
distal	(42) 54.0 ± 2.5	(0)	(3) 50.7 ± 6.2	(3) 76.4 ± 9.2	(36) 52.4 ± 2.5
middle	(42) 60.7 ± 2.3	(4) 57.0 ± 4.6	(13) 61.3 ± 3.2	(10) 65.0 ± 5.9	(15) 58.2 ± 3.7
proximal	(42) 69.5 ± 2.8	(15) 65.0 ± 4.2	(14) 71.3 ± 5.6	(9) 74.1 ± 3.9	(4) 68.0 ± 9.6
<u>$R = \delta/2r_i$ wall to lumen ratio</u>					
all 3 segments	(126) 0.968 ± 0.100	(19) 0.132 ± 0.006	(30) 0.189 ± 0.008	(22) 0.500 ± 0.052	(55) 1.867 ± 0.158
distal	(42) 2.075 ± 0.199	(0)	(3) 0.220 ± 0.044	(3) 0.606 ± 0.036	(36) 2.353 ± 0.195
middle	(42) 0.548 ± 0.063	(4) 0.110 ± 0.013	(13) 0.179 ± 0.011	(10) 0.545 ± 0.094	(15) 0.987 ± 0.068
proximal	(42) 0.278 ± 0.038	(15) 0.138 ± 0.006	(14) 0.191 ± 0.008	(9) 0.416 ± 0.062	(4) 0.801 ± 0.195

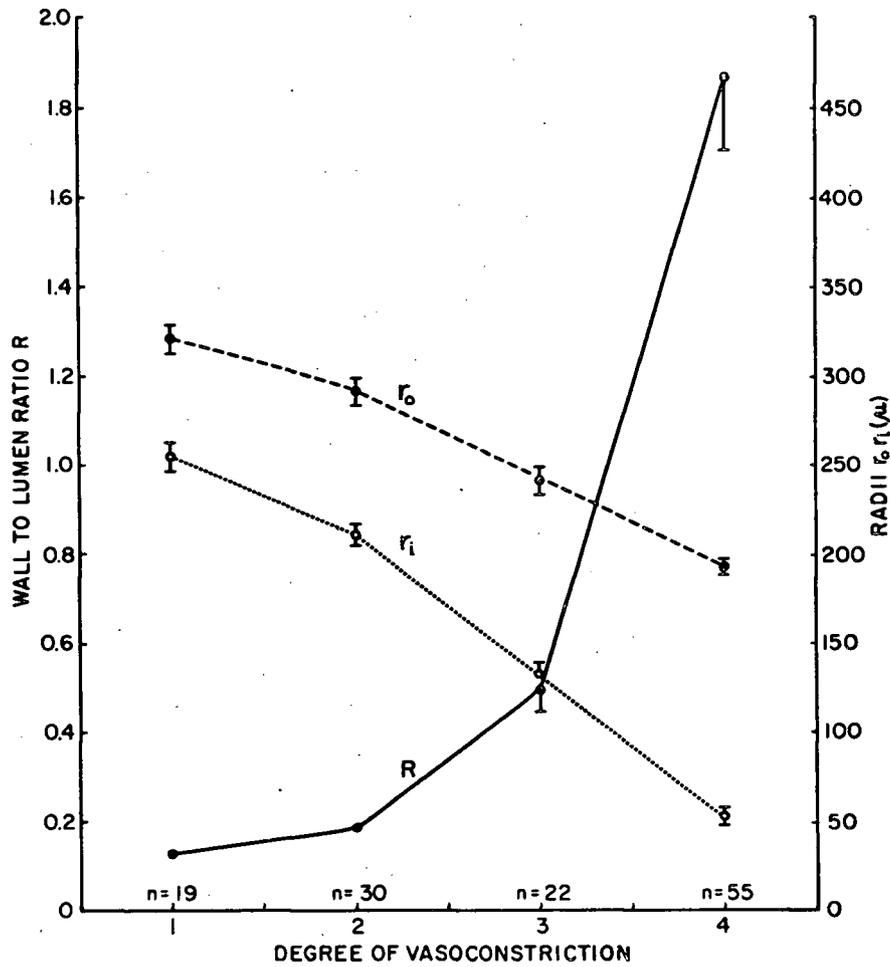


Fig. 2 Relation between wall to lumen ratio, R , inner and outer radii, r_i and r_o , and the degree of vasoconstriction, 1, 2, 3, or 4, for the rat tail artery. The number of artery sections is given by n .

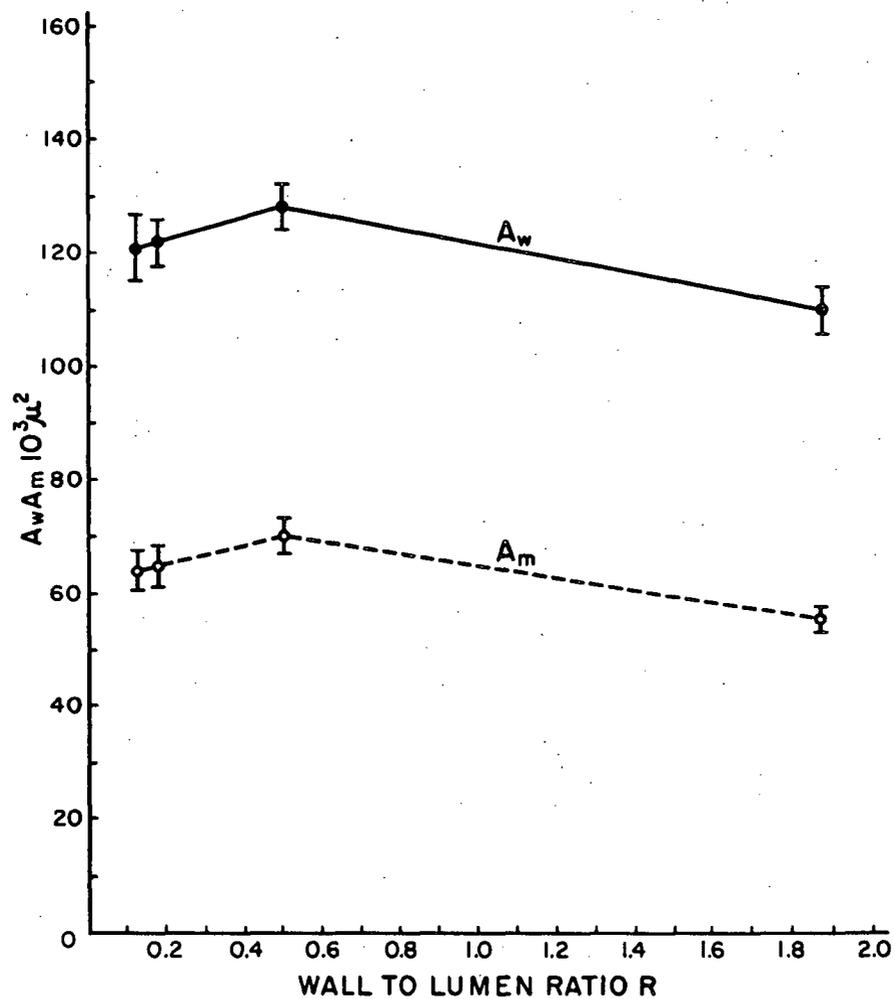


Fig. 3 Relation between the cross-sectional areas of the rat tail artery wall, A_w , and media, A_m , and the wall to lumen ratio, R .

the main increase in constriction, i.e. the greatest increase in R, was associated with a decrease in both the wall area and the media area. This decrease was also reflected in the correlation coefficients (for n = 126) between:

1. A_w and R: $r = -0.412$ ($p < 0.001$); A_m and R: $r = -0.444$ ($p < 0.001$)
2. A_w and states 1,2,3,4: $r = -0.218$ ($p < 0.02$)
 A_m and states 1,2,3,4: $r = -0.171$ ($p < 0.05$)
3. A_w and r_i : $r = +0.392$ ($p < 0.001$); A_m and r_i : $r = +0.466$ ($p < 0.001$)
4. A_w and r_o : $r = +0.609$ ($p < 0.001$); A_m and r_o : $r = +0.616$ ($p < 0.001$)

These significant correlations mean that the more constricted arteries (with larger R, greater degree of constriction, and smaller inner and outer radii) had smaller wall and media cross-sectional areas. The media decreased its area by $8.7 \times 10^3 \mu^2$, or 14%, between relaxed and fully constricted states, and by $15.2 \times 10^3 \mu^2$, or 22%, between the moderately and fully constricted states. Although its overall decrease of $11 \times 10^3 \mu^2$, or 9%, between the relaxed and fully constricted states was not significantly different from zero, the whole wall decreased its area by $18 \times 10^3 \mu^2$, or 14%, between the moderately and fully constricted states. Over 80% of this decrease in the wall area can be accounted for by the decrease in the media area. Since the length of these segments was constant, the decrease in the cross-sectional area means that vasoconstriction was associated with a decrease in the volume of the artery wall of about 14%.

As shown in the columns of Table I, the artery decreases in size distally. Since the distal segments were more constricted than the proximal segments, it might be thought that this taper is the cause of the observed decrease in wall and media areas. However, the separate consideration of

the 3 segments indicates that the decrease in the wall area was associated with vasoconstriction, not with the wall taper.

Fig. 4 shows the inverse relationship between the area of the media, A_m , and the wall to lumen ratio, R , for the distal segments. These segments were obtained from the tail artery before any drugs were applied. The larger values of R were definitely associated with the smaller values of A_m ($n = 42$, $r = -0.479$, so $p < 0.01$). A significant correlation was also obtained for A_w vs R for the distal segments, and for A_m vs R for the middle segments. (On the other hand, there was no correlation between R and the areas for the proximal artery segments. The distended appearance of many of these proximal segments suggested that they may have reacted to the treatment of the middle segments.) These relationships mean that the taper of the tail artery was not the cause of the observed decrease in the wall cross-sectional area. Rather, the walls of the more constricted arteries had smaller cross-sectional areas, and hence, smaller volumes.

The different vasoconstrictive agents did not have the same effect on the dimensions of the middle arterial segments. Having only 6 segments in each group meant that trends and not highly significant differences were observed. Two of the 7 groups of middle segments seemed different in their geometrical response to constriction:

1. The group treated with a non-pressor dose of angiotensin was unique. It was the only group for which the correlation coefficient was positive for R vs A_w and for R vs A_m . This suggested that after a non-pressor dose of angiotensin, arteries which had constricted had larger cross-sectional areas than arteries which were less constricted--the opposite to the observation for all 126 arteries and for two of the segments.

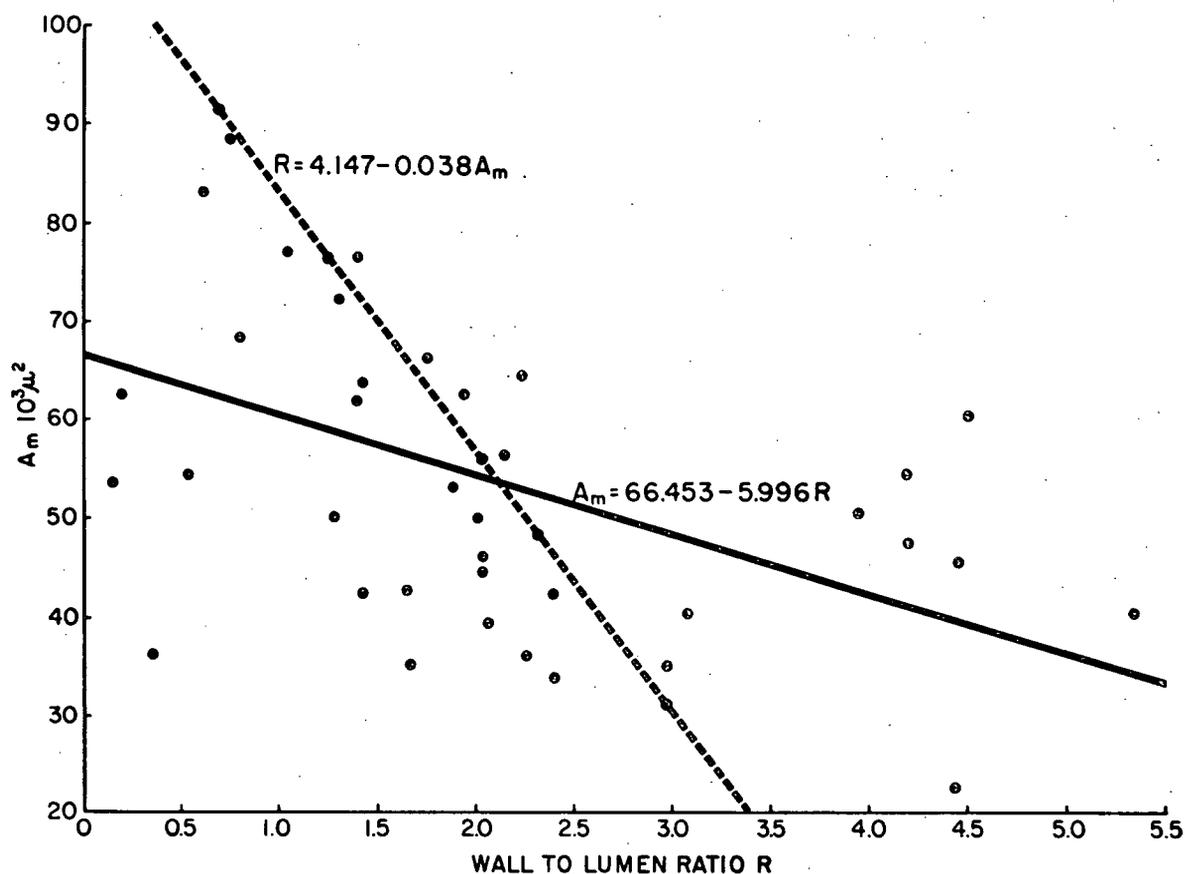


Fig. 4 Relation between the cross-sectional area of the artery media, A_m , and the wall to lumen ratio, R , for the distal segments of the rat tail artery.

2. Some of the arteries were fully constricted even though they showed no pressure rise to a non-pressor dose of PLV-2. These arteries were unusual. Of the 15 fully constricted middle segments, 3 were given PLV-2 in a non-pressor dose. Table II shows that the outer radii and wall area of these 3 were larger than those for the other 12 segments. The difference between these 2 groups was significant only at $p < 0.05$. These differences mean that the fully constricted arteries which were given a non-pressor dose of PLV-2 had larger wall volumes than expected.

TABLE II. Outer radius (μ) and cross-sectional area ($10^3 \mu^2$) of the wall of fully constricted middle segments of the rat tail artery.

	All segments	PLV-2 (non-pressor) segments	other 12 segments
r_o	(15) 203 ± 6	(3) 225 ± 8	(12) 198 ± 6
A_w	(15) 114 ± 5	(3) 135 ± 7	(12) 109 ± 5

These two points suggest that non-pressor doses of angiotensin and PLV-2 alter the geometry of constricted arteries in a manner different than other vasoconstrictive agents. There is a suggestion that they increase the volume of the constricted artery wall. In spite of this fact, omitting one or both of these groups of 6 arteries did not significantly alter the R vs A_w (and A_{III}) relationships for the 126 sections or for the middle arterial segments.

3. Effect of Fixation and Embedding on Arterial Dimensions

Since the above findings depended upon measurements made from histological sections, it is important to know the effect of preparative handling on the arterial dimensions. The only measureable factor was the effect on the outer radii of the vessels. This determination led to an estimate of the shrinkage caused by the freezing, substitution, and embedding processes (see 14).

The outer radii of the arteries in situ were measured: (a) from projections of photographs, and (b) through a microscope with a micrometer scale. These values were compared to those calculated from the histological

sections. These 3 outer radii will be labelled as: $r_o(\text{film})$, $r_o(\text{observed})$, and $r_o(\text{histology})$, respectively. The 3 values of r_o can be compared since the measurements were all made in approximately the same spot on the artery segments: $r_o(\text{film})$ was measured in the middle of the photographs, $r_o(\text{observed})$ was determined for the middle of the segment, and $r_o(\text{histology})$ was calculated for sections cut from the middle of the segments. The maximum errors in these values were: $\pm 10 \mu$ in $r_o(\text{film})$, $\pm 25 \mu$ in $r_o(\text{observed})$, and $\pm 2 \mu$ in $r_o(\text{histology})$. The average values of the 3 outer radii are presented in Table III.

TABLE III. Outer radius, r_o (in μ), of the rat tail artery from in situ photographs, in situ observations, and histological sections.

	overall	proximal segments	middle segments*	distal segments
$r_o(\text{film})$	(157)** 310 ± 6	(40) 373 ± 8	(41) 317 ± 9	(41) 232 ± 5
$r_o(\text{observed})$	(159)** 290 ± 5	(40) 355 ± 7	(41) 287 ± 10	(42) 223 ± 5
$r_o(\text{histology})$	(126) 244 ± 5	(42) 291 ± 7	(42) 247 ± 8	(42) 194 ± 5

* post-drug measurements

** includes pre-drug measurements of the middle segments

1. The differences between $r_o(\text{film})$ and $r_o(\text{observed})$ were significant (by the Student's t-test) only for the proximal artery segments (which had the largest diameters). That these 2 in situ values should be different was not unexpected, but why the average values of $r_o(\text{film})$ were larger than those of $r_o(\text{observed})$ can not be explained.

2. The values of $r_o(\text{histology})$ were significantly smaller than those of either $r_o(\text{film})$ or $r_o(\text{observed})$. The size of the differences indicated

that the arteries did in fact shrink during preparation of the histological sections.

The extent of the shrinkage in the sections can be estimated best by comparing the cross-sectional areas of the artery wall plus lumen, A_0 , before and after the histological preparation. The average values of A_0 , calculated from the corresponding values of r_0 , are given in Table IV.

TABLE IV. The cross-sectional area of the rat tail artery wall plus lumen, A_0 (in $10^3 \mu^2$), from in situ photographs, in situ observations, and histological sections.

	overall	proximal segments	middle segments	distal segments
A_0 (film)	(157) 318 ± 11	(40) 444 ± 18	(41) 327 ± 19	(41) 173 ± 8
A_0 (observed)	(159) 280 ± 10	(40) 403 ± 16	(41) 273 ± 19	(42) 160 ± 7
A_0 (histology)	(126) 199 ± 9	(42) 273 ± 12	(42) 201 ± 14	(42) 122 ± 7

The differences between all 3 values were significant (i.e. $p < 0.02$):

$$A_0(\text{histology}) - A_0(\text{film}) = -119 \times 10^3 \mu^2, \text{ a change of } -37\%$$

$$A_0(\text{histology}) - A_0(\text{observed}) = -81 \times 10^3 \mu^2, \text{ a change of } -29\%$$

The average decrease in the cross-sectional area of the artery wall plus lumen after freezing, dehydration, and embedding was 33%.

It is to be remembered that this shrinkage of 1/3 was in the cross-sectional area of the whole section, not just in the artery wall. However, it was possible to measure the inner radius of the artery wall, r_1 , from the photographs of 3 segments. Since the cross-sectional area of the wall is $A_w = \pi(r_0^2 - r_1^2)$, it was possible to compare A_w for the arteries in situ with A_w calculated from the histological sections. The changes in cross-

sectional area (in $10^3 \mu^2$) after histological preparation of these 3 arteries were: (a) A_o : + 1.8, A_w : + 59.3, (b) A_o : -24.2, A_w : +42.7, (c) A_o : -40.4, A_w : -15.8. While it is not possible to make a definitive statement about 3 arteries, these values do indicate that there need not be a correlation between the decrease in A_o and the decrease in A_w . In addition, since the lengths of the artery segments were not measured after fixation and embedding, any changes in length could not be determined. Consequently, the shrinkage in the cross-sectional area of the artery sections can not be extended to a decrease in the volume of the section, or of the wall itself.

4. Discussion

The cross-sectional areas of the walls of relaxed and constricted arteries have been measured before. However, either no change in wall area with vasoconstriction was observed, or the changes were not mentioned. Using his image-splitting technique, Baez measured the wall radii of 14 microvessels in vivo (2). Although he found no consistent change in wall area after constriction with epinephrine or norepinephrine, he did observe an increase in wall area after vasodilation with acetylcholine. Wiederhielm reported that 43 measurements (S.D. \pm 10%) from photographs showed no significant difference between the wall areas of relaxed and constricted arterioles (7). On the other hand, there is some support for a decrease in arterial wall volume during vasoconstriction. From X-ray photographs, Ticker and Sacks (9) measured the radii and lengths of human brachial and dog thoracic arteries inflated with air. With increased intraluminal pressure, the wall volume decreased. From electronmicrographs of mouse intestinal smooth muscle, Rhodin (8) measured the intercellular spacing and the extracellular space. The values were 1500 A and 11% for the relaxed muscles and 600 A and

5% for contracted muscles. Hinke (6) measured the cross-sectional areas of perfused and non-perfused rat tail arteries from histological sections. Although not mentioned by him, the wall to lumen ratios of these vessels show that the non-perfused arteries, which were more constricted (ratio = 1.22), had a smaller cross-sectional wall area ($46.5 \times 10^3 \mu^2$) than the perfused arteries (ratio = 0.35 and area = $56.5 \times 10^3 \mu^2$). Recently, Phelps and Luft (15) compared the appearances of relaxed and constricted frog arterioles. Two of their electronmicrographs show relaxed and constricted portions of the same arteriole. Planimeter measurements of these figures reveal that the constricted vessel (wall to lumen ratio = 0.738) had an 18% smaller wall cross-sectional area than the relaxed vessel (ratio = 0.218).

Baez suggested that the inner radius decreased more than the outer radius during constriction because of a "tethering action afforded by the tissue structures surrounding the microvessel" (2). Since the same observation was made in this study for arteries separated from their surroundings, this suggestion seems unlikely. The increase in wall thickness is more likely due to the mechanism of constriction of the vascular wall (see 16).

A decrease in the cross-sectional area and volume of the artery wall during constriction suggests that the wall lost some of its water when it constricted. This suggestion will be examined in the next chapter.

5. Summary

1. Freeze substitution was a good method for fixing arteries in various states of constriction.

2. The process of freezing, dehydrating, and embedding the arteries was associated with a decrease of 1/3 in the total cross-sectional area of the artery wall plus lumen.

3. From a classification of 4 states of constriction, the average outer diameter decreased from 642 μ for the relaxed rat tail artery to 388 μ for the fully constricted artery--a decrease of 40%. The diameter of the lumen decreased from 510 to 105 μ --a decrease of 80%.

4. The wall to lumen ratio was a good index of the state of constriction of these arteries.

5. In all states of constriction, the media accounted for about 50% of the artery wall cross-sectional area. Since the adventitia was very loose, the % by weight was probably higher.

6. The length of these tethered arterial segments did not change after vasoconstrictive agents were applied. Thus, changes in the artery wall volume were reflected in changes in the cross-sectional area.

7. The more constricted arteries had significantly smaller wall and media cross-sectional areas than less constricted arteries. Vasoconstriction was associated with a decrease in the volume of the artery wall of about 14%.

8. Such a decrease in wall volume suggests that there are movements of fluid out of the constricting artery wall.

9. Non-pressor doses of angiotensin and PLV-2 altered the geometry of constricted arteries in a manner different than other vasoconstrictive agents.

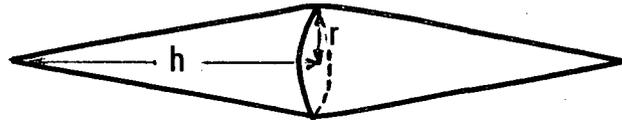
B. CHANGES IN DIMENSIONS OF SMOOTH MUSCLE CELLS DURING VASOCONSTRICTION

Very little has been done on characterizing the geometrical changes of contracting smooth muscle cells. A few dimensional measurements have been made (8,15,17), but comparison of relaxed and contracted cells has been mainly qualitative. Baez (17) found a 32 to 70% increase in the thickness

of smooth muscle cells of the constricting rat arteriole--the greater increase for the greater dose of norepinephrine. Using a model for the smooth muscle cell and measurements from histological sections, a more detailed study of the changes which the vascular smooth muscle cell undergoes during constriction was attempted.

1. Mathematical Model for the Smooth Muscle Cell

As a model for the smooth muscle cell, consider 2 right circular cones joined at their bases:



For the dimensional parameters of the cell, let:

length = 2h

volume = $V = \frac{2}{3} \pi r^2 h$

diameter at center = 2r

surface area = $A = 2\pi r(r^2 + h^2)^{1/2} \approx 2\pi rh$

The approximation for the surface area is justified since the dimensions of the smooth muscle cells of a small artery are such that $h^2 \gg r^2$ (8). This approximation will be examined later. During contraction of the smooth muscle cell these variables will change. Let Δ refer to the changes in these variables. For x, any cellular variable, $\Delta x = x$ (after contraction) - x (before contraction). Also the average value of x is then $\bar{x} = \frac{1}{2}(2x + \Delta x)$. With these definitions, it is possible to express the changes in the cell surface area and volume as functions of the cellular radius and half-length.

Change in cell surface area:

$$\Delta A = \Delta(2\pi rh) = 2\pi(\bar{r}\Delta h + \bar{h}\Delta r) = 2\pi(r\Delta h + h\Delta r + \Delta r\Delta h)$$

So,
$$\frac{\Delta A}{A} = \frac{\Delta h}{h} + \frac{\Delta r}{r} + \frac{\Delta r}{r} \frac{\Delta h}{h} \dots \dots \dots (1)$$

This equation is for the % change in the cell surface area as a function of the % changes in the cell radius and length.

Change in cell volume:

$$\begin{aligned} \Delta V &= \Delta\left(\frac{2}{3}\pi r^2 h\right) = \frac{2\pi}{3}(r^2 \Delta h + 2r \bar{h} \Delta r) \\ &= \frac{2\pi}{3} \left[\Delta h (r^2 + 2r \Delta r + (\Delta r)^2) + \Delta r (2rh + h \Delta r) \right] \end{aligned}$$

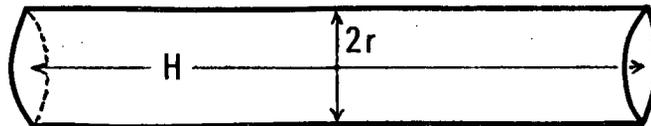
$$\text{So, } \frac{\Delta V}{V} = \frac{\Delta h}{h} + \frac{2\Delta r}{r} + \frac{2}{r} \frac{\Delta r}{h} + \left[\frac{\Delta r}{r}\right]^2 \left[1 + \frac{\Delta h}{h}\right] \dots \dots \dots (2)$$

Substituting equation (1) into equation (2):

$$\frac{\Delta V}{V} = \frac{2\Delta A}{A} - \frac{\Delta h}{h} + \left[\frac{\Delta r}{r}\right]^2 \left[1 + \frac{\Delta h}{h}\right]$$

This equation shows that if the % change in the cell surface area is zero, the % change in the cell volume need not be.

As an alternative model of the smooth muscle cell, consider a right circular cylinder:



Let the cell parameters be:

length = H

volume = V = $\pi r^2 H$

diameter = 2r

surface area = A = $2\pi r H$

Change in cell surface area:

$$\Delta A = \Delta(2\pi r H) = 2\pi(\bar{r} \Delta H + \bar{H} \Delta r) = 2\pi(r \Delta H + H \Delta r + \Delta r \Delta H)$$

$$\text{So, } \frac{\Delta A}{A} = \frac{\Delta H}{H} + \frac{\Delta r}{r} + \frac{\Delta r}{r} \frac{\Delta H}{H}$$

This equation is the same as equation (1) since: $\frac{\Delta H}{H} = \frac{\Delta(2h)}{2h} = \frac{\Delta h}{h}$

Similarly for the change in cell volume:

$$\frac{\Delta V}{V} = \frac{\Delta H}{H} + \frac{2\Delta r}{r} + \frac{2\Delta r}{r} \frac{\Delta H}{H} + \left[\frac{\Delta r}{r} \right]^2 \left[1 + \frac{\Delta H}{H} \right]$$

This equation is the same as equation (2). For the 2 models, ΔA and ΔV are different, but the % changes in A and V are the same. This can be shown by substituting 2h for H in the above 2 equations, so that:

$A(\text{cyl}) = 2 A(\text{cones})$ and $\Delta A(\text{cyl}) = 2\Delta A(\text{cones})$, so that:

$$\left(\frac{\Delta A}{A} \right)_{\text{cyl}} = \left(\frac{\Delta A}{A} \right)_{\text{cones}}$$

$V(\text{cyl}) = 3 V(\text{cones})$, and $\Delta V(\text{cyl}) = 3\Delta V(\text{cones})$, so that:

$$\left(\frac{\Delta V}{V} \right)_{\text{cyl}} = \left(\frac{\Delta V}{V} \right)_{\text{cones}}$$

In addition to giving the magnitude of the changes in the cell area and volume, equations (1) and (2) contain information on the signs of these changes. Since $\Delta r > 0$ and $\Delta h < 0$, the signs of ΔA and ΔV will depend on the relative magnitudes of r , h , Δr , and Δh . But for a given artery, the values of the radius and length of the smooth muscle cells in the relaxed state (i.e. r and h) are fixed, at least within a fairly narrow range. Thus, the magnitude of Δr and Δh determine the signs of ΔA and ΔV . It is possible to construct graphs relating these variables. For example, once values for r and h have been chosen, $\Delta A/A$ from equation (1) can be plotted against $\Delta h/h$ for a family of curves of different Δr values, or against $\Delta r/r$ for a family of different Δh values. Similarly for $\Delta V/V$ from equation (2). Values for r and h are determined in the following section.

2. Measurement of Cell Dimensions

Methods

To obtain the values of the relaxed smooth muscle cell radius, r , and

length, $2h$, for the rat tail artery, and to estimate the physiological range of Δr and Δh (i.e. the difference between the relaxed and contracted values), several of the histological sections from the study described above were used. The stained arterial cross-sections were photographed and projected onto a table top where the total magnification was 2900 x. The cell half-length (from the middle of the nuclei to the end of the cell) and maximum width through the nuclei of relaxed and contracted cells were measured with a ruler. Cells without nuclei in the plane of the section and cells cut in cross-section were ignored. Some of the sections contained both relaxed and contracted cells so that comparison of the dimensions in the 2 states was more meaningful. In addition, the areas of individual cells in the arterial cross-section were measured with a planimeter to determine the better model: double cones or cylinder. The area of 1/2 of a cell was compared with the calculated areas of a triangle (rh) and a rectangle ($2rh$), since they are the projections onto 2 dimensions of a cone and a cylinder, to see which was the closest fit.

Results

The dimensions (\pm S.E.) of the relaxed and contracted smooth muscle cells are given in Table V.

TABLE V. Half-lengths and radii (in μ) of relaxed and contracted smooth muscle cells of the rat tail artery.

	relaxed cells	contracted cells	Δ
half-length, h	(24) 40.3 ± 1.4	(70) 20.9 ± 0.5	$\Delta h = -19.4$
cell radius, r	(87) 1.76 ± 0.03	(83) 3.46 ± 0.07	$\Delta r = +1.70$

From these values, $\frac{\Delta h}{h} \times 100 = -48\%$ and $\frac{\Delta r}{r} \times 100 = +97\%$.

The area of the half-cell was best approximated by the area of a triangle for both relaxed and contracted cells. So, the double cone model is the better fit for these smooth muscle cells. The appearance of the cells agreed with this, although the taper at the extremities of the cells was more pronounced in the relaxed than the contracted cells.

3. Fitting the Experimental Data to the Model

With the understanding that the results are only approximate, the values of the cell radius and half-length were substituted into the equations for the surface area and volume of the double cone model. The values obtained are given in Table VI.

TABLE VI. Surface area (μ^2) and volume (μ^3) of relaxed and contracted smooth muscle cells of the rat tail artery.

	relaxed cells	contracted cells	Δ
surface area, A	446	454	$\Delta A = +8$
volume, V	261	524	$\Delta V = +263$

From these values, $\frac{\Delta A}{A} \times 100 = +2\%$ and $\frac{\Delta V}{V} \times 100 = +101\%$.

So, the model indicates that contraction of these vascular smooth muscle cells was associated with no change in the cell surface area but a doubling of its volume. The approximation made for the surface area of the double cone [i.e. $(h^2 + r^2)^{\frac{1}{2}} = h$] is poorest for the contracted cells. But the values were only 1.5% too small, so the approximation seems justified. If 2 different models were used to represent the relaxed and contracted cells (considering the contracted cells as ellipsoids is the only other realistic possibility), then the % changes in A and V become even larger.

The model predicts the % changes in the surface area and volume of the muscle cells as functions of the % changes in cell length and radius during vasoconstriction. Figs. 5, 6, 7, and 8 show these changes. The curves were obtained from equations (1) and (2) by substitution of the experimental values for the relaxed cell half-length, $h = 40.3 \mu$, and radius, $r = 1.76 \mu$ into these equations. The points on the graphs are those derived from Table VI. Constriction in the rat tail artery would be represented by a straight line drawn from the mid-axes point to the point derived from the data. The graphs show, as mentioned above, that the signs of ΔA and ΔV are determined by the signs of Δr and Δh . Figs. 5 and 6 show: (a) For a given decrease in cell length, the greater the cell thickening, the more positive the change in cell surface area. (b) For a given increase in cell thickness, the greater the cell shortening, the more negative the change in cell surface area. Fig. 7 and 8 show: (a) For a given decrease in cell length, the greater the cell thickening, the more positive the change in cell volume. (b) For a given increase in cell thickness, the greater the cell shortening, the more negative the change in cell volume. (c) An increase in the cell volume is not necessarily accompanied by contraction, or vice versa.

4. Discussion

These cells are about the same size as other vascular smooth muscle cells. Baez (17) found that smooth muscle cells in relaxed rat arterioles were from 2.08 to 2.78 μ thick. Using frog arterioles, Phelps and Luft (15) reported that the relaxed cells were 100 μ long and 9 μ at the widest point. Keatinge (18) found the smooth muscle cells of sheep carotid arteries were 60 to 100 μ long and 2.3 μ wide. Rhodin (8) noted that ". . . muscle fibers

Figs. 5 to 8. The % changes in surface area, $\Delta A/A$, and volume, $\Delta V/V$, of the double cone model of the vasular smooth muscle cell as functions of the % changes in cell half-length, $\Delta h/h$, and radius, $\Delta r/r$. These graphs were calculated from equations (1) and (2) in the text. The values for the half-length and radius of the relaxed smooth muscle cells of the rat tail artery ($h = 40.3 \mu$ and $r = 1.76 \mu$) were substituted into these equations to obtain the 4 families of curves. Note that the scale of the horizontal axis is different in Figs. 6 and 8 than in Figs. 5 and 7. The points on the graphs are those derived from Table VI.

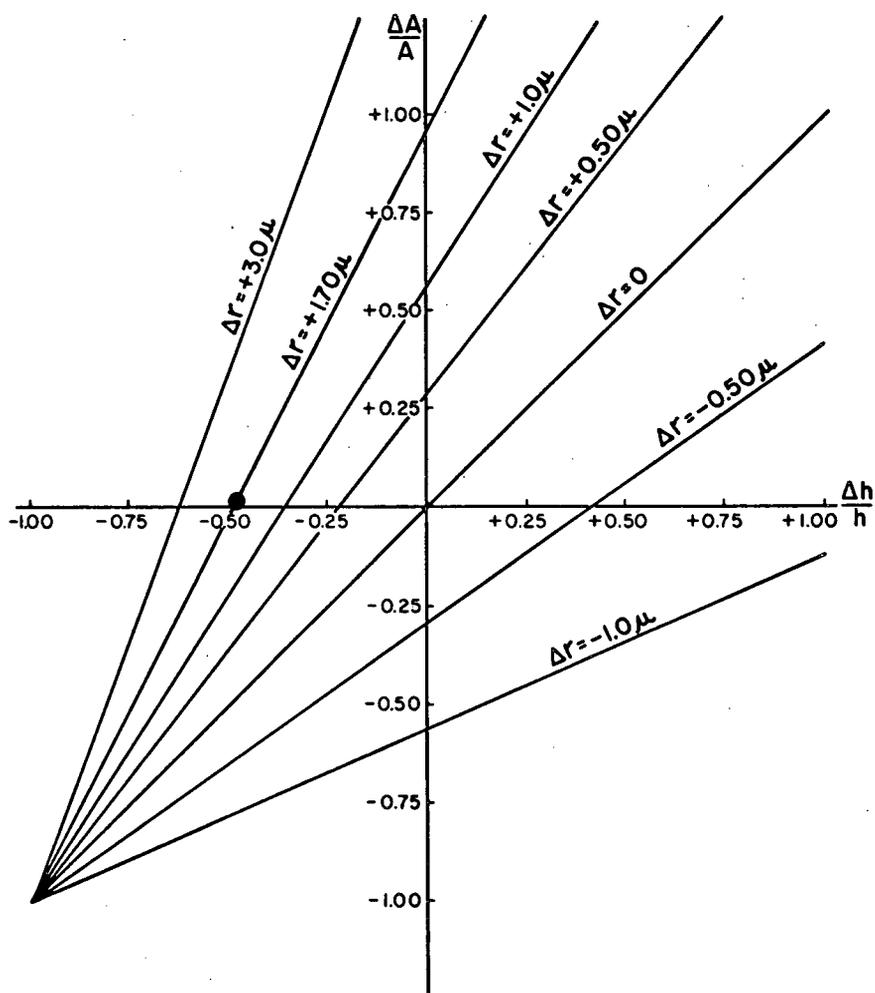


Fig. 5 Cell surface area changes vs cell length changes

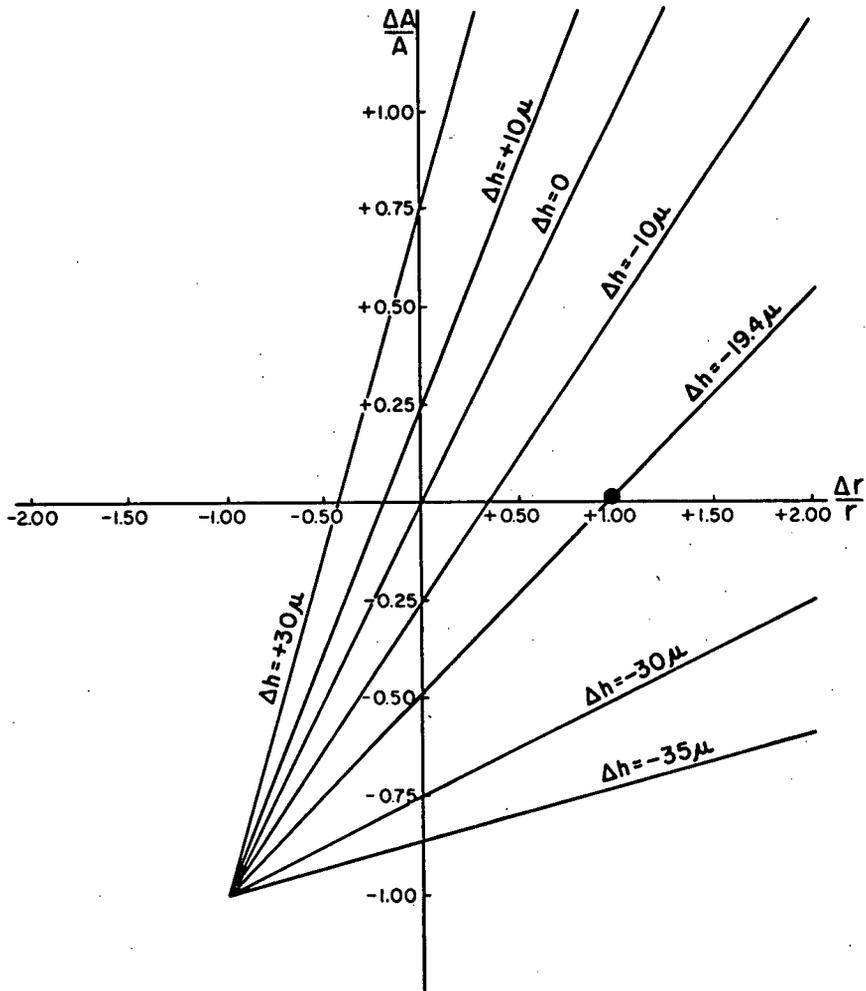


Fig. 6 Cell surface area changes vs cell radius changes

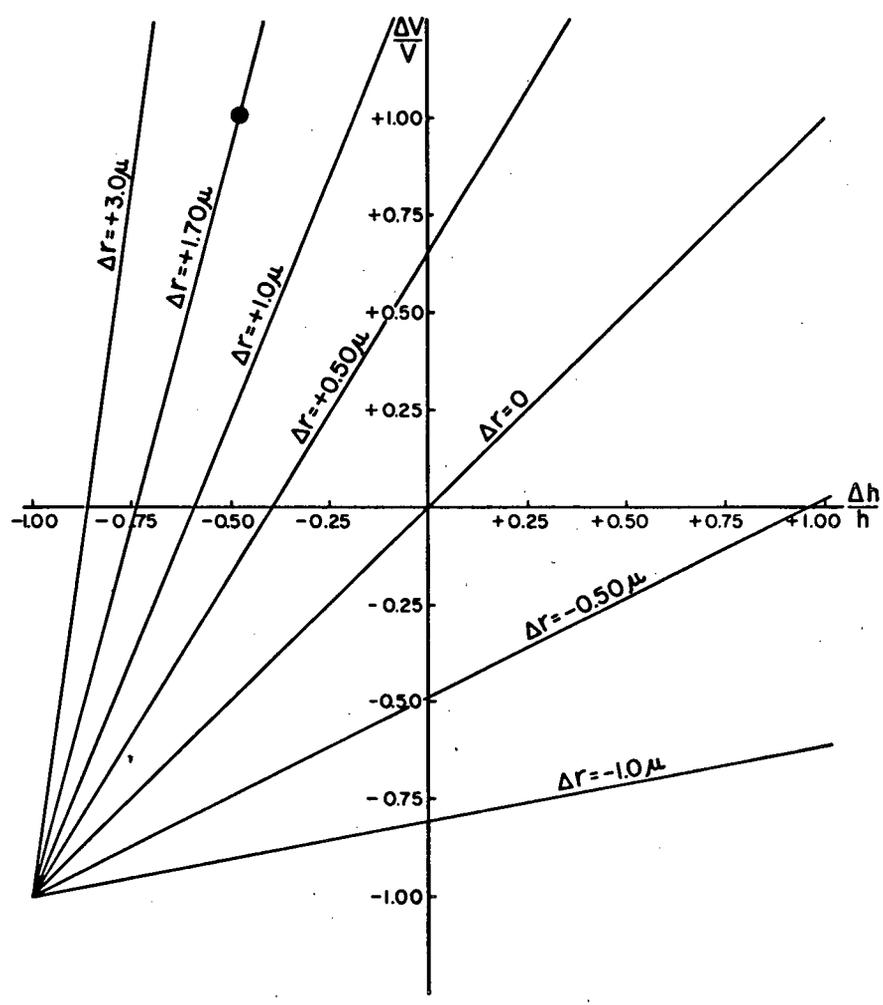


Fig. 7 Cell volume changes vs cell length changes

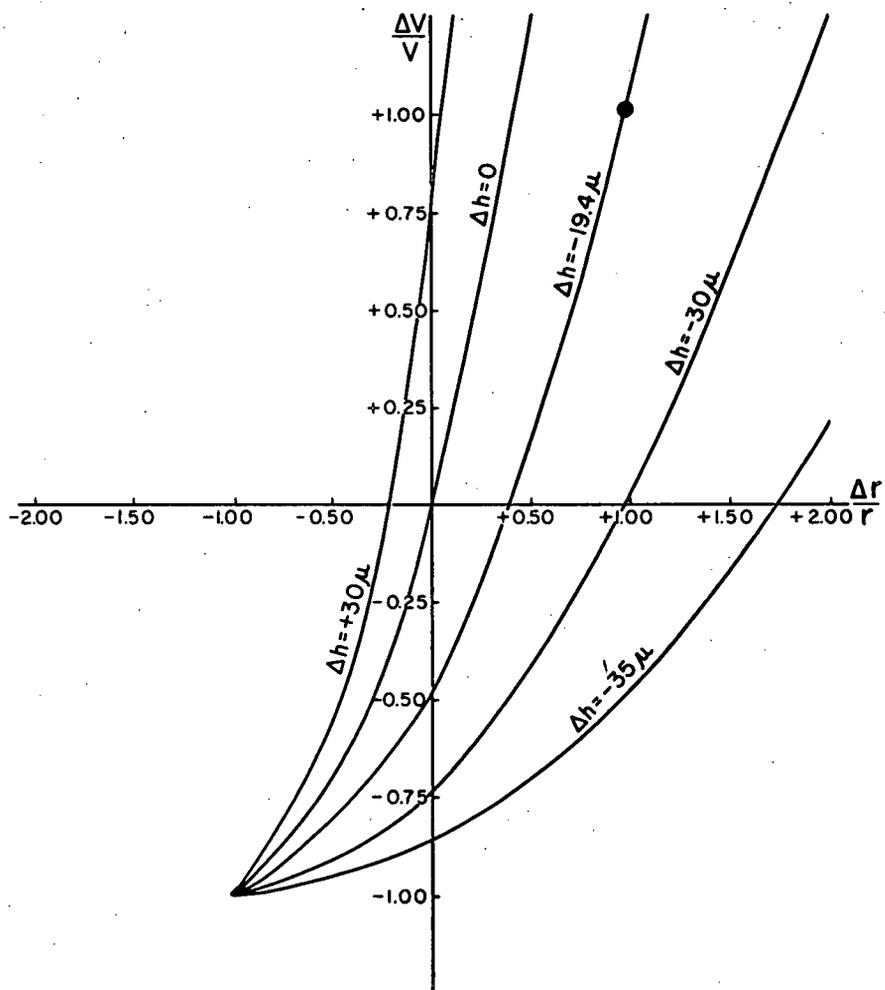


Fig. 8 Cell volume changes vs cell radius changes

of small arteries have a cross diameter in the central cell region of about 1.5 to 2.5 μ and an average length of about 60 μ . . . the total surface area of the cell is about 400 μ^2 plus another 100 μ^2 for numerous inpocketings and pinocytotic vesicles."

The double cone model was a better fit for these rat tail artery smooth muscle cells than the cylinder model. This is in contrast to Rhodin's finding that the ". . . smooth muscle cell in the relaxed state has the shape of a cylinder rather than that of a double cone" (8). However, Phelps and Luft (15) observed that the relaxed cells of a frog arteriole were spindle shaped and the contracted cells had "a roughly cubic shape."

The constant surface area during contraction of vascular smooth muscle cells refers to the surface area of the double cone model--not necessarily to that of the cells themselves. The model does not include the inpocketings in the smooth muscle cell membranes. Changing the number of inpocketings could alter the surface area--depending on the mechanism: (a) if an inpocketing were closed off, then the surface area of the cell would be decreased, while that of the model would be constant, (b) on the other hand, if all the cell membrane in the inpocketing came to the outside of the cell, then the surface area of the model would be increased while that of the cell would be constant. Since changes in the surface area of the smooth muscle cell would probably involve the inpocketings and pinocytotic vesicles, this observation of constant surface area of the model during vasoconstriction, refers to the "envelope" of the cell, not its actual surface area. To include the inpocketings, measurements of the surface areas of vascular smooth muscle cells would have to be made from electron micrographs.

The increase in the cell volume seems extraordinarily large. But the model really only qualitatively indicated that vasoconstriction was associated with an increase in the volume of the smooth muscle cell. It is assumed that the quantity of solids in the cells remained constant (19). If the density of bound water were less than that of free water, an increase in the amount of bound water in the contracting smooth muscle cell would increase its volume, but only slightly. This means that a movement of water into the contracting muscle cell was the cause of its volume increase. Reasons why water would move into the contracting cell will be discussed in Chapter VI.

An increase in the volume of contracting smooth muscle cells would agree with the effects of non-isosmotic solutions on vascular tissue. Hypotonic solutions cause cell swelling and vasoconstriction (19), while hypertonic solutions cause cell shrinkage and vasodilation (20). This interrelationship between cell volume and the degree of vascular tension suggests that the same relation may hold for drug induced tension changes. To explain ionic movements, Friedman (21) has suggested that the smooth muscle cell volume may increase during vasoconstriction. A 5% increase in cell water was observed for the rat tail artery constricted with norepinephrine (22). In addition, although Jonsson (19) suggested that active tension in the rat portal vein opposed cell swelling in hypotonic solutions, he found 7 to 11% increases in the cell water of veins constricted with norepinephrine. It should be noted that these small increases were determined from inulin and sucrose distributions which may not accurately reflect extracellular space changes during vasoconstriction (see Chapter III).

These predictions from geometric models and experimental measurements are first approximations. The changes in volume or surface area of the vascular smooth muscle cell may turn out to be different than those predicted. If so, it would still remain to explain exactly how the changes in geometry of the contracting cell differ from those of the models.

5. Summary

1. Geometrical models of the vascular smooth muscle cell provide a good framework for understanding dimensional changes of the contracting cell.

2. During constriction of the rat tail artery, the smooth muscle cell length decreased by half and the radius at the cell center doubled: the relaxed cells were 80 μ long and 3.5 μ wide while the contracted cells were 40 μ long and 7 μ wide.

3. These cells were best approximated by 2 right circular cones joined at their bases.

4. This model of the cell provided a means of relating the changes in cell volume and surface area to the changes in cell length and radius.

5. The model indicated that the surface area of the smooth muscle cells was constant while the volume of the cells increased during vasoconstriction.

6. The increase in volume implied that the contracting muscle cells gained water.

CHAPTER III
CHANGES IN WATER CONTENT OF ARTERY WALL
DURING CONSTRICTION

The decrease in the cross-sectional area of the constricted artery wall, discussed in the previous chapter, suggests the existence of a decrease in the water content of the constricting artery wall. A few studies have demonstrated such a water loss from contracted smooth muscle, but they dealt mainly with changes in Na and K and were not followed up (1,2). In addition, an extracellular decrease associated with vasoconstriction has been measured (3). Considerable effort has been expended in analyses of the ionic content of arteries (4,5,6) and their exchanges during constriction (1,7,8). On the other hand, comparison of the water content of arteries in two different states, whether normotensive and hypertensive or relaxed and constricted, is quite a difficult procedure. Extreme care in handling the arteries and adequate controls are required. This study was designed to clearly establish any changes in the hydration of the constricting artery wall. The study of ionic and extracellular changes was secondary to this main goal.

A. METHODS

After incubation, fluid trapped in the artery lumen interferes with the determination of the water content. The contribution of this fluid varies with the lumen size, i.e. the degree of constriction. This variable was eliminated by perfusing intraluminally with an O₂/CO₂ mixture. Use of a particular artery from an inbred strain of rats still results in a large individual variation in water content. This problem was overcome by cutting the tail arteries into proximal and distal halves so the control and test

samples were from the same artery. The test samples were alternately proximal or distal halves.

The following incubation experiments differ in the treatment of the test samples and the analyses after incubation. The initial procedure was common to most of the experiments. The ventral tail artery of the anesthetized rat was exposed and cannulated with P.E. 50 polyethylene tubing at its midpoint. The distal half-artery was then flushed with Krebs solution, removed from the tail bed and transferred with its cannula to either a test or control test tube. The proximal half-artery was next cannulated at its proximal end, flushed with Krebs solution, removed from its bed, and placed in the other test tube. Thus each rat in an experiment contributed 1 test and 1 control half-artery. Both test tubes contained Krebs solution aerated with 95% O₂ and 5% CO₂ at 37°C. After a half-artery had been incubated for 3 hours, the polyethylene tubing carrying the gas mixture into the solution was connected to the cannula of that artery. The artery was then perfused with O₂/CO₂ for 7 to 10 minutes. Intravascular pressure was monitored with a Statham transducer connected at a T-joint to the tubing carrying the gas mixture to the artery. The pressure was only an estimate of the wall tension since an open perfusion system was used. After gaseous perfusion the control arteries were removed for analysis. The test arteries, handled similarly, were induced to constrict with various vasoactive agents for various times, then removed for analysis. Maximal pressor doses of the following vasoconstrictive agents were used: norepinephrine (levarterenol bitartrate, Winthrop), high K solution (55 meq K/liter, identical to Krebs solution except 50 mM KCL replaced 50 mM NaCl), and PLV-2 (phenylalanine² - lysine - vasopressin, Sandoz).

The chemical composition of the Krebs solution, in meq/liter, was: Na 150, K 5.0, Ca 4.2, Mg 2.4, Cl 124, HCO_3 25.0, H_2PO_4 1.2. It also contained 2 g/liter of dextrose and 40 g/liter of polyvinyl pyrrolidine (a plasma protein substitute). The osmolarity of the Krebs solution was 295 mosmoles/liter.

The water content of the arteries was determined from the wet and dry weights (9) and expressed as ml water/100 g fat free dry weight. As defatting the arteries decreased their weight by a negligible amount, it was omitted on occasion. Some of the experiments involved analysing the Na and K contents of wet ashed arteries (9); these results were expressed as meq/100 g fat free dry weight. The distribution of inulin in the artery, as an estimation of the extracellular space, was determined in two ways: (a) chemical; the Krebs solution for incubation contained 300 mg% purified inulin. The inulin space of the arteries was measured by the method of Friedman et al. (9).

(b) isotope; the arteries were incubated in Krebs solutions containing between 0.01 and 0.06 $\mu\text{curies/ml}$ of C^{14} - Inulin. The C^{14} content of the arteries was determined by liquid scintillation. Cl was determined by potentiometric titration with silver nitrate.

These experiments involved differences between the static water content determinations of arteries in non-constricted (control) and constricted (test) states. (The control arteries could not be classed as relaxed since there was no method of ascertaining this, but they were definitely less constricted than the test arteries.) Although no dynamic measurements of the hydration of a constricting artery were made, the differences in water content are referred to as "changes", for easier understanding of what was happening to the artery

wall as it constricted. In the results that follow, Δ refers to the change in a parameter with vasoconstriction, i.e. the test value minus the control value. Using control and test samples from the same artery meant that the results could be significant in two ways: (a) the number of differences for the pairs of half-arteries which had the same sign (+ or -) can be significant--calculated from the χ^2 distribution corrected for continuity; (b) the difference, Δ , between the average content of test and control arteries can be significantly different from zero--calculated from the Student's t test. This difference was included in the tables when it was significant at levels of $p < 0.02$ or $p < 0.05$.

B. NOREPINEPHRINE INDUCED VASOCONSTRICTION

Norepinephrine was selected as the vasoconstrictive agent for the first set of experiments because it easily induces fairly strong, short-lived constrictions in muscular arteries, and because its action on these arteries has been well studied (10-14). Five groups of experiments were done: (1) Water Content, (2) Water and Ion Content, (3) Varying Durations of Constriction, and (4) Inulin Space, and (5) Chloride Content. The first two groups were rather preliminary while the third forms the bulk of the results. The different experimental conditions in these experiments meant that the water content of the control arteries in the different groups cannot be compared. The differences between the absolute values provide the most interesting information.

1. Water Content

After the addition of norepinephrine (NE) to the incubating solution, a pressure increase could be observed when O_2/CO_2 passed through the artery segment with its branches patent (see Fig. 11). The first few arteries were

treated with 2 μg NE/ml, the remainder, 3 μg NE/ml. The test arteries were removed from the Krebs solution when the peak of constriction was observed on the pressure recording, and blotted between 2 pieces of filter paper. The average duration of constriction (\pm S.E.) for the 6 test arteries in this group was 127 ± 38 seconds. Five of the 6 test half-arteries had smaller total water contents than their corresponding control halves (5/6 is not statistically significant according to the chi-square test). The average values for the two groups are shown in Table VII.

TABLE VII. Water contents (ml/100 g dry wt) of rat tail arteries removed at peak of norepinephrine induced constriction after 3 hours of aerobic incubation.

		control arteries	test (NE) arteries	Δ	$\dagger p < 0.05$
total H_2O	(6)	283 ± 11	254 ± 12	$- 29 \pm 11^\dagger$	
% H_2O	(6)	73.8 ± 7	71.6 ± 0.9	$-2.1 \pm 0.8^\dagger$	

() number of half-arteries \pm S.E.

1. This experiment showed it was possible to observe a difference in water contents of non-constricted control and constricted test arteries with this procedure.
2. Constriction induced by norepinephrine was associated with a decrease of about 10% in the water content of the artery wall.

2. Water and Ion Content

This study involves a more detailed chemical analysis of the rat tail artery. The experiments differ only in the extent of handling and the duration of constriction. After removal from solution the arteries were cut into

small pieces alternately placed in two groups in a weighing bottle. One of the groups was analysed for the water, Na, and K contents, the other for the inulin space by the chemical method. These inulin space determinations were unsatisfactory in all 3 experiments since the amount of tissue available, about 4 mg wet weight, was too small.

Test arteries removed at peak of NE constriction

The first few test arteries were treated with 3 μ g NE/ml solution, the remainder, 4 μ g/ml. Upon removal from solution, the arteries were blotted between two pieces of filter paper. Nine rats were used in this experiment. The average duration of constriction (\pm S.E.) was 97 ± 15 seconds. All 9 constricted test half-arteries had smaller water contents than their corresponding control halves (all 9 differences negative is significant at $p < 0.004$). Of the 8 pairs of Na and K values, all 8 test arteries had less Na ($p < 0.008$) but only 4 of the 8 had less K (not significant by the chi-square test). The average results of this experiment are given in Table VIII.

TABLE VIII. Water contents (ml/100 g dry wt), Na and K contents (meq/100 g dry wt) of rat tail arteries removed from solution at the peak of NE constriction after 3 hours of aerobic incubation.

		control arteries	test (NE) arteries	Δ *($p < 0.02$)
total H ₂ O	(9)	203 ± 6	152 ± 10	$- 50 \pm 9 *$
% H ₂ O	(9)	67.2 ± 0.5	59.3 ± 1.9	$- 7.5 \pm 1.9*$
total Na	(8)	41.1 ± 2.2	29.5 ± 2.0	$-11.9 \pm 3.3*$
total K	(8)	11.8 ± 1.1	11.6 ± 1.3	

1. Water Vasoconstriction induced by norepinephrine for about 100 seconds was associated with a 25% decrease in the arterial water content. The decrease was significant both between the individual halves and between the averages for the halves.
2. The Na and K contents of the non-constricted control arteries were about 5 meq/100 g dry wt higher and lower respectively, than the values found in the literature for the rat tail artery (15,16). This suggests that there was a 1:1 exchange of cellular K for Na during the 3 hours of incubation, i.e. the smooth muscle cell metabolic Na-K pump was operating at less than its "normal" level of activity.
3. Sodium Vasoconstriction was associated with a 29% decrease in the Na content of the artery wall. An indication of the source of this Na lost during constriction can be obtained by assuming all the lost water came from the free ECS (i.e. the extracellular compartment which is in chemical equilibrium with the external medium and is roughly equivalent to the inulin space). This assumption would mean that the 50 ml water leaving the free ECS would have "carried" with it about 7.5 meq Na/100 g dry wt. A minimum of $11.9 - 7.5 = 4.4$ meq Na then, must have come from other compartments. If not all the lost water came from the free ECS, then more than 4.4 meq Na must have come from compartments other than the free ECS. This assumption and calculation says nothing about the movement of Na^+ into the contracting smooth muscle cells, but deals only with the movement of Na out of the whole artery wall during vasoconstriction.
4. Potassium Changes in the K content of the artery wall during constriction could not be measured with this technique.

Test arteries removed after 60 seconds of NE constriction

In this and all the subsequent experiments there was an attempt to obtain larger and more "normal" water contents (15,17) by gentler handling of the arteries. Instead of being blotted, the artery was simply placed on filter paper while its cannula was removed. Four μg NE/ml was added to the incubating solutions of the test arteries. Eight rats were used in each of these two experiments.

Of the 8 test half-arteries, 7 had less total water (not significant), all 8 had less total Na ($p < 0.008$), and 6 had less total K (not significant) than their corresponding control halves. The average results (\pm S.E.) of this experiment are given in Table IX.

TABLE IX. Water content (ml/100 g dry wt), Na and K contents (meq/100 g dry wt) of rat tail arteries constricted with norepinephrine for 60 seconds after 3 hours of aerobic incubation.

		control arteries	test (NE) arteries	Δ *($p < 0.02$)
total H ₂ O	(8)	292 \pm 9	231 \pm 15	- 61 \pm 13*
% H ₂ O	(8)	74.4 \pm 0.6	69.3 \pm 1.3	- 5.0 \pm 1.1*
total Na	(8)	43.7 \pm 1.8	34.8 \pm 1.8	- 8.9 \pm 1.7*
total K	(8)	13.9 \pm 1.0	13.5 \pm 1.2	

After 60 seconds of constriction, the artery wall lost about 21% of its water, about 20% of its Na, and none of its K. If it is again assumed that all of the lost water came from the free ECS, then the movement of 61 ml water should have carried with it 9.1 meq Na. Since the observed difference was -8.9 meq Na, this assumption indicates that all of the lost Na

could have come from the free ECS. It should be noted that 61 ml water leaving the free ECS would have also carried about 0.3 meq K along with it.

Test arteries removed after 120 seconds of NE constriction

Of the 8 test half-arteries, 6 had less total water and less total Na (not significant) than their corresponding control halves. Four of the 7 test K values were less than their control values (not significant). The average contents are given in Table X.

TABLE X. Water contents (ml/100 g dry wt), Na and K contents (meq/100 g dry wt) of rat tail arteries constricted with norepinephrine for 120 seconds after 3 hours of aerobic incubation.

		control arteries	test (NE) arteries	Δ *(p < 0.02)
total H ₂ O	(8)	264 \pm 14	219 \pm 18	- 45 \pm 19 *
% H ₂ O	(8)	72.2 \pm 1.0	67.7 \pm 2.0	
total Na	(8)	44.0 \pm 1.6	42.4 \pm 1.4	
total K	(7)	13.5 \pm 0.8	11.9 \pm 1.0	

After 120 seconds of constriction, the artery wall lost about 17% of its water. The decreases in the Na and K contents were not significant.

Summary

1. The water losses after 60, 97, and 120 seconds of vasoconstriction were 61, 50, and 45 ml water/100 g dry wt respectively. That is, the amount of water lost from the constricting artery wall decreased between 1 and 2 minutes. This suggests that after 1 minute of constriction, water returned to the wall, i.e. the original water content was being restored. The following experiment is an attempt to confirm this suggestion.

2. There was no pattern to the loss of Na from the constricting artery wall. The movement of Na involved 20 to 30% of the total wall Na. Most of this loss could be due to the movement of the lost water from the free ECS.

3. The slight decrease in the K content of the constricting artery was not statistically significant.

3. Varying Duration of Vasoconstriction

A more extensive study of the changes in arterial water and ionic contents after different durations of constriction was performed. Since no inulin spaces were measured, the arteries were not cut into small pieces as in the previous experiments. Four experiments were performed in which the test arteries were treated with 4 μ g NE/ml solution and allowed to constrict for 15, 30, 60, or 120 seconds. Eight rats were used in each experiment. The averaged results of these 4 timed experiments are given in Table XI.

1. Water All 32 constricted test half-arteries had less total water than their corresponding non-constricted control halves ($p < 0.001$). The greatest water loss was at 30 seconds of constriction. The average % decreases in water at 15, 30, 60, and 120 seconds of constriction were 18%, 23%, 17% and 13% respectively. The overall average water loss was 18%.

2. Sodium Of the 30 pairs of Na values, 27 of the test arteries had less Na than their controls ($p < 0.001$). The greatest Na loss was also at 30 seconds of constriction. The average % Na losses were 13%, 16%, 9%, and 13% respectively. The overall average Na loss was 13%.

3. Potassium The K contents were determined only for the 8 pairs of arteries in the 30 sec experiment. For 5 of these 8 pairs, the K content was less

TABLE XI. Water content (ml/100 g dry wt) and Na content (meq/100 g dry wt) of arteries after 15, 30, 60, and 120 seconds of norepinephrine-induced constriction in vitro during perfusion with O₂/CO₂ after 3 hours of aerobic incubation.

time (sec)	total H ₂ O			% H ₂ O			total Na		
	control	test	Δ	control	test	Δ	control	test	Δ
15 (8)	359 ± 14	292 ± 11	-66 ± 18	78.0 ± 0.7	74.3 ± 0.8	-3.7 ± 1.1	38.2 ± 0.7	34.1 ± 0.7	-4.8 ± 1.0
30 (8)	349 ± 12	270 ± 11	-80 ± 22	77.6 ± 0.6	72.8 ± 0.9	-4.9 ± 1.4	41.7 ± 1.1	35.0 ± 1.4	-6.7 ± 1.9
60 (8)	381 ± 9	317 ± 7	-64 ± 8	79.2 ± 0.3	76.0 ± 0.4	-3.3 ± 0.3	37.6 ± 1.1	34.1 ± 0.9	-3.5 ± 1.7 [†]
120 (8)	377 ± 12	327 ± 11	-50 ± 10	78.9 ± 0.5	76.5 ± 0.6	-2.5 ± 0.5	39.2 ± 0.7	34.1 ± 0.7	-5.1 ± 0.8
average (32)	367 ± 6	302 ± 6	-65	78.5 ± 0.3	74.9 ± 0.4	-3.6	39.2 ± 0.6	34.3 ± 0.5	-4.9

[†] p < 0.05, all other Δ are significant at p < 0.01.

in the constricted test halves than in the controls (not significant). There was also no significant difference between the average K contents (\pm S.E.): control arteries, 15.8 ± 0.7 meq K/100 g dry wt; test (NE) arteries, 13.4 ± 1.0 meq K/100 g dry wt. The results of the water and Na analyses are discussed in more detail in the following section.

4. Discussion

Changes in water content

1. Comparison of Tables IX, X and XI shows that the water loss was relatively constant at 60 seconds (-64 and -61 ml water/100 g dry wt) and at 120 seconds (-50 and -45) of vasoconstriction, even though the absolute water content of the control arteries was quite different. This suggests that the size of the water loss from the wall was dependent on the duration of constriction, not on the original water content. It should be noted that the severity of constriction could not be estimated since the perfusion pressures recorded during constriction were mainly dependent upon the route of O_2/CO_2 escape from the lumen.

2. Averaging the values for the 2 experiments involving 60 seconds and 120 seconds of vasoconstriction yields water losses (\pm S.E.) of 63 ± 7 ml at 60 seconds and 48 ± 10 ml at 120 seconds.

3. The pattern of water loss from the constricting artery wall is shown in Fig. 9. The values for 15 and 30 seconds are from Table XI, while the values for 60 and 120 seconds are the above averages. The values at zero water loss are for the control arteries. Although there were no significant differences between the 4 water losses, after 30 seconds of constriction, there seems to be a smaller amount of water lost. This suggests that water left the artery wall during the first 30 seconds of NE induced vasoconstriction, then slowly returned to the wall.

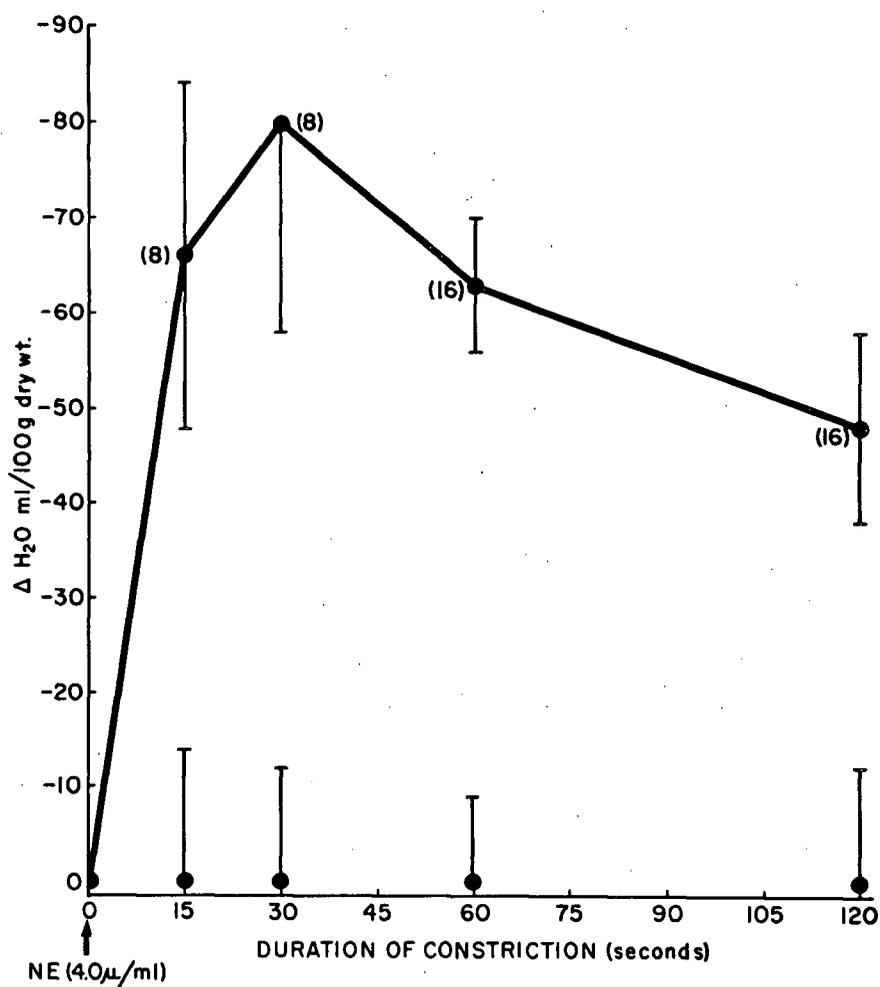


Fig. 9 Changes in H_2O content of rat tail artery wall during norepinephrine induced constriction in vitro with O_2/CO_2 perfusion. The bars represent 1 S.E. The points with zero ΔH_2O are the control values. The numbers in parenthesis are the number of arteries used.

Changes in Na content

1. The total Na contents of the 60 and 120 second control arteries in Table XI are smaller than the previous values in Tables IX and X. This fact, plus the higher total K content in this 30 second experiment, suggest that the arteries were more viable in these later experiments.
2. Although the Na losses in the two 60 second and the two 120 second experiments were not similar, for consistency the values were averaged (\pm S.E.): -6.2 ± 1.3 meq Na at 60 seconds and -3.4 ± 0.9 meq Na at 120 seconds of constriction.
3. These averaged values and the values from Table XI for 15 and 30 seconds of constriction are given in the lower curve of Fig. 10 which shows the pattern of Na loss from the constricting artery wall. There is no significant difference between any of the 4 values of ΔNa , but there is a suggestion that the Na movements are similar to the water movements: the artery wall loses Na for the first 30 seconds of constriction, then slowly regains it.
4. The upper curve in Fig. 10, $\Delta(\text{Na})_{\text{ECS}}$, is a calculated curve. The values on this curve represent the Na losses which would have occurred if the observed water losses from Fig. 9 were all from the free ECS. The calculations were the same as those made above. In Fig. 10, the observed Na loss is smaller than the calculated Na loss.
5. The 2 curves in Fig. 10 can best be understood by using the overall average values ($n = 48$) for the H_2O and Na losses. The average $\Delta\text{Na}_{\text{ECS}} = -9.2$ meq Na/100 g dry wt. That is, the loss of 61 ml of water from the free ECS would have carried 9.2 meq Na with it. However, only 4.8 meq Na actually left the constricting artery wall. There are 2 possible explanations of this

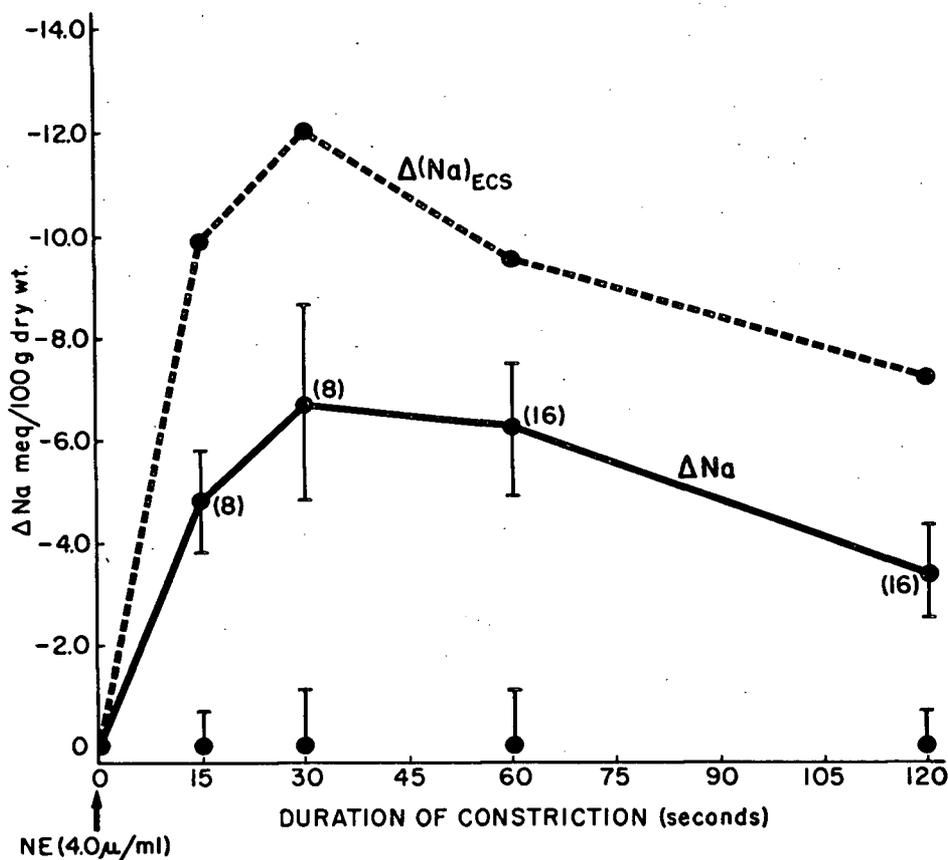


Fig. 10 Changes in Na content of rat tail artery wall during norepinephrine induced constriction *in vitro* with O_2/CO_2 perfusion. The bars represent 1 S.E. The points with zero ΔNa are the control values. The number in parenthesis is the number of arteries used. See the text for an explanation of the $\Delta(Na)_{ECS}$ curve.

situation: (a) Half the Na in the 61 ml of free ECS water which left the wall, moved from the free ECS to other wall compartments. This might include Na^+ which entered the contracting smooth muscle cells. (b) Some of the water which left the constricting wall came from compartments other than the free ECS. To carry out 4.8 meq Na, about half the observed water loss, 32 ml free water/100 g dry wt, would have been required. A combination of these 2 explanations would also explain the situation. This discussion is, of necessity,

simplistic, since the compartments of the artery wall are not isolated and certainly there would be osmotic adjustments of water between them.

6. The Na concentration of the fluid leaving the constricting artery wall was 70 to 100 meq/liter.

Changes in intravascular pressure

1. The pattern of water and Na losses from the constricting artery wall was similar to the profile of the intravascular pressure during NE induced vasoconstriction: a rapid rise followed by a slow decline.

2. The pressure recordings, however, showed that the intravascular pressure had reached a plateau only in some of the 120 second experiments (see Fig. 11). This suggests that the water and Na had left the artery wall and were returning while the pressure was still increasing in the lumen of the perfused constricting artery.

3. There was no correlation between the final pressure or the increase in pressure and the total water content or the decrease in water content.

5. Inulin Space

Inulin is generally regarded as the best marker for estimating the volume of fluid in the extracellular space (ECS) (18-23). For this study, the fluid assumed to be in chemical equilibrium with the external medium is designated as the free ECS. It will be assumed that the free ECS is approximated by the volume of distribution of the inulin molecule at the same concentration as in the external medium.

The above experiments have indicated that water was lost from the constricting artery wall. To substantiate these experiments and to indicate the source of the lost water, estimates of the extracellular space were required. The previous attempts to determine this space by the distribution of inulin failed because the artery samples were too small. Using larger tissue

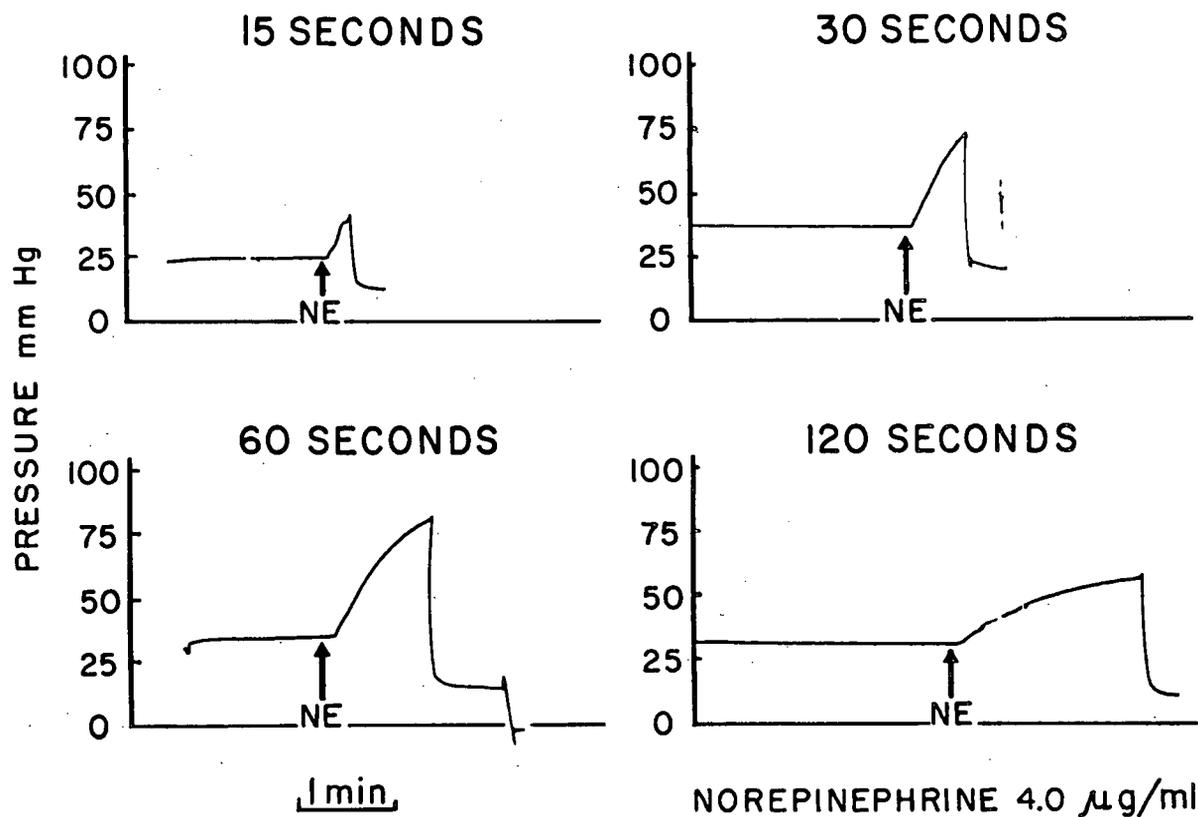


Fig. 11 Varying durations of norepinephrine induced constriction of rat tail arteries perfused in vitro with O_2/CO_2 .

samples, two inulin space experiments were performed. To permit the determination of the arterial water content, C^{14} - Inulin was used as the extracellular space marker. This method uses dry tissue so that wet and dry weights can be determined. The arteries were incubated for 30 minutes instead of 3 hours. In a series of experiments with Miss M. Mar, rat tail arteries were incubated in C^{14} - Inulin Krebs solution for 20, 40, 60, and 120 minutes. There was no difference between the inulin spaces or the water contents of these 4 groups of arteries. Thus, equilibration of the rat tail artery in the C^{14} - Inulin Krebs solution has apparently occurred within 30 minutes. This agrees with the equilibration time for other tissues (22).

Whole arteries

There were 2 other procedural differences in this experiment. (a) Only small rats (250 g) were available, so the whole tail artery was required for enough tissue for the analysis. (In the previous experiments, rats weighing about 400 g were used.) This meant that no comparison of paired test and control halves was possible and 16 rats were used for the 8 test and 8 control arteries. (b) The arteries were not perfused with the O₂/CO₂ mixture in order to determine the effect on the difference in water content between the constricted test and non-constricted control arteries. Consequently, the arteries were not cannulated. As before, the test arteries were allowed to constrict for 2 minutes after the addition of 4 µg NE/ml to the test artery solutions. The averaged results of this experiment are given in Table XII. The % inulin space is the % of the total H₂O, not the % of wet weight, as used by some authors (for example, 22).

TABLE XII. Water and inulin space (ml/100 g dry wt) of rat tail arteries constricted for 2 minutes with norepinephrine after 30 minutes of aerobic incubation.

		control arteries	test (NE) arteries	Δ	*p < 0.02
total H ₂ O	(8)	268 ± 6	252 ± 5		
inulin space	(8)	90.4 ± 3.6	76.1 ± 3.5	-14.3*	
% inulin space	(8)	33.4 ± 1.2	30.8 ± 1.6		

1. There was no significant difference between the test and control water contents. This suggests that the absence of the O₂/CO₂ perfusion did allow varying amounts of water to be left in the lumen, obscuring the difference between the 2 groups.

2. The % inulin space of the control arteries, 33.4%, agrees with the values in the literature (6).

3. The inulin space decreased by about 16% during NE induced vasoconstriction.

Half-arteries

This experiment followed the above procedure except that the arteries were divided in half so paired analysis could be done. In addition, the arteries were perfused with O₂/CO₂ so that a significant water content difference could be observed. Seven rats weighing about 400 g were used. All 7 test half-arteries had less water than their controls ($p < 0.02$), and 5 test arteries had smaller inulin spaces than their control halves (not significant). The average results of this experiment are given in Table XIII.

TABLE XIII. Water and inulin space (ml/100 g dry wt) of rat tail arteries constricted for 2 minutes with norepinephrine after 30 minutes of aerobic incubation.

		control arteries	test (NE) arteries	Δ *p < 0.02
total H ₂ O	(7)	298 \pm 4	261 \pm 8	-37 \pm 9*
inulin space	(7)	70.8 \pm 2.2	65.9 \pm 2.6	
% inulin space	(7)	24.9 \pm 0.9	25.0 \pm 1.0	

1. The fact that the water loss was significant in this experiment, but not in the previous one, indicates that inaccuracies due to water trapped in the lumen may obliterate differences.

2. The water loss associated with the NE induced constriction was about 12% of the water content of the control arteries.

Discussion

1. There are indications that the inulin space decreased during non-repinephrine induced vasoconstriction. Of 18 pairs of arteries, 13 of the test arteries had smaller inulin spaces than their corresponding controls ($p < 0.1$, not significant according to the χ^2 test). The average change in the inulin space for these 18 pairs of arteries was -17.3 ± 9.5 ml/100 g dry wt (not significantly different from zero). This 20% ECS decrease agrees with the observations of Turker et al. (3).

2. The large standard errors of the inulin space values meant that decreases in the inulin space smaller than about 15% could not be observed.

3. The % inulin space was constant during constriction: of the 18 pairs of values, 9 test arteries had smaller % inulin spaces than their controls. This means that the % decrease in the non-(free ECS) was also constant.

4. In addition to the usual problem of how inulin is distributed in the extracellular space, there are other problems using inulin as the ECS marker during vasoconstriction. Conformational changes in the extracellular solids or permeability changes in the smooth muscle cell membranes (24) associated with constriction may result in an altered distribution of inulin. The fluid changes associated with the onset of constriction may occur too quickly for the inulin molecule to assume a new equilibrium distribution. These possibilities imply that the decreases in inulin space during constriction are only qualitative measurements.

6. Chloride Content

Since the constricting artery underwent a decrease in ECS and a loss of Na unaccompanied by K, it seemed that measurement of the predominately

extracellular wall Cl content would give additional information on the distribution of the H₂O loss associated with vasoconstriction.

Rat tail arteries were incubated for 90 minutes in Krebs solution aerated with O₂/CO₂. These arteries were not divided in half, cannulated or perfused with O₂/CO₂. Consequently, intravascular pressure measurements were not made. Two groups of 6 arteries were used: (a) non-constricted control arteries which were removed for analysis after pre-incubation, and (b) constricted test arteries which had 2 µg/ml of norepinephrine added to the media and were allowed to constrict for 15 minutes. The averaged results are given in Table XIV.

TABLE XIV. Water (ml/100 g dry wt) and ion content (meq/100 g dry wt) of rat tail arteries constricted with norepinephrine for 15 minutes after 90 minutes of aerobic incubation.

	non-constricted	constricted (NE)	Δ *p < 0.02
total H ₂ O	342 ± 6	283 ± 4	-59*
total Na	41.0 ± 0.4	32.8 ± 0.5	-8.2*
total K	23.3 ± 0.5	22.3 ± 0.5	
total Cl	34.9 ± 0.5	29.1 ± 0.6	-5.8*

n = 6 for each value ± S.E.

1. The constricted arteries had 17% less H₂O, 20% less Na, no change in K, and 17% less Cl than the non-constricted arteries.

2. The ratio of Na loss to Cl loss, 1.41, is approximately equal to the ratio of Na and Cl concentrations in the external media, 150/124 = 1.21.

3. The Na carried out with 59 ml of ECS fluid would have been 8.9 meq Na/100 g dry wt, only slightly larger than the observed Na loss.

4. The above 2 points suggest that the water loss associated with constriction is a movement of water and electrolytes, mainly NaCl, from the extracellular fluid of the constricting artery wall.

7. Summary of Results of Norepinephrine Induced Vasoconstriction

1. The H₂O and Na losses from the rat tail artery constricted in vitro with norepinephrine are shown in Table XV. The % H₂O loss agrees with that found for the contracted uterus (1) and constricted aorta (2). Most of the observed changes in Na content associated with constriction have not been significant (2, 10, 25), although Daniel found a significant loss of Na and water from the contracted uterus (26).

TABLE XV. Overall average H₂O (ml/100 g dry wt) and Na (meq/100 g dry wt) contents of rat tail arteries constricted in vitro with norepinephrine. The ratio of constricted test arteries with smaller H₂O or Na than their corresponding control halves.

		control	test (NE)	Δ	% change	ratio	signif.
H ₂ O	(78)	307	256	-51	-16%	66/70	p < 0.001
Na	(55)	40.9	34.9	-6.0	-15%	49/54	p < 0.001

2. In Table VIII, the observed Na loss was greater than the ΔNa_{ECS} calculated from the water loss. In Tables IX and XIV, they were the same, while in Fig. 10, the observed Na loss was less than the ΔNa_{ECS} . The inulin space data suggested that only part of the water loss was from the ECS, while the Cl data suggested all the water loss could have been from the ECS. It thus seems possible that most of the H₂O lost from an artery wall constricted with norepinephrine, came from the extracellular space as a predominately NaCl solution. The remainder of the Na present in this volume of extracellular fluid may have entered the contracting smooth muscle cells.

3. There may have been a slight decrease in the arterial K content during NE constriction. Less K than their controls was observed for 19/31 test arteries (not significant). The overall average K values (\pm S.E.) for the control (n = 33) and test arteries (n = 31) were 13.7 ± 0.5 and 12.6 ± 0.6 meq K/100 g dry wt, respectively (non-significant difference). These non-significant changes in the K content of the constricting artery are in agreement with some findings (2,27) but in conflict with others in which large K decreases during constriction were observed (10,25).

4. The pattern of water, Na and intravascular pressure for the NE constricted arteries shows that the H₂O and Na losses had occurred and equilibrium was being restored before the increasing intravascular pressure had reached a plateau. This suggests that the ion and water changes were associated with the onset of vasoconstriction.

C. HIGH K INDUCED VASOCONSTRICTION

Norepinephrine induced constriction is associated with a loss of water from the artery wall. The question now arises: Is this water loss associated only with the action of norepinephrine, or is it common to all vasoconstrictive processes? A commonly used pressor agent is a solution with a high potassium concentration, called a high K solution, made by replacing some of the NaCl in a physiological solution with either KCl or K₂SO₄. The action of high K solutions on vascular tissue has been well studied and appears to have a different mode of action than norepinephrine (14,27-34). This experiment was performed to determine if high K induced constrictions were also associated with a loss of water from the wall of the rat tail artery.

As in the NE experiments, the arteries were divided into test and control halves and equilibrated in Krebs solution. The test arteries were

perfused with O_2/CO_2 for 5 to 10 minutes while the pressure was monitored, transferred while still recording to high K solution for 2 minutes, then removed for analysis. The control arteries were then similarly perfused and removed. The high K solution was the same as the Krebs solution except that 50 meq NaCl/liter were replaced by 50 meq KCl/liter, so the concentrations were 100 meq Na/liter and 55 meq K/liter. The osmolarity was 328 mosm/liter for the high K solution and 332 for the Krebs solution. The concentration of C^{14} - Inulin for the extracellular space determination was the same in both solutions. The arteries were analysed for water content and inulin space. Consequently, not enough tissue was available for Na and K measurements. Three experiments were performed: (1) Arteries from 8 rats were pre-equilibrated for 30 minutes in normal Krebs solution. Four of the 8 test arteries did not constrict in the high K solution--as observed from the pressure recordings--and were grouped with the control arteries as non-constricted arteries. (2) The same procedure as in (1) was used except the C^{14} - Inulin concentration was doubled. (3) Arteries from 12 rats were equilibrated for 3 hours in normal Krebs solution instead of 30 minutes as in the above two experiments.

The water content results of these 3 experiments are averaged in Table XVI. The only satisfactory inulin space values (\pm S.E.) were from experiment (1), in ml/100 g dry wt: non-constricted arteries (12), 77.1 ± 3.6 , i.e. $26.5 \pm 1.1\%$ and constricted arteries (4), 67.7 ± 5.8 , i.e. $27.4 \pm 3.7\%$. The differences between the two groups were not significant. The standard errors were so large, the inulin space would have to decrease by more than 25% to be observed. The % inulin space was essentially constant.

Table XVI shows that the water losses associated with high K induced vasoconstriction were about the same size (-14%) as those associated with

norepinephrine induced constriction (-16%).

TABLE XVI. Water content (ml/100 g dry wt) of rat tail arteries constricted for 2 minutes in high K solution and the ratio of constricted test arteries with smaller water content than their corresponding non-constricted control halves after aerobic incubation.

Experiment	non-constricted	constricted	Δ	% change	ratio	signif.
1.	(12) 291 \pm 6	(4) 254 \pm 13	-37*	-13%	4/4	none
2.	(8) 323 \pm 8	(8) 247 \pm 12	-76 \pm 10*	-23%	8/8	p < 0.008
3.	(12) 308 \pm 9	(12) 279 \pm 9	-29 \pm 12 [†]	- 9%	9/12	none
average	(32) 306	(24) 264	-42	-14%	21/24	p < 0.001

() number of half-arteries [†] S.E. * p < 0.02 [†] p < 0.05

D. SYNTHETIC VASOPRESSIN, PLV-2, INDUCED VASOCONSTRICTION

As a further test of the relationship between vasoconstriction and water loss from the artery wall, a synthetic vasopressin, PLV-2, was used to constrict the rat tail arteries. Some work has been done on the effects of PLV-2 on the ionic exchanges in the rat aorta (35).

The half-arteries were incubated for 3 hours in Krebs solution (332 mosm/liter) containing C¹⁴ - Inulin. During the O₂/CO₂ perfusion of the test arteries at the end of this incubation period, while the intravascular pressure was recorded, 40 millipressor-units PLV-2/ml were added to the media of the test arteries. They were allowed to constrict for 2 minutes before removal for analysis of their water and inulin contents. The control arteries were treated similarly but were not induced to constrict. Twelve rats were used in this experiment. The averaged results are given in Table XVII.

TABLE XVII. Water content and inulin space (ml/100 g dry wt) of rat tail arteries constricted for 2 minutes with PLV-2 and ratio of constricted test arteries with smaller amounts than their corresponding control halves after 3 hours of aerobic incubation.

		control	test (NE)	Δ	% change	ratio	signif.
total H ₂ O	(12)	293 \pm 9	255 \pm 11	-38 \pm 14*	-13%	10/12	p < 0.04
inulin space	(12)	105 \pm 4	102 \pm 7			8/12	none
% inulin space	(12)	35.9 \pm 1.0	39.7 \pm 1.7	+3.8 \pm 1.1*	+11%	1/12	p < 0.006

() number of half-arteries \pm S.E. * p < 0.02

1. The water loss associated with PLV-2 induced vasoconstriction was in the same range as the water loss associated with high K and norepinephrine induced constrictions.

2. There was no change in the inulin space during PLV-2 constriction. In addition, the % inulin space increased by more than 10%. Such an increase would be expected if the water lost from the constricting artery wall originated in compartments other than the free ECS, so that the amount of water in the inulin space relative to the total water would be increased. This suggests that the effect of PLV-2 induced constriction on the artery wall is quite different than the effect of high K and norepinephrine.

E. DISCUSSION

The rat tail artery wall lost water during constriction induced by norepinephrine, high K, or PLV-2. Of the 106 pairs of half-arteries, 97 of the constricted test arteries had less water than their corresponding non-constricted control halves (p < 0.001). The overall average H₂O contents

were (n = 118): control arteries, 306 ml H₂O/100 g dry wt, and test arteries, 258. The average H₂O loss associated with vasoconstriction was thus, 48 ml, or 16% of the control water content. There have been several studies which indicated there was a loss of water from the constricting artery wall. In 1956, Tobian and Fox (10) found a slight, non-significant decrease in the water content of femoral arteries constricted in situ with norepinephrine. Daniel's early studies showed no significant change in hydration of the constricted rat aorta (25, 36). However, he observed water losses, or decreases in water gain, in uterine strips contracted with vasoactive agents or metabolic inhibitors (1,26,37,38). These strips lost 10 to 17% of their total water. Daniel suggested that "contraction causes loss of substantial quantitation of interstitial fluid in smooth muscle allowed to contract isotonicly" (1). Headings and Rondell (27) found dog carotid arteries given epinephrine lost 2.3% H₂O. The data of Friedman et al. (35) show that perfusion of a synthetic vasopressin, PLV-2, was associated with a slight non-significant decrease in the water content of the rat aorta. On the other hand, Henry et al. (39) found a 25% increase in the inulin space of the rabbit aorta in vitro after the application of adrenaline. Similar inulin space increases were observed by his associates for the rat diaphragm (40) and trout dorsal muscle (41) given adrenaline. The Somlyos (24) suggested that since Henry's work conflicted with that of Daniel and that of Rorive (discussed below), "epinephrine may increase intracellular penetration of inulin in the rabbit aorta". Although this suggestion may be true, it is possible that the data of Henry et al. do not apply to the constricted aorta. They were interested in the effect of adrenaline on the turnover rate of free nucleotides, not constriction, and used extremely small doses

of adrenaline: 0.006 $\mu\text{g/ml}$ solution and 0.04 $\mu\text{g/ml}$. Supporting this possibility is the observation by Türker et al. of a 10 to 30% decrease in inulin space of carotid artery and uterus strips constricted with angiotensin (3). They suggested that since the strips contracted, the decrease in extracellular space could be due to a "squeezing out effect". The work of Rorive et al. (2) provides a clear demonstration of a water loss associated with vasoconstriction. They found an increase in the percentage dry weight of the rat aorta given norepinephrine (5 $\mu\text{g/ml}$ bath) or angiotensin. Their data show the constricted aorta lost 10 to 16% of its water. They suggested that the loss of water may have been due to "repartition des ions entre les different compartments tissulaires". Recently, Rorive (42) observed a significant percent dry weight increase and an inulin space decrease for the rat aorta constricted in a high K solution (20 meq K/liter). His values show that about 3/4 of the H_2O loss could be explained by the inulin space decrease. Why constriction is associated with a loss of arterial water will be discussed in Chapter VI. It is of some interest to consider the changes, caused by constriction, in the physical parameters of the entire rat tail artery wall. These changes can be estimated from the average water content and average water loss.

(a) wet weight of control half-artery (n = 149):		13.8 \pm 0.3 mg
(b) wet weight of whole non-constricted artery:	2 x 13.8 =	27.6 mg
(c) % water in control artery (n = 118):	100(306/406) =	75.5%
(d) water content of non-constricted artery:	0.755 x 27.6 =	20.8 mg
(e) a loss of <u>16%</u> of the water in the artery wall meant a change of:	-0.16 x 20.8 =	-3.3 mg water

(f) % change in weight of whole artery:	$100(-3.3/27.6) =$	-12%
(g) wet weight of constricted artery:	$27.6 - 3.3 =$	24.3 mg
(h) if density of lost fluid was 1.00 g/cc, then volume change of constricting artery was:		<u>-3.3 μl</u>
(i) density of artery (43,44):		1.06 g/cc
(j) volume of control artery, V_w :	$27.6/1.06 =$	26.0 μ l
(k) volume of constricted artery:	$26.0 - 3.3 =$	22.7 μ l
(l) % change in volume of artery wall during constriction:	$100(-3.3/26.0) =$	<u>-13%</u>
(m) % volume change from histological sections in Chapter II:		-14%
(n) density of constricted artery:	$24.3/22.7 =$	1.07 g/cc
(o) constriction of tail artery by NE, high K, or PLV-2 is associated with a change in the density of the wall of:		+0.01 g/cc

There was a small non-significant loss of K from the norepinephrine constricted rat tail artery. Tobian and Fox found a large K loss from the dog femoral artery constricted with NE (10), as did Daniel et al. for the rat aorta (25). On the other hand, Rorive et al. found no change in K for the rat aorta constricted with NE (2), and Headings and Rondell observed a slight gain in K for the dog carotid artery constricted with epinephrine (27). It should be noted that the proportion of smooth muscle cells in the aorta is smaller than in muscular arteries or the uterus. Consequently, changes in K, assumed to be predominately intracellular, would be much more

difficult to observe in the aorta. However, there have been indications that epinephrine and norepinephrine may cause constriction without membrane depolarization (27,30,45), or even with membrane hyperpolarization (46). This means that the loss of cellular K usually associated with depolarization and contraction in striated muscle, need not occur in NE induced vasoconstriction. Shibata and Briggs (46) explained the hyperpolarization and the large increases in K efflux and influx which their group had observed in the rabbit aorta given epinephrine (28), as due to an increase in membrane permeability to K caused by epinephrine, while "contraction results from an action of epinephrine which involves Ca". In short, if norepinephrine causes constriction without depolarizing the vascular smooth muscle cell membrane, it is not surprising that no change in K was observed in this study.

The norepinephrine induced constriction of the rat tail artery was associated with a loss of 15% of the arterial Na. In the literature, there is no clear picture of changes in Na content of the constricting artery wall. Non-significant changes in Na content were observed in the rat aorta (2,25) and dog femoral artery (10) constricted with norepinephrine. The slight increases in Na found (10,25) did not correlate with the loss of K observed simultaneously. On the other hand, Daniel found a loss of Na from the contracted uterus (1,26,37) and commented that "contractions in smooth muscle were accompanied by a decrease in Na, Cl and water in some instances obscuring cellular uptake of Na" (1). Without entering the 'Ca vs Na as the current carrying ion' debate, it seems that even if Na did enter vascular smooth muscle cells during NE constriction, the quantity of Na involved would be quite small (36). In this study, the Na lost from the constricting rat tail artery apparently accompanied the loss of extracellular water as suggested by Daniel (1).

F. ERROR IN CALCULATING INNER RADIUS FROM ISOVOLUMETRIC ASSUMPTION

Frequently in the literature, the inner and outer radii of an artery wall are measured in the relaxed state and the cross-sectional area of the wall is calculated. The artery is then constricted and its outer radius measured, but not the inner radius since it is not easily discernible in the constricted state. The inner radius for this constricted state is then calculated from the outer radius and the cross-sectional area, using the assumption that constriction is isovolumetric (for example, see 47,48). In the light of the indication of a loss of about 13% of the volume of the artery wall during constriction, it is of interest to see what error is introduced into the calculation of the inner radius by this isovolumetric assumption, $\Delta V_w = 0$.

If ()' refers to variables in the constricted state and () refers to variables in the relaxed state, then:

$$V_w = \pi L(r_o^2 - r_i^2) \quad \text{and} \quad V_w' = \pi L(r_o'^2 - r_i'^2)$$

$$\text{so, } V_w' - V_w = \Delta V_w = \pi L(r_o'^2 - r_i'^2 - r_o^2 + r_i^2)$$

$$\text{rearranging: } r_i' = \sqrt{r_o'^2 - r_o^2 + r_i^2 - \Delta V_w / \pi L} \quad \dots\dots\dots (1)$$

The size of the error introduced by assuming that the last term in equation (1) is zero can be observed in Fig. 12. The two curves were calculated from equation (1) by arbitrarily considering the case of constrictions in which the outer radius decreased by 50%, i.e. $r_o' = \frac{1}{2} r_o$. It was also assumed, for the sake of these calculations, that: $r_i = \frac{7}{8} r_o$ for values of the outer radius from 200 to 1000 μ . The lower curve is for isovolumetric constriction, the upper curve for a 13% decrease in the wall volume-- which means that for a constant length of the arterial segment, there was a

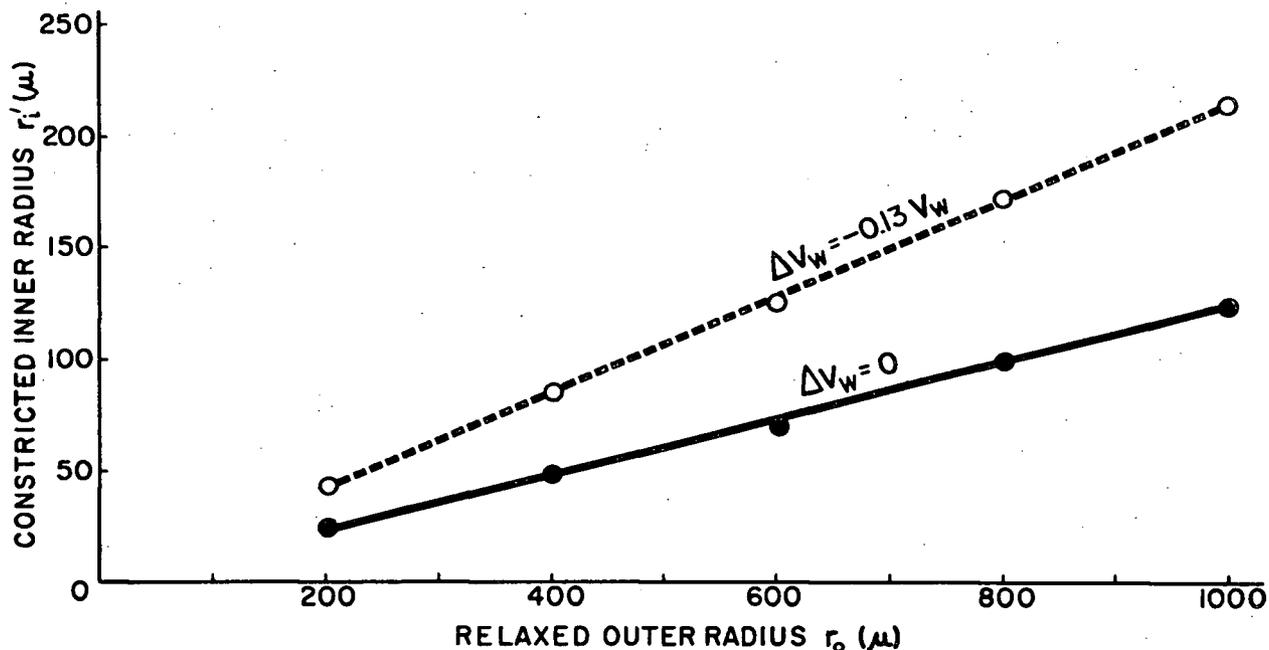


Fig. 12 Inner radius of a constricted artery calculated from the constricted outer radius and the relaxed inner and outer radii, with and without a 13% decrease in the volume of the constricted artery wall.

13% decrease in the cross-sectional area of the wall. The isovolumetric assumption always results in a calculated inner radius of the constricted artery that is too small: the error is about 42% for the example shown in Fig. 12. This means that the artery really constricted considerably less than indicated by this assumption.

G. EXAMINATION OF POSSIBLE EXPERIMENTAL ARTIFACTS

The finding of decreased arterial hydration during constriction is contrary to the generally accepted view of isovolumetric constriction. For this finding to be acceptable, the possibility that the observed loss of

water was an experimental artifact has to be dismissed. In this regard, there are 3 objections which can be raised to claim the observed water loss was due to: (1) changes in the artery after 3 hours in vitro, (2) water left in the lumen, and (3) the effect of the O_2/CO_2 perfusion on the arterial water content.

1. Effect of Relaxation of Constricted Arteries on Water Content

There is some indication that incubation of arteries causes them to gain water slowly, i.e. either they imbibe water or are recovering from the trauma of excision. If it is assumed that vasoconstriction causes only an extra load of water, acquired during equilibration, to be expelled from the wall, then subsequent relaxation will leave them, at least for a time, with less water than their control halves. On the other hand, if the water loss is directly a function of vasoconstriction, then arteries constricted then relaxed should have the same amount of water as the untreated controls. To resolve this question, arteries were constricted and then allowed to relax.

Rat tail arteries were divided in half and incubated for 3 hours. The test half-arteries were perfused with O_2/CO_2 , treated with 4 $\mu\text{g/ml}$ of norepinephrine, and allowed to constrict for 2 minutes. These constricted test arteries were then transferred to fresh Krebs solution and allowed to relax for 25 minutes while the intravascular pressure was monitored. The controls were perfused with O_2/CO_2 and removed with the test arteries for water measurement. Fourteen arteries were used in this experiment.

For most constricted test arteries, the intravascular pressure returned to normal after about 10 minutes in the fresh Krebs solution (see Fig. 13), so certainly the test arteries were relaxed after 25 minutes. Seven of the 14 test half-arteries had less water than their corresponding

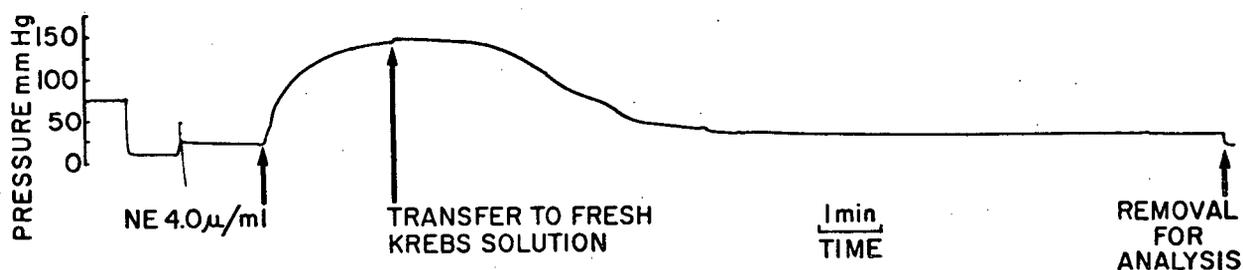


Fig. 13 Intravascular pressure of a rat tail artery constricted in vitro with norepinephrine, then allowed to relax, while being perfused with O_2/CO_2 .

control halves. The averaged values (\pm S.E.) showed a similar non-significant difference: control arteries, 314 ± 6 ml water/100 g dry wt, and test arteries, 301 ± 7 , i.e. both had the same amount of water. This means that the loss of water during constriction was not an artifact due to simple removal of some acquired extra water. It should be noted that the test arteries could not have gained back any loaded water in the 15 minutes of incubation after they were relaxed, since 15 minutes is too short a time for this to happen. The experiment mentioned above showed there was no difference in water content of rat tail arteries incubated for 20 or 120 minutes. In addition, there was no correlation between the average incubation times and the water contents of the control arteries from Table XI. In conclusion,

the loss of water from the artery wall during constriction and its subsequent return during relaxation were independent of any incubation artifacts.

2. Water Remaining in the Lumen after Gaseous Perfusion

Could water left in the lumen after O₂/CO₂ perfusion explain the water loss during vasoconstriction? This remaining water could be considered as either a constant volume of fluid or a layer of fluid of constant thickness. Here, constant refers to comparison of the non-constricted control and the constricted test arteries. A constant volume of fluid left in the lumen would not affect the control and test arteries differently, so it could not be the cause of the observed water loss. A boundary layer of fluid of constant thickness containing less fluid in the constricted state, due to the smaller lumen, might give the impression of less water in the constricted arteries. However, this possibility does not seem too likely:

$$\text{Volume of boundary layer fluid} = V_b = 2\pi L y r_i$$

where: L = length of artery segment; y = boundary layer thickness;
r_i = lumen radius.

Assuming $\Delta y = 0 = \Delta L$, where Δ is the change caused by constriction, then, $\Delta V_b = 2\pi L y \Delta r_i$. Since, $\Delta r_i < 0$, then $\Delta V_b < 0$. The question is then reduced to: Can a decrease in the boundary layer volume account for the observed loss of water by the constricting artery? The r_i values from Table I can be used for a rough indication of Δr_i : r_i was 255 μ and 55 μ for the relaxed and fully constricted arteries, respectively. So, $\Delta r_i = -200 \mu$. For L = 10 cm, $\Delta V_b = -1.20 y$ (for ΔV_b in ml and y in cm). The water loss for a 10 cm length of rat tail artery was calculated to be 3.3 μ l. If the change in boundary layer volume, $\Delta V_b = -3.3 \mu$ l, then the

thickness of the layer, $y = 27.5 \mu$. That is, a boundary layer of fluid 27.5μ thick would be required to explain the observed water loss. But this would mean that the constricting artery, with a lumen diameter of 110μ , would contain only a core of O_2/CO_2 , 55μ in diameter, filling only half of the lumen. Since the humidity of the O_2/CO_2 was zero, this is wholly unrealistic.

A variation in the amount of fluid left in the lumen thus cannot be used to rationalize the water loss observed in constricted arteries.

There is additional support for this conclusion: if a significantly large layer of fluid remains in the lumen, it would evidently be smaller in the distal than in the proximal half-arteries. The values for the control water content of the two halves were accordingly compared. Control arteries with a water content (\pm S.E.) in the range of 300 ml/100 g dry wt were selected: proximal control arteries ($n = 32$), 295 ± 4 ml water/100 g dry wt, and distal control arteries ($n = 31$), 313 ± 6 . The 18 ml difference was significant. Certainly then, the distal control arteries do not have less water than the proximal control arteries.

3. Effect of Gaseous Perfusion on Arterial Water Content

Although adequate controls were used, the perfusion of arteries with O_2/CO_2 was hardly physiological and some comment is necessary to show that it did not produce the observed water loss during vasoconstriction.

Evaporation from the artery wall during O_2/CO_2 perfusion depends on the vapour pressure of the wall fluid. At $37^\circ C$ the vapour pressure of water is about 47 mm Hg (49). The effects of solutes and pressure on this value are less than 1% (50). The wall fluid vapour pressure is affected by the meshwork of endothelial cells and internal elastic lamina separating the

wall from the lumen. The extent of this effect depends essentially on the shape of the meniscus of the fluid in the "cracks" of the meshwork. For a convex/concave surface, the vapour pressure is altered by about $\pm 10\%$ (see 51,52). An arterial fluid vapour pressure between 42 and 52 mm Hg would cause some evaporation from the artery wall during O_2/CO_2 perfusion. The following is a maximal estimation of this evaporation.

Rate of evaporation from a rat tail artery

A non-constricted artery was left to evaporate in a weighing bottle on a 6-place Mettler balance and its weight was recorded every 1 or 2 minutes. The artery lost 3.5 ml water/100 g dry wt/minute for the first 40 minutes. This evaporation occurred from the adventitial and luminal surfaces, but the in vitro artery was exposed to O_2/CO_2 only on its luminal surface. From Table I, the inner and outer radii of a relaxed rat tail artery were 255 and 321 μ , so for a 10 cm length of artery, the inner and outer surface areas would be 1.6 and 2.0 cm^2 , respectively. Actually, the surface area of the porous adventitia would be considerably larger. The maximum evaporation rate from the luminal surface during O_2/CO_2 perfusion might thus be estimated as 2 ml/100 g dry wt/minute. The perfusion time for the control arteries was about 4 or 5 minutes, compared to 5 or 6 minutes for the test arteries. This time difference could result in a hydration difference of no more than 4 ml water/100 g dry wt--much less than the observed 48 ml water difference. In reality, the evaporation loss would probably be even less because of osmotic replacement of any evaporated water. The arteries were incubated in Krebs solution and the artery wall is quite permeable to water (see Chapter IV), ensuring no dehydration of the luminal surface of the gas perfused arteries.

Another factor diminishing the possible role of evaporation is that evaporation depends upon the area exposed to the O_2/CO_2 . The constricted test arteries have a smaller luminal surface area than the non-constricted control arteries. [If r_i was 255 μ and 55 μ for the relaxed and fully constricted arteries respectively (from Table I), then constriction was associated with an 80% decrease in luminal surface area.] So, the test arteries should lose less water per minute by evaporation than the control arteries, resulting in a higher water content than the control arteries--the opposite of what was observed.

In general, if O_2/CO_2 perfusion did significantly affect the arterial hydration, then presumably the longer the perfusion time, the greater the effect. However: (1) There was no correlation between the water content and the perfusion time for either the control or test arteries. (2) In the relaxation experiment, the test arteries were perfused for 30 minutes and the controls for 5, yet both had the same water content. (3) The water content of control arteries perfused with O_2/CO_2 , 306 ml/100 g dry wt, was about the same as that obtained for arteries perfused with Krebs solution (17).

H. SUMMARY

1. Vasoconstriction, induced by strong pressor doses of norepinephrine, high K, or PLV-2, was associated with a 16% decrease in the H_2O content of the rat tail artery wall.

2. In the norepinephrine induced constrictions, this finding of a water loss was supported by a decrease in the Na and Cl contents and the inulin space of the artery wall.

- (a) Most of the H₂O loss was due to a decrease in the extracellular fluid volume.
- (b) The size of the H₂O loss depended upon the duration of constriction, not upon the original water content.
- (c) The H₂O and Na losses were greatest 30 seconds after vasoconstriction began.
- (d) Before the plateau of the increasing intravascular pressure was reached, the water and Na began to return to the artery wall, i.e. the H₂O and Na movements were associated with the onset of vasoconstriction.
- (e) The K content and the % inulin space remained essentially constant during NE constriction.

3. In the PLV-2 induced constrictions, the inulin space remained constant while the % inulin space increased. This indicates that, in contrast with the other 2 agents, almost all the lost water came from inulin-inaccessible compartments of the artery wall.

4. About the same water loss was associated with vasoconstriction induced by all 3 agents, although no comparison could be made between their vasoconstrictive actions.

CHAPTER IV
HEMODYNAMIC AND PERMEABILITY CHANGES
DURING VASOCONSTRICTION

The morphological and biochemical studies in the previous two chapters have demonstrated a loss of a few microliters of fluid from the constricting rat tail artery wall. Experiments were designed to determine if this altered wall hydration would affect the hydrodynamics of the constricting artery. In addition to the expected observations, it was discovered that the permeability of the artery wall was considerably altered during constriction. Three experiments were performed during vasoconstriction induced by norepinephrine: pressure and flow measurements, varying intravascular pressure, and dye perfusion. Although constant pressure perfusion is often used in hydrodynamic experiments (for example, 1,2), it was decided to follow Burton's advice to use constant flow perfusion (3).

A. PRESSURE, FLOW AND LUMEN VOLUME

Hemodynamics has been the subject of numerous reviews (4-9). Some work has been done comparing the elastic and pressure-flow properties of relaxed and constricted arteries (1,2,10-14). There have also been observations on the effect of vasoconstrictive agents on blood flow and pressure (for example, 15-18). However, these studies have been concerned with the altered hemodynamics of the system once vasoconstriction was established. They have not been concerned with flow and pressure during the onset of constriction. The major problem has been to obtain accurate estimates of flow. The present study used a flowmeter which was very sensitive to small flow changes in the

hope of detecting the addition of wall fluid to the lumen during constriction.

1. Methods

A 12 cm segment of the tail artery was exposed and its collaterals tied. It was tested for leaks by perfusion with Krebs solution to which Evans blue dye had been added. There were two subsequent procedures for this artery: (a) it remained in situ with its exposed surface kept moist with Krebs solution, or (b) it was removed from the tail bed and placed in a perspex chamber at 37°C. Perfusion was by a constant infusion pump (B. Braun) with two syringes: both contained Krebs solution, and one also contained norepinephrine (4 µg/ml) (NE). Vasoconstriction was induced using a 4-way switch to change the perfusion from Krebs solution to NE-Krebs solution. Topical application of NE was also used for some in situ arteries. The pressure gradient down the artery was monitored with two Statham transducers connected by T-joints to the P.E. 50 polyethylene tubing proximal and distal to the artery. The flow rate of the effluent was determined using a special photocell-flowmeter. The effluent passed through coils of polyethylene tubing wound around light pipes attached to a photocell. The only path for light from a DC lamp to reach the photocell was through a thin line along the coils. A small bubble was inserted into the system between the artery and the flowmeter. Each time the dark meniscus of the leading edge of the bubble crossed the line along the coils of the flowmeter, its passage was recorded as a vertical deflection on the polygraph output from the photocell. The rate of flow out of the artery was determined as follows: The separation of the polygraph deflections, representing the passage of the bubble through 1 coil of the flowmeter, was measured during a control run (i.e. without NE). From the polygraph chart speed and the pump rate, the volume of one

coil was determined. For measurements during vasoconstriction, the number of coils, including fractions, traversed in 15 seconds was determined. From the volume of 1 coil, the flow rate for this 15 second interval was then calculated. The flow measurements were accurate to $\pm 0.02 \mu\text{l}/\text{sec}$, or $\pm 0.6\%$. For this method to be that accurate, the velocity of the bubble had to be equal to the average velocity of the fluid. The size of the bubble used was not too small, since it would move in the axial stream at greater than the average velocity, or too large, since it would have a different viscosity than the perfusing solution (5). It has been estimated that the maximum deviations of the bubble velocity from the blood flow, for a wide range of flows, are $\pm 5\%$ (19). The presence of this flowmeter in the system meant that the intravascular pressure was fairly high, about 44 mm Hg at the midpoint of the artery, while the pressure gradient down the artery was about 8 mm Hg during the control runs. The pressure and flow characteristics of the NE induced constrictions were determined in 14 rat tail arteries.

2. Results

The pressure gradient and the effluent flow rate for a typical artery constricted with norepinephrine are shown in Fig. 14. At 30 to 90 seconds after the onset of constriction there was an increase in the otherwise constant flow rate and an increase in the increasing pressure gradient. These anomalies were observed for all the constrictions, although their sizes varied considerably. The source of the anomalies lies in the arteries themselves because the flow rate of the infusion pump was constant during these experiments. The pump operated through a series of gears which prevented any "backlash" from the experimental system of the artery, tubing and flowmeter onto the infusion pump.

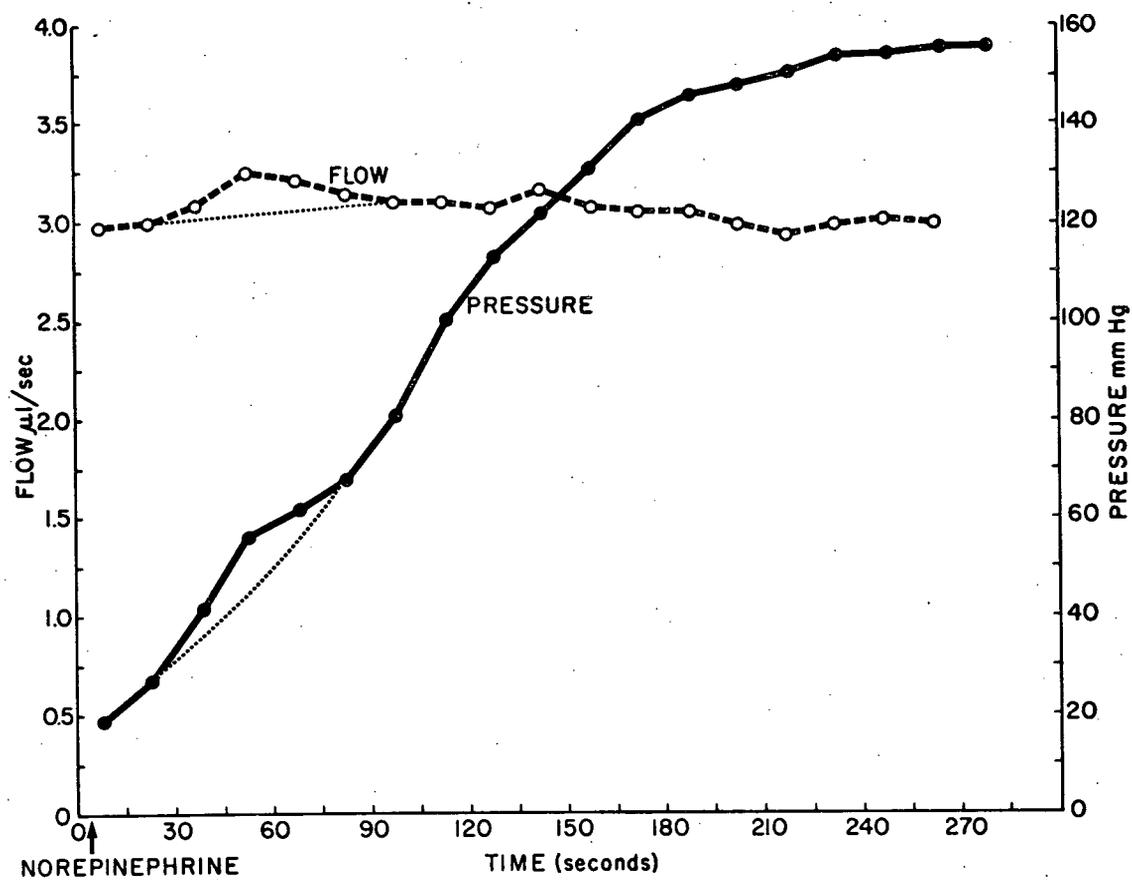


Fig. 14. Effluent flow rate, F , and pressure gradient, P , for a rat tail artery constricted in situ with norepinephrine.

3. Discussion

Could fluid "squeezed out" of the lumen of the constricting artery have been the source of the increase in flow rate and the "hump" in the pressure rise? The concept of "squeezing out" implies that there was a relatively static amount of fluid stored in the lumen which was expelled during constriction. But, the time course for constriction (a few minutes) was much longer than the time required for fluid to move through the lumen (a few seconds), so there could be no sudden shift of fluid out of the lumen. Over the few minutes of constriction, the decrease in the cross-sectional area of the lumen, A_1 , was constantly balanced by the increase in the velocity through the lumen, \bar{v} , since the flow rate, $F = A_1\bar{v}$, remained constant. When the artery constricted, the volume of the whole system (artery lumen plus polyethylene tubing) decreased, so less time was required for transit through the system. But the increase in velocity was only through the artery lumen, not through the rest of the system. Changes inside the system could alter the cross-sectional areas, the velocities, and the volumes, but not the flow rates.

The anomalies in the curves of Fig. 14 can be explained by a movement of fluid into the constricting lumen. The source of this fluid could have been: (a) the artery wall--which would agree with the results of the previous two chapters, or (b) the fluid surrounding the artery--which would indicate that the permeability of the artery wall was altered during constriction, to allow fluid to pass through it into the lumen. Of course, these two possibilities are not mutually exclusive. The size of the increase in flow indicates that fluid passed into the lumen from the surroundings. The maximum increase in flow in Fig. 14 was $0.25 \mu\text{l}/\text{sec}$ --about

10 x that expected from the loss of wall fluid. Also, the area under the flow curve in Fig. 14 indicates that over 20 μ l of fluid was added to the lumen. The whole artery wall had only 20 to 30 μ l of water.

The increase in flow in Fig. 14 was accompanied by a "hump" in the increasing pressure gradient. This is to be expected from Poiseuille's equation for laminar flow through rigid tubes, by which $P \propto F/V^2$, where: P = pressure gradient down the artery segment, F = outflow from the artery, and V = lumen volume. Although Poiseuille's equation is for equal inflow and outflow, the above relationship is probably still true as a first approximation when there is a small amount of fluid added to or removed from the perfusate. This relation means that a decrease in V or an increase in F would cause an increase in P. A combination of these two events during vasoconstriction would result in the observed pressure pattern in Fig. 14.

4. Summary

1. There was a slight increase in the outflow from the perfused artery constricted with norepinephrine. This increase was associated with a "hump" in the increasing pressure gradient.

2. These changes indicate that there was an increase in wall permeability during vasoconstriction which allowed fluid to enter the lumen from the fluid surrounding the artery.

B. EFFECT OF INTRAVASCULAR PRESSURE

The previous section suggested that wall tension changes caused an alteration in the permeability of the artery wall. Sawyer and Valmont have reported that the aorta and vena cava are permeable to Na and Cl (20). It

has been suggested that since the net ion movements are in different directions for these 2 vessels, the ion movements may depend on the luminal pressure (21). It thus seemed reasonable to examine the effect of the intravascular pressure on vascular permeability.

Most studies involving variable intraluminal pressure have been concerned with demonstration of a vascular myogenic response (22,23). The studies which considered the effect of pressure on permeability have been concerned with capillary filtration (see 24), or transport through other membranes (25-28). There has also been some work on the effect of luminal pressure on constriction (29,30).

Pressure and flow were measured during norepinephrine induced constriction in the rat tail artery at either "high" or "low" intravascular pressure.

1. Methods

The above procedure for perfusing rat tail arteries in situ was used. The arteries were constricted by the addition of 0.2 μg norepinephrine in a 10 second perfusion of NE-Krebs solution at 0.200 ml/min while the pressure gradient was monitored. The flow rate of the effluent was determined by collecting the effluent every 20 seconds in weighing bottles and measuring the volumes with a 100 μl syringe. This method was less accurate than that of the photocell-flowmeter: the volume readings were $\pm 1 \mu\text{l}$ and the time recordings were ± 1 second. Consequently, the error in an average flow of 0.180 ml/min for a 20 second interval in which 60 μl was collected was ± 0.012 ml/min or about 6.7%. When multiple constrictions were induced in the same artery, at least 30 minutes equilibration was allowed between constrictions.

The arteries were constricted at two different basal intravascular pressures: low and high. (The intravascular pressure, taken as the pressure in the lumen at the midpoint of the artery segment, should not be confused with the pressure gradient, the difference in the pressure between the two ends of the artery segment, used in Poiseuille's equation.) For low intravascular pressures, the polyethylene tubing distal to the artery was at the same level as the artery. For high intravascular pressures, the distal tubing was raised about 50 cm above the level of the artery. This increased the intravascular pressure without producing a large change in the pressure gradient. The average intravascular pressures were: low, 3mm Hg, and high, 41 mm Hg.

2. Results

The pressure gradient patterns for the constricting tail artery preparations with low and high intravascular pressures are presented in Fig. 15. These curves represent the average results of 4 norepinephrine-induced constrictions. There was a significantly larger increase in the pressure gradient during perfusion at the low intravascular pressure (+ 140 mm Hg) than at the high intravascular pressure (+ 84 mm Hg). The time courses of the pressure increases were the same but the relaxation was faster for the high pressure case. The same result was obtained on 1 artery when constrictions at high and low intravascular pressures were induced.

The average flow patterns for 8 low and 4 high intravascular pressure constrictions are given in Fig. 16. It shows that:

1. The "base line" flow before and after constriction was significantly less for perfusion at high intravascular pressure than at low pressure. The average flow rates (\pm S.E.) before constriction were: (a) low intra-

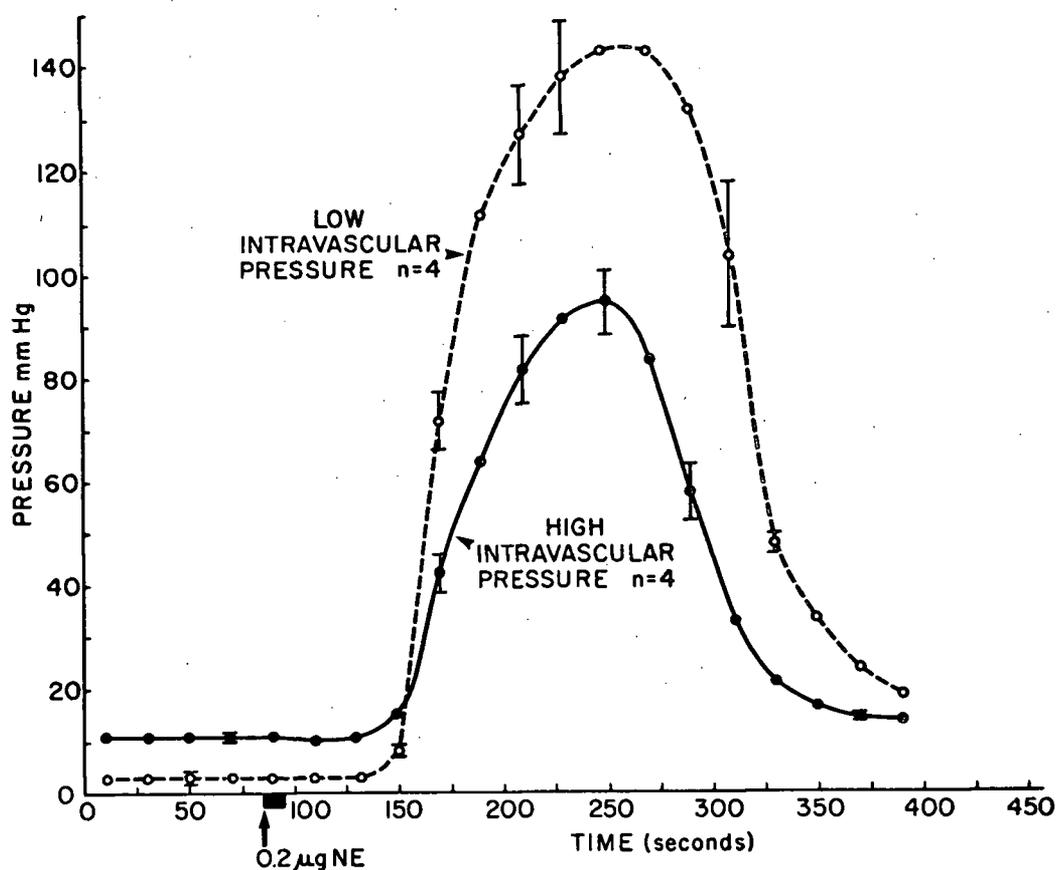


Fig. 15 Pressure gradient, P , down rat tail arteries constricted *in situ* with norepinephrine (NE) at high and low intravascular pressure. The vertical bars represent the S.E.

vascular pressure ($n = 44$), 0.182 ± 0.002 ml/min, and (b) high intravascular pressure ($n = 24$), 0.171 ± 0.001 ml/min. In addition both effluent flow rates were less than that of the infusion pump: ($n = 8$), 0.200 ± 0.002 ml/min --from calibrations at the 0.200 ml/min setting of the pump.

2. The changes in the effluent flow rates were remarkably different during vasoconstriction.

(a) The flow during the low pressure constrictions decreased considerably, reaching its lowest point when the pressure was one-half its peak

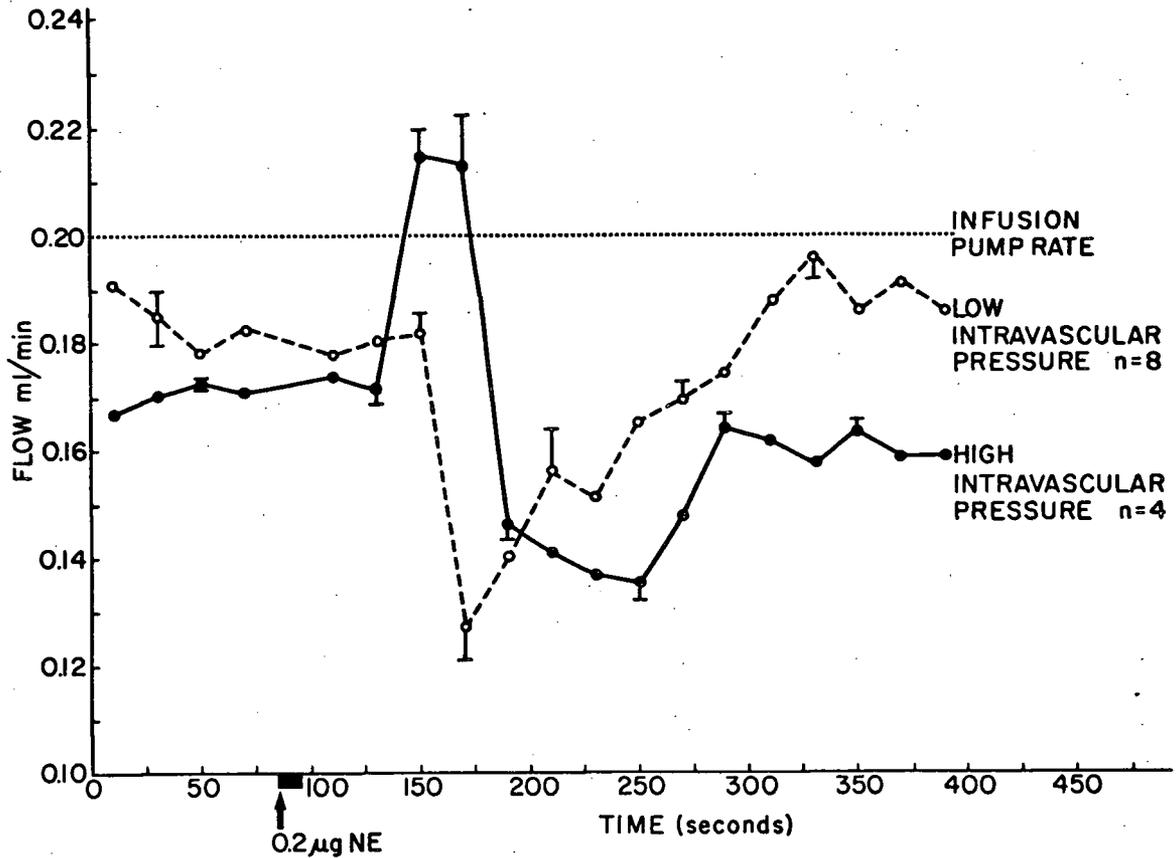


Fig. 16 Effluent flow rate, F , for rat tail arteries constricted *in situ* with norepinephrine (NE) at high and low intravascular pressure. The vertical bars represent the S.E.

value. The flow did not return to its previous value until after the peak of constriction. The area under the flow curve revealed that $55\mu\text{l}$ less fluid passed through the artery over 150 seconds.

(b) The flow during the high pressure constrictions was biphasic: the flow increased during the initial rise in pressure, returned to its previous value when the pressure was one-half its peak value, and decreased to its lowest point when the peak pressure gradient was attained. The

initial increase in flow represented about 25 μl more fluid in the effluent in 50 seconds (5 μl of which, over 30 seconds, could not have come from the pump), while for the later decrease in flow, there was about 40 μl less fluid in the effluent over 110 seconds. These losses and gains of fluid could not be explained by leaks in the system. They were real and repeatable in each of the constrictions.

3. Discussion

The smaller peak pressure gradient for higher intravascular pressures was also observed by Nicholas and Hughes (30). They found an inverse relationship between the pressor response to norepinephrine and the resting blood pressure. On the other hand, Sparks and Bohr (31) found contraction of artery strips, in response to a standard electrical stimulus, increased with stretch until an optimal length, after which the response decreased. The high intravascular pressure results were obtained in circumstances similar to those in the above photocell-flowmeter experiment. The intravascular pressures in the incubation experiments of Chapter III were between those in the high and low pressure cases.

The flow results can be explained only if the in situ rat tail artery preparation was permeable to the perfusing Krebs solution. The arteries were all tested for leaks from the tied collaterals and there were no leaks from the constant flow pump assembly. The permeability of the artery wall was affected by the intravascular pressure and by vasoconstriction.

1. Before constriction, fluid passed out of the lumen, through the artery wall and into the surrounding fluid. These losses were $0.200 - 0.182 = 0.018 \text{ ml/min} = 0.3 \mu\text{l/sec}$ during perfusion at low intravascular

pressures and 0.5 μ l/sec at high pressures. (That the outflow was less than the inflow was suspected from the results of the previous section, but the control runs were not of a sufficient length to comment.) This indicates that there was a simple filtration process through the artery wall which increased when the pressure in the artery lumen was increased. A similar observation was made by Wilens and McClusky for excised human iliac arteries and veins (32).

2. During the NE-induced vasoconstriction and the resulting increase in the pressure gradient, the permeability of the artery wall was drastically altered. (a) At low intravascular pressures, the permeability increased so that even larger amounts of fluid left the lumen. (b) At high intravascular pressures, initially the permeability not only decreased but 5 μ l of the fluid was added to the lumen, and then the permeability increased so that large amounts of fluid left the lumen. In both cases, as the artery relaxed, the permeability returned to its pre-constriction level and fluid continued to pass from the lumen to the surroundings.

3. There was no simple inverse correlation between the intravascular pressures and the flow rate before and during the constrictions. The situation was made complex by the opposite behavior of the flow in the two pressure cases during the initial rise in pressure.

There is no direct evidence from this experiment for possible changes in the hydration of the artery wall during constriction. The large movements of fluid right through the artery wall completely obscured any small movements out of the wall. A possible exception was the addition of about 5 μ l of fluid to the lumen in the initial phase of the pressure rise with the high intravascular pressure. The direction of the fluid movement through

the wall could have reversed or the fluid could have come from the artery wall itself. These two possibilities cannot be distinguished from this experiment.

Possible causes of the changes in artery wall permeability are discussed in Chapter VI.

4. Summary

1. The higher the intravascular pressure, the smaller the pressure response to norepinephrine.
2. The wall of the perfused tail artery was quite permeable: fluid passed out of the lumen, through the artery wall, and into the surrounding fluid.
3. This free passage of fluid was very much affected by the pressure in the artery lumen and by the state of tension in the wall.
4. The higher the intravascular pressure, the greater the permeability of the wall, i.e. the greater the loss of fluid out through the artery wall.
5. When the artery constricted at low intravascular pressures, the permeability increased, but at high intravascular pressures, it decreased then increased.
6. There may have been a decrease in the hydration of the artery wall during constriction at high intravascular pressures.

C. DYE DILUTION

The wall water loss and the permeability changes during vasoconstriction suggested a more direct estimation of fluid movement. Perfusion of a dye during constriction would provide a medium which would "amplify" to an observable level any movements of fluid in or out of the lumen. The best and most commonly used dye for vascular perfusion is T 1824, Evans blue

(33,34,35). This dye has several advantages for this particular study:

(a) It leaves the blood stream only slowly (35); (b) For concentration calculations from optical density measurements (see 36), Evans blue obeys the Lambert-Beer Law for strict proportionality between optical density and concentration (34); (c) In view of the Na movement accompanying the wall water loss, it is important that the optical density of Evans blue is not affected by variations in NaCl concentration (34).

The % transmission of Evans blue in a Krebs solution perfusing the rat tail artery was continuously monitored during constriction using a flow-through microcell on a Zeiss spectrophotometer.

1. Methods

The same in situ preparation of the rat tail artery was used as in the above two experiments. The arteries were perfused with Krebs solution to which Evans blue dye had been added (12.5 mg/liter) (282 mosm/liter). Norepinephrine was added to the artery either internally or topically. The effluent passed through a 20 μ l flowthrough cell in a Zeiss spectrophotometer, set at 600 m μ , the absorption peak for Evans blue (35). The intravascular pressure proximal to the artery was monitored. This pressure and the % transmission of the effluent were displayed on a polygraph. Fifty-one constrictions were induced in 13 tail arteries.

2. Results

One of the responses of this in situ artery preparation to the addition of norepinephrine is shown in Fig. 17. The pressure increase was accompanied by an increase in the % transmission (% T) of the effluent. This meant that the Evans blue in the perfusate must have been diluted. The extent of the

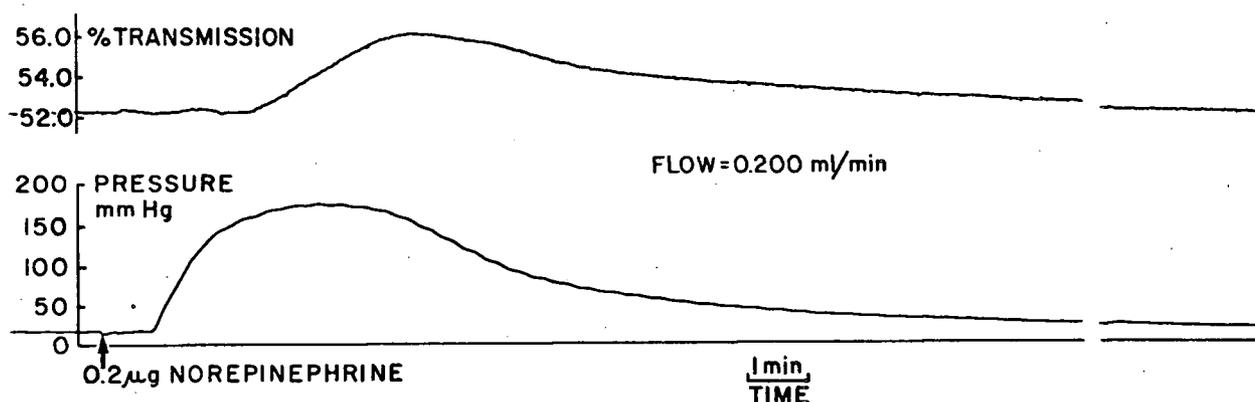


Fig. 17 Intravascular pressure and % transmission of the effluent from a rat tail artery in situ after the addition of norepinephrine.

dye dilution was calculated from the % T curve:

Over the 500 seconds of the % T increase, the average % T was 54.7%. From Beer's law for this solution and spectrophotometer cell, the corresponding concentration was $42 \log(1/0.547) = 11.0$ μ moles/liter. The concentration before the NE was added was 11.8 μ moles/liter. In 500 seconds, the volume of fluid normally passing through the lumen would be $0.200 \text{ ml/min} \times 500/60 \text{ min} = 1.667 \text{ ml}$. It is assumed that the amount of dye in this volume was constant. Thus, the true volume of fluid in the 500 seconds must have been $1.667 \times 11.8/11.0 = 1.912 \text{ ml}$.

So, the amount of water added to the perfusant was about 121 μ l. From a calibration run, this method of determining the added volume from the area under the curve was accurate to about $\pm 10\%$.

All the other NE-induced constrictions showed % T patterns similar to that in Fig. 17. The changes in % T were not artifacts. The Evans blue dye did not enter the artery wall. In fact, the % T decreased slightly during the runs, i.e. the solutions became slightly more concentrated, not less. The % T was not significantly affected by changes in the flow rate through the spectrophotometer cell.

3. Discussion

As with the previous experiments, the addition of over 100 μ l of fluid to the lumen of the constricting artery can only mean that the permeability of the artery wall was altered during constriction. This permeability change can be explained in 1 of 3 ways:

1. Before the NE was added, fluid was leaving the lumen and passing into the fluid surrounding the artery. Constriction was then associated with a decrease in the permeability of the wall, so that less fluid passed out of the lumen, resulting in a less concentrated effluent. (This was the cause of the initial flow change in the high pressure constriction in the previous experiment.)

2. Before the NE was added, fluid was entering the lumen from the surroundings. Constriction then increased the wall permeability so that even more fluid entered the lumen, resulting in a more diluted effluent.

3. Before the NE was added, no fluid entered or left the lumen through the wall. Constriction then increased the wall permeability and fluid moved into the lumen, resulting in a diluted effluent.

There is evidence that explanation #2 applies to the changes observed in Fig. 17. Calculation of the molar extinction coefficient (in Beer's law) for the Evans blue-Krebs solution in the absence of the artery, gave dye

concentrations which were too low when the artery was present. (The dye concentration was 12.5 mg/liter = 13.0 μ moles/liter, compared to 11.8 μ moles/liter for Fig. 17.) This indicates that even before the NE was added, fluid was passing through the artery wall and entering the lumen. Constriction increased this passage.

In the previous experiment, before constriction fluid moved out of the lumen, while in this experiment, before constriction fluid moved into the lumen. The direction of flow through the wall was probably affected by the osmolarity of the solution perfusing the lumen and bathing the exterior of the artery and by the intravascular pressure in the two experiments. (In each experiment, the same solution was used inside and outside the artery.) In any case, it seems unlikely that there are large transmural fluid movements before constriction for arteries in vivo. These experiments were not designed to determine the "normal" wall permeability, but rather the effect which vasoconstriction had on the wall permeability.

4. Summary

1. The Evans blue perfusing the arteries was diluted during vasoconstriction.
2. Before the addition of norepinephrine, fluid passed through the artery wall from the surroundings into the lumen.
3. During constriction, the permeability of the wall was drastically increased--allowing fluid to pour into the lumen.

D. SUMMARY

1. Norepinephrine-induced vasoconstriction in the rat tail artery had profound effects on its hemodynamic properties and permeability.

2. There was a suggestion that the hydration of the artery wall decreased during constriction at "high" intravascular pressure.

3. The walls of these arteries were quite permeable to fluid.

4. The intravascular pressure and vasoconstriction affected this permeability very drastically.

5. Higher intravascular pressures increased the permeability of the artery wall.

6. Vasoconstriction either caused an increase in wall permeability, or a decrease followed by an increase in permeability.

7. How typical are these rat tail arteries of muscular distributing arteries can be argued. But certainly any studies on perfused arteries (for example, monitoring the concentration of ions in the effluent), should take this question of permeability into account.

8. That the permeability of the wall was affected both by the pressure in the artery lumen and tension in the artery wall suggests that the balance of forces that normally determine the permeability of the wall--the steady state which the wall maintains with its surroundings--was dependent upon the intravascular pressure and was upset by the changes associated with vasoconstriction.

CHAPTER V

EXPERIMENTALLY INDUCED ALTERATIONS IN THE
HYDRATION OF THE ARTERY WALL

The experiments discussed above have indicated that the hydration as well as the transmural permeability of the artery wall are considerably altered when the artery constricts under the influence of several vasoactive agents. The artery wall can be viewed as a network of components which are all intimately connected and act as a unit to regulate the volume of blood that passes through the artery lumen. This network consists of endothelial and smooth muscle cells and the extracellular macromolecules: collagen, elastin, and protein-polysaccharide complexes. The above experiments examined the effects of active changes in the wall produced by vasoconstriction. The contents of the vascular wall can also be altered passively. In this study, passive changes were induced in the artery wall by (a) varying the composition of the external ions and by (b) cooling then rewarming the arteries.

A. EFFECT OF VARYING EXTERNAL IONIC COMPOSITION

The anionic groups of the extracellular material (the paracellular matrix) of the artery wall bind Na and other ions (1-7). It is possible to change the counter-ion bound to these groups by changing the ionic composition of the solution in which the artery is incubated (6). Such an ion exchange may alter the configuration of the paracellular matrix and consequently alter the hydration of the artery wall (7).

To test the ion exchange properties of the rat tail artery and their effect on the physical properties of the artery, a series of experiments were performed using the techniques of Chapter III. Arteries were incubated in isosmotic solutions of different ionic composition while their intravascular pressure was monitored, then analysed for their water and ion content.

1. Methods

After the ventral tail artery of the rat was exposed, its distal and proximal halves were cannulated by inserting polyethylene tubing at the mid-point and proximal end of the artery. The distal half-artery was flushed with the solution in which it was to be incubated, removed from the tail bed and placed with its cannula in that particular solution. The proximal half-artery was similarly flushed, removed and placed in a second solution.

Five solutions of different ionic composition were used as incubation media for the arteries: in meq/liter, solution 1: NaCl 143, KCl 5.0, MgSO₄ 2.4, CaCl₂ 4.2; solution 2: KCl 5.0, MgSO₄ 2.4, CaCl₂ 4.2, lactose; solution 3: MgSO₄ 2.4, CaCl₂ 4.2, lactose; solution 4: CaCl₂ 4.2, lactose; solution 5: lactose. Solutions 2 to 5 had one cation less than the solution numerically before it. The amounts of lactose added made the solutions isosmotic (290 mosm/liter). Since zero sodium solutions preclude the use of a conventional NaHCO₃-NaH₂PO₄ buffer system, the solutions were buffered with Tris-HCl. One-tenth the usual Tris concentration, 0.7 g/liter (5 mM), was used to minimize both the contribution of chloride ions and the unknown effect of the Tris cation on the extracellular matrix of the arterial wall. This low buffering capacity meant that the solutions could not be aerated with O₂/CO₂; instead 100% oxygen was used, resulting in a pH of 7.0 at 37°C.

Four experiments were performed, using a different pair of solutions in each: solutions 1 and 2, 2 and 3, 3 and 4, 4 and 5. Eight rats were used in

each experiment. Both solutions in an experiment contained 4 distal and 4 proximal half-arteries. After an artery had been incubated for 3 hours, it was perfused with oxygen for 7 to 10 minutes to remove fluid from the lumen and produce a basal tension in the artery wall. The polyethylene tubing carrying oxygen into the solution was connected to the cannula of the artery. The intravascular pressure during this gas flow was monitored by a Statham transducer connected just proximal to the artery. (The gas perfusion was essentially constant for all the arteries so the pressures recorded for arteries in different solutions could be compared.) The artery was then removed from solution, blotted, and analysed for its water and ion contents as described in Chapter III. A Techtron atomic absorption spectrometer was used for the ion analysis.

2. Results

Table XVIII shows the water (see Fig. 18), ion content, and intravascular pressure for arteries incubated in one of five solutions. Arteries in solutions 3 and 4 (with no monovalent cations) had a greater divalent ion content, smaller water content and greater intravascular pressure than arteries in solution 1. The only significant difference between the arteries in solutions 3 and 4 was the gain in Ca, equal to one-half the loss of Mg. In solution 5, where H^+ was the only cation, the water and pressure values were about the same as in solution 1. It should be noted that these five solutions were isosmotic. So, for example, arteries in solution 2 which lost Na and presumably Cl, would not have lost water because of an osmotic imbalance.

There was an inverse relationship between the water content and the Ca + Mg content: for the 60 arteries in the five solutions, the correlation coefficient was -0.495 ($p < 0.001$). This suggests that the quantity of

TABLE XVIII. Intravascular pressure, water and ion contents (\pm S.E.) of rat tail arteries incubated in one of 5 isosmotic solutions.

Solution	Pressure (mm Hg)	H ₂ O (ml/100 g dry wt)	Na ⁺	K ⁺ (meq/100 g dry weight)	Mg ⁺⁺	Ca ⁺⁺
1. (all ions) (8)*	65 \pm 5	286 \pm 13	43.6 \pm 1.7	12.5 \pm 1.2	3.39 \pm 0.15	2.19 \pm 0.11
Δ^\dagger	+ 62	-33	-41.4	-2.4	+0.81	+0.80
2. (no Na ⁺) (12)	127 \pm 12	253 \pm 5	2.20 \pm 0.28	10.1 \pm 0.4	4.20 \pm 0.09	2.99 \pm 0.12
Δ	+32	-24	-1.17	-3.9		
3. (no Na ⁺ , K ⁺) (16)	159 \pm 8	229 \pm 2	1.03 \pm 0.14	6.19 \pm 0.36	4.27 \pm 0.10	3.24 \pm 0.09
Δ					-2.73	+1.46
4. (no Na ⁺ , K ⁺ , Mg ⁺⁺) (16)	148 \pm 14	253 \pm 3	0.78 \pm 0.08	5.67 \pm 1.29	1.54 \pm 0.04	4.70 \pm 0.14
Δ	-72	+55	+0.50		+0.50	-3.73
5. (lactose) (8)	76 \pm 18	290 \pm 8	1.28 \pm 0.15	6.01 \pm 0.32	2.03 \pm 0.07	0.97 \pm 0.07

* Number of arteries

† Difference between arterial contents ($p < 0.02$).

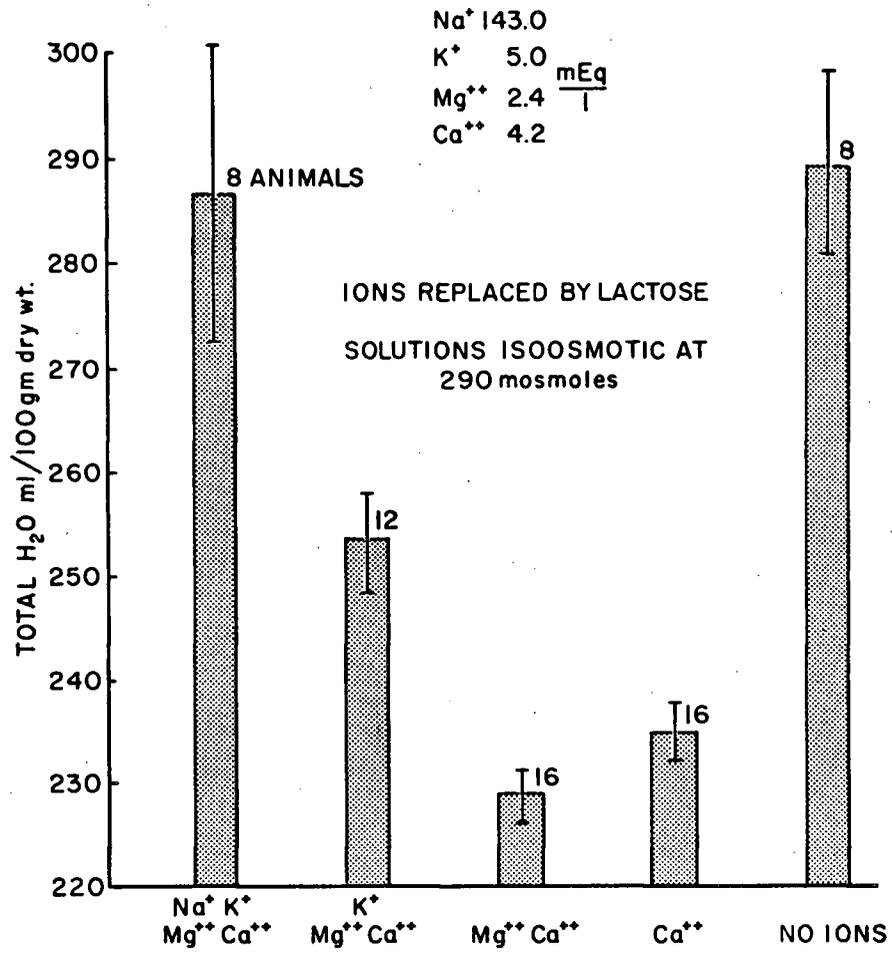


Fig. 18 Water content of rat tail arteries equilibrated in 1 of 5 isosmotic solutions of different ionic composition.

divalent cations was associated with changes in the hydration of the artery wall. An inverse relationship was also observed between the water content and the intravascular pressure. For all 60 arteries, the correlation coefficient was -0.59 ($p < 0.001$). In addition, the intravascular pressure was directly related to the Ca + Mg content ($r = 0.40$, $p < 0.01$) and to the Ca content ($r = 0.46$, $p < 0.001$). It was noted that the proximal half-arteries had smaller water contents than the distal halves in all five solutions, but the difference was only significant ($p < 0.01$) for the arteries in solution 5.

3. Discussion

There are two possible explanations for the observed results which are not mutually exclusive.

1. The different ionic composition of the solutions caused changes in the membrane potential of the vascular smooth muscle cells. The cells of arteries in solution 2 (with no Na), compared to those in solution 1 (all ions), were contracted and probably depolarized (8,9 and calculations from the Goldman equation). This constriction might thus explain: (a) the increased content of Ca, since Ca apparently enters the contracting smooth muscle cell (10), (b) the increased intravascular pressure, and (c) the decreased H₂O content associated with constriction (see Chapter III). It is unlikely that all of the 1 meq increase in total Ca between solutions 1 and 3 was intracellular, since that would mean the cell Ca concentration had increased by about 6 meq/liter cell H₂O--assuming the cells contained 2/3 of the total water. In addition, since the inulin space decreases in low Na solutions (7), the increase in cell Ca would be even larger. However, even a small increase in intracellular Ca could probably account for all of the constriction.

2. Some of the gain in arterial Ca in solution 2 could have been bound extracellularly. Using an ion exchange process, Palatý et al. found that the sum of the increases in the Ca and Mg arterial contents in the low Na solutions was approximately equal to the amount of Na bound extracellularly--about 5 meq/100 g dry wt for the rat tail artery (6). In the present study, the total divalent ion increase between solutions 1 and 2 was 1.61 meq/100 g dry wt, well within the estimates of Palatý et al. (6) and others (1,4,11,12) of the extracellularly bound Na. This suggests that changes in the paracellular matrix of the artery wall may have played a role in the changes in Table XVIII. These changes can be qualitatively explained by considering the paracellular matrix as an ion exchanger. In general, the swelling of a given ion exchange resin is dependent upon the degree of cross-linking in the resin, and upon the valency, size and external concentration of the counter-ion (13). If the counter-ions are mainly monovalent, then the extracellular matrix of the artery wall is extended and relatively swollen--as were the arteries in solutions 1 and 5. With divalent counter-ions, the degree of cross-linking is increased so the matrix becomes tighter and the increased elastic forces cause the extracellular matrix to shrink. The same results have been shown for polyelectrolyte gels (14,15). Bozler has found that the stiffness and opacity of frog stomach muscle in dilute solutions of CaCl_2 and MgCl_2 are increased strongly (16,17). For the rat tail arteries, the 20% decrease in wall water content between solutions 1 and 3 may be partly due to an ion exchange process that replaces monovalent ions with divalent ions as the counter-ions to the anionic groups of the protein-polysaccharide complexes in the paracellular matrix. The increased intravascular pressure for arteries in the divalent solutions could be explained if the ion exchange process, which resulted in conformational

changes in the paracellular matrix and hydration changes in the artery wall, also profoundly affected the physical response of the artery to perfusion.

These results cannot be compared to those for hypertensive arteries in which an increase in water content (18,19) is associated with an increase in the elastic stiffness (20), since in hypertensive arteries the polysaccharide matrix is actually increased (21).

4. Summary

1. Rat tail arteries were incubated in isosmotic solutions of different ionic composition.

2. Removing monovalent ions from the solution resulted in a greater divalent ion content, a smaller water content and a greater intravascular pressure of the arteries.

3. The water content of the arteries was smaller when: (a) the Ca + Mg content was greater, and (b) the intravascular pressure was greater.

4. In addition to definite changes in the tension of the vascular smooth muscle cells, there may have been changes in the paracellular matrix of the artery wall: an ion exchange process may have altered the configuration of the matrix and the hydration of the wall.

B. EFFECT OF COOLING AND REWARMING

1. On the Water and Ion Content of the Vascular Wall

Cold acts as an inhibitor of cellular metabolism. Tissues cooled to 2°C for an extended period of time gain sodium and water and lose potassium (22). If the tissues are rewarmed to 37°C, the processes are reversed as the metabolism of the cell is reactivated. The effects of cooling and rewarming on sodium and potassium exchanges have been studied using many

smooth muscle tissues (23,24), including vascular smooth muscle (5,25-29). Friedman et al. found that the curve of the Na efflux from a rewarmed artery could be divided into 2 components: a fast (complete within 15 minutes of rewarming) component, apparently unaccompanied by K and unaffected by idodoacetate, and a slower metabolic component coupled 1:1 with K. They suggested that this temperature sensitive fast Na efflux represents the effect of temperature on either Na bound to the anionic groups of the extracellular matrix or Na leaving the smooth muscle cells through channels independent of the Na-K pump--perhaps accompanied by Cl.

Although cooling and rewarming is an artificial procedure, it has provided considerable information on the normal behavior of Na and K in the artery wall. It might also help in understanding the behavior of water in the artery wall; in particular the effect of various factors on vascular hydration. In this regard, a series of experiments was performed in an attempt to answer several questions:

1. What is the effect of temperature on the extrusion of water from the rewarming artery?
2. What effect does a sodium gradient between the arterial wall and the rewarming solution have on this extrusion of water?
3. Is there any relationship between the water extrusion and the fast non-K linked sodium extrusion?

Since water can not be studied using the isotope or flow-through electrodes techniques other workers have employed in their cooling-rewarming experiments, it was decided to simply incubate the arteries at various temperatures and determine their water contents from wet and dry weights.

(a) Methods

The rat tail arteries were cooled overnight at 2°C, then incubated for

15 minutes in solutions of different sodium concentration at a given temperature between 2° and 37°C. This procedure allowed the water and ion content of the arteries to be determined at distinct points during the rewarming process. Fifteen minutes was chosen as the incubation time, rather than a few hours, so the changes that occurred during the extrusion of the non-K linked, temperature-sensitive sodium component would not be obscured by the effect of the slower metabolic exchanges of Na and K.

The tail arteries of the rats were excised after being flushed with Krebs 140 solution (140 refers to the Na concentration in meq/liter--similarly for the other solutions). The arteries were cut in half, placed in flasks of Krebs 140 solution and kept in the refrigerator overnight at 2°C. The arteries were transferred to fresh Krebs 140 solutions, aerated with oxygen, and incubated for a further 2 hours at 2°C. Fifteen half-arteries were removed for analysis of water and ions at this point. Five groups of 8 half-arteries were then transferred to Krebs 140 solutions, aerated with O₂, at 8°, 10°, 20°, 30°, or 37°C for 15 minutes of incubation and then analysed. Another group of 8 half-arteries was incubated for 3 hours at 37°C so that the effect of the complete rewarming process could be determined. The Na and K contents of the arteries were determined using a Techtron absorption spectrophotometer.

To examine the effects of the sodium gradient, after overnight cooling in Krebs 140 solution, the arteries were: (a) transferred to Krebs 100 solution and the above procedure repeated using Krebs 100 throughout the rewarming, (b) transferred to Krebs 0 solution at 5°, 8°, 10°, or 37°C for 15 minutes. (Solutions 1 and 2 from the previous section were used for the Krebs 140 and Krebs 0 solutions in this experiment.) The solutions were aerated with oxygen. Lactose replaced the NaCl in these solutions.

(b) Results

Effect of Temperature

The changes in the arterial water and ion content during rewarming in Krebs 140 solution are shown in Figs. 19 to 21 and Table XIX. Arteries rewarmed in Krebs 140 solution did not lose a significant amount of water during the 15 minute rewarming periods between 2° and 37°C, although they lost 34 ml water/100 g dry weight during the 3 hours at 37°C. (All the subsequent water and ion values will be per 100 g dry weight.) The arteries lost Na and gained K when rewarmed in Krebs 140 solution. Since the arteries were equilibrated for 2 hours, these changes were due solely to the effect of temperature. In the 15 minute rewarming periods between 2° and 37°C, there was an increase of 2.86 meq K. It will be assumed that this increase in K was due to the effect of temperature on the metabolic Na-K pump of the vascular smooth muscle cells. The activity of the pump increased with the temperature, resulting in a slight restoration of the ionic gradients during these 15 minute rewarming periods. The arteries lost 6.08 meq Na during these periods. The exchange of Na and K by the pump in vascular smooth muscle cells is probably in a 1:1 ratio (5,29). The difference between the Na lost and the K gained shows there was 3.22 meq of extra Na lost from the artery wall between 2° and 37°C. This extra lost Na was not accompanied by K, and was presumably independent of the metabolic Na-K pump, although quite dependent upon the temperature. Table II also shows that most of the Na extruded from the artery wall during the 15 minute rewarming periods left between 10° and 20°C, while most of the K returned between 20° and 30°C. So, the loss of this extra Na component was not only independent of K, but also occurs at a lower temperature than the metabolic Na-K exchanges. During the 15 minute rewarming periods only 1/3 of the total regained K re-

TABLE XIX. Changes in H₂O (ml/100 g dry wt), Na and K (meq/100 g dry wt) contents of rat tail arteries at different rewarming temperatures in 3 solutions with 140, 100, or 0 meq Na/liter. Δ is given when $p < 0.05$.

	Temperature range	Δ H ₂ O	Δ Na	Δ K
Krebs 140				
	2° to 10°			-0.739
	10° to 20°		-10.1*	+1.56
	20° to 30°			+2.40
	30° to 37°			
Subtotal	2° to 37°		-6.08	+2.86
	37° to 37° (3 hr)	-23.6	-5.48	+7.06
Total	2° to 37° (3 hr)	-34.0	-11.6	+9.91
Krebs 100				
	2° to 10°			} +0.51
	10° to 20°	-15.5	-6.00	
	20° to 30°	+19.2		+2.15
	30° to 37°	-26.1		
Subtotal	2° to 37°	-19.4	-8.79	+3.27
	37° to 37° (3 hr)		-6.09	+5.24
Total	2° to 37° (3 hr)	-30.2	-14.9	+8.50
Krebs 0				
	5° to 8°			
	8° to 10°	-44.5		
	10° to 37°		-7.59	+1.86
Subtotal	5° to 37°	-67.6	-8.23	+2.53

* 8° to 20°C

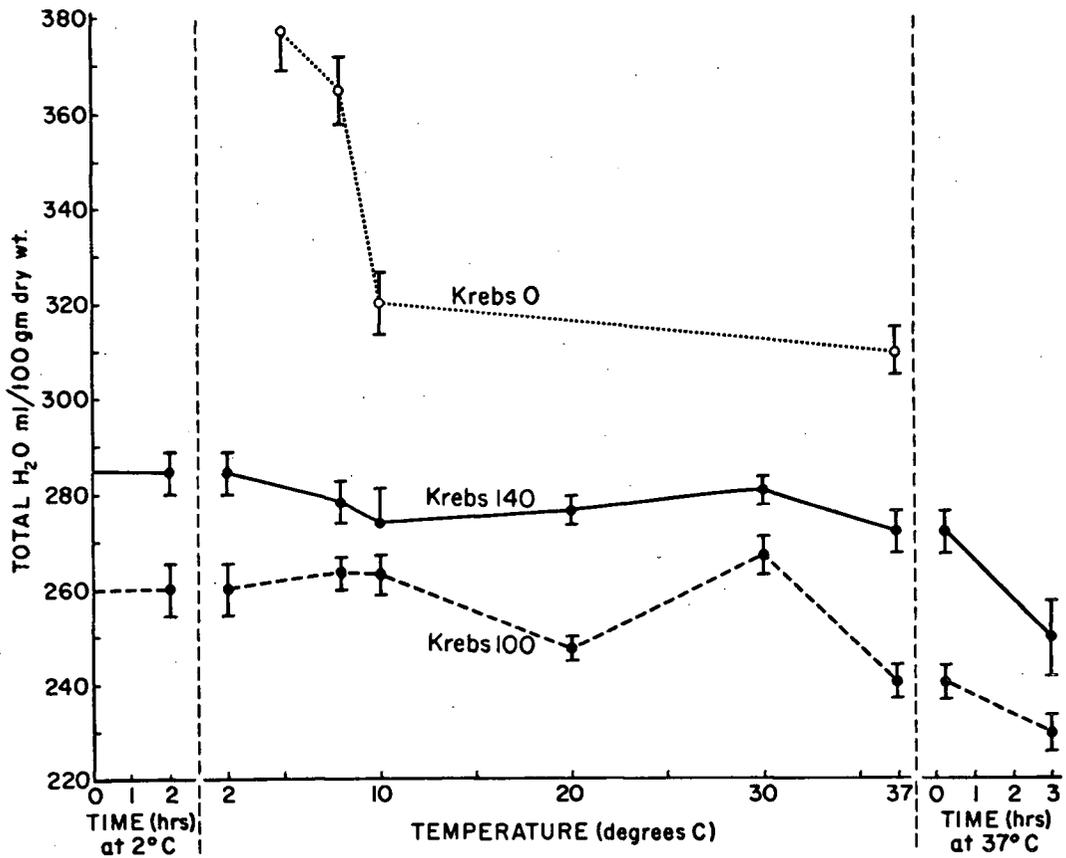


Fig. 19 Effect of temperature during rewarming on the H_2O content of the rat tail artery cooled for 18 hours at $2^\circ C$. The arteries were transferred from the solution at $2^\circ C$ to one of the solutions between $2^\circ C$ and $37^\circ C$ for 15 minutes of rewarming. Three rewarming solutions with different Na concentrations were used. Each point represents 8 arteries except at $2^\circ C$ where $n = 15$.

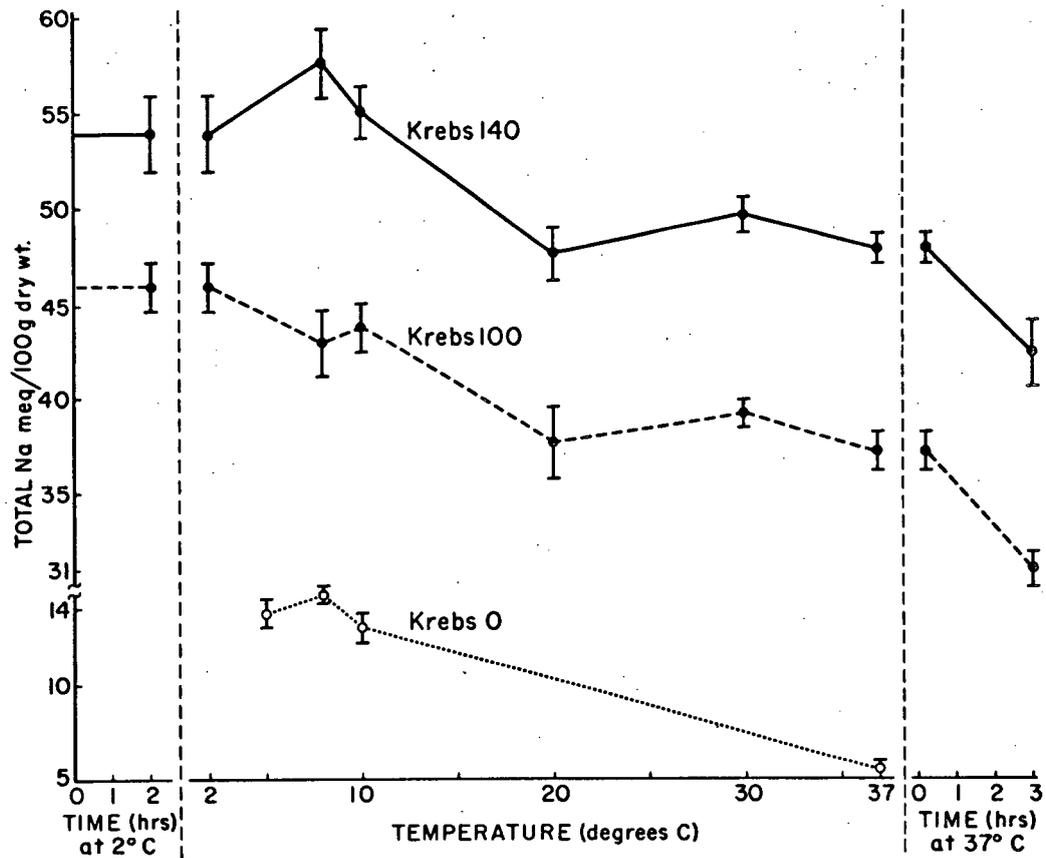


Fig. 20. Effect of temperature during rewarming on the Na content of the rat tail artery cooled for 18 hours at 2°C. The arteries were transferred from the solution at 2°C to one of the solutions between 2° and 37°C for 15 minutes of rewarming. Three rewarming solutions with different Na concentrations were used. Each point represents 8 arteries except at 2°C where n = 15.

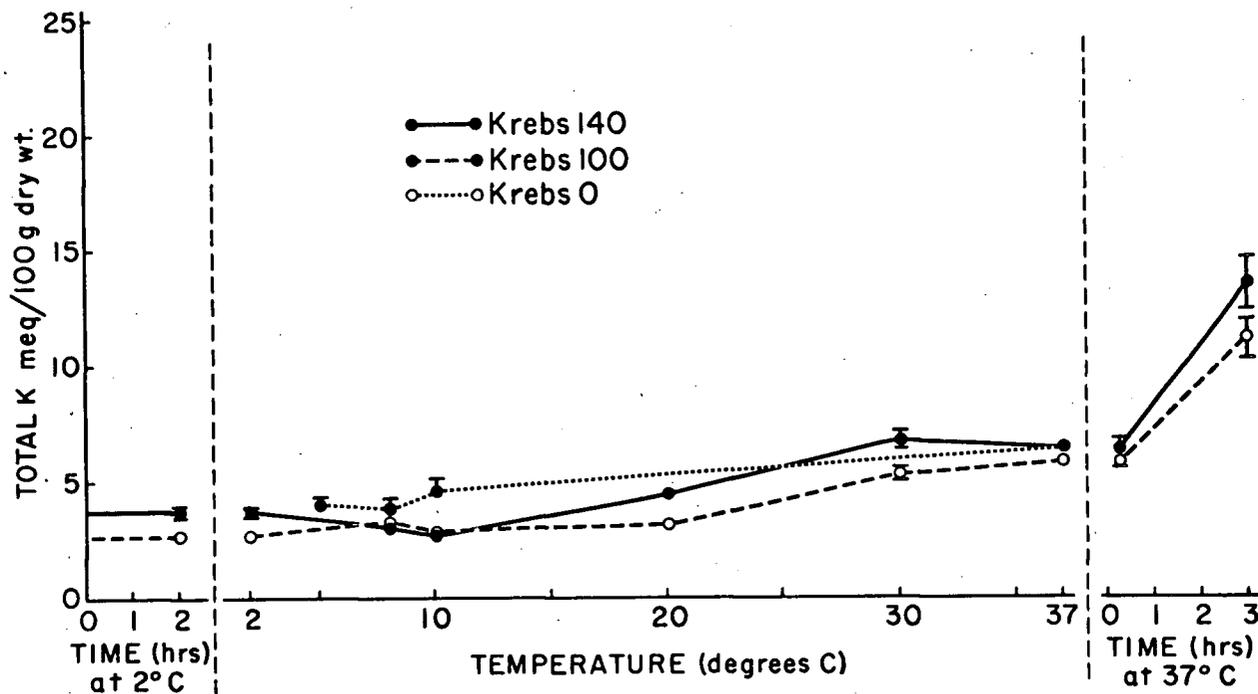


Fig. 21 Effect of temperature during rewarming on the K content of the rat tail artery cooled for 18 hours at 2°C. The arteries were transferred from the solution at 2°C to one of the solutions between 2° and 37°C for 15 minutes of rewarming. Three rewarming solutions with different Na concentrations were used. Each point represents 8 arteries except at 2°C where n = 15.

turned, while 1/2 of the total extruded Na left. This indicates that the Na-K pump requires the extended incubation period at 37°C to become fully operating. It also indicates that the 15 minute rewarming periods at temperatures between 2° and 37°C are not long enough periods of time for the metabolic Na-K pump to restore the ionic gradients upset by overnight cooling.

During the 15 minute rewarming periods in Krebs 100 solution, the arteries lost about 19 ml water/100 g dry wt. At 30°C, there was a significant

relative maximum, or "hump" in the water content. (This maximum was also present for arteries in Krebs 140 solution, but was not significant.) As shown in Table XIX, compared to changes in the arteries rewarmed in Krebs 140 solution, the K changes were about the same, while there was more Na lost from arteries in Krebs 100 solution.

Arteries rewarmed in Krebs 0 solution showed a dramatic loss of 45 ml water between 8° and 10°C. Between the 15 minute incubations at 5°C and at 37°C, there was a loss of about 68 ml water. No information was obtained on the relative maximum at 30°C. These arteries also gained about the same amount of K and lost more Na than the arteries rewarmed in Krebs 140. Because there was no equilibration period for these arteries, the Na loss on rearming may simply represent Na extruded from the artery in 0 Na solution.

Effect of the External Na Concentration

The arterial hydration was very dependent upon the temperature and this temperature dependence was radically affected by the external Na concentration. The 3 different initial values of the water content in Fig. 19 are probably due to the different osmolarities of the 3 solutions: Krebs 0, 290; Krebs 140, 313; Krebs 100, 329 mosm/liter. The initial values are inversely related to the osmolarity as would be expected. The temperature at which most of the water was extruded from the rearming artery was lower when the external Na concentration was lower. Fig. 22 shows that the amount of water lost during the 15 minute rearming periods (2° to 37°C) was greater when the external Na concentration was lower. Since the Na-K pump was only minimally effective during these short rearming periods, this suggests that some other mechanism was mainly responsible for the changes in the hydration of the rearming artery wall.

The different initial values of the Na content for arteries in the 3 solutions in Fig. 20 simply reflect the different Na concentrations of their bathing media. The greater loss of arterial Na when the external Na concentration was lower will be discussed below. There does not seem to be any effect of external Na concentration on the activity or the temperature of operation of the metabolic Na-K pump. The K gained between 2° and 37°C in 15 minute rewarming periods was essentially the same for all 3 solutions. There was no real difference between the percentages of the total Na and K movements during the 15 minute rewarming periods in Krebs 140 and Krebs 100 solutions. Nor did the external Na concentration affect the temperature at which the Na-K pump "came into action".

Extrusion of the Non-K linked Na Component

The external Na concentration affected the extrusion of the extra non-K linked Na component. It was this effect that resulted in the different losses of Na in the 3 solutions, and not any effect on the Na-K pump. The size of this extra Na component released during the 15 minute rewarming periods between 2° and 37°C is presumably the difference between the Na lost and the K gained in the interval (see Table XIX): Krebs 140 solution: $6.08 - 2.86 = 3.22$ meq Na; Krebs 100 solution: $8.79 - 3.27 = 5.52$ meq Na; Krebs 0 solution: $8.23 - 2.53 = 5.70$ meq Na. However, the true size of this extra Na component is smaller than these values because of the accompanying extrusion of water from the rewarmed artery wall. Some of this extruded water probably came from the free extracellular space (ECS) which is in equilibrium with the external solution and consequently has the same Na concentration. Inulin space measurements were not done, so the amount of water that came from the free ECS is not known. For the purpose of calculation, suppose that all of the extruded water came from the free ECS.

Subtraction of the amount of Na that would have been carried out with this water will give the maximum correction to the size of the extra non-K linked Na component. The values thus obtained will be the minimum amounts, i.e. the amounts of Na released, unaccompanied by K, from compartments of the rewarming artery other than the free ECS. The above uncorrected values are the maximum amounts, i.e. they include Na lost from the free ECS.

Krebs 140 solution: The non-significant decrease in the arterial water content of $284 - 272 = 12$ ml water between 2° and 37°C (see Fig. 19) will be considered as a real decrease for these rough calculations. Twelve ml of free ECS water would have carried out $0.14 \times 12 = 1.68$ meq Na and $0.005 \times 12 = 0.06$ meq K. The minimum size of the extra Na component would have been $6.08 - 1.68 - 2.86 - 0.06 = 1.43$ meq Na.

Krebs 100 solution: The 19.4 ml of lost water would have carried out 1.94 meq Na and 0.097 meq K from the free ECS. The minimum size of the extra Na was $5.52 - 1.94 - 0.097 = 3.48$ meq Na.

Krebs 0 solution: Since it is the minimum size of the extra Na component that is of interest, assume that the maximum trace Na in this solution was 2 meq Na/liter. The minimum amount of Na in the extra component released was $8.23 - (0.002 \times 67.6) - 2.53 - (0.005 \times 67.6) = 5.23$ meq Na.

These minimum and maximum values of the extra non-K linked Na component released during rewarming are shown in Fig. 22.

It is interesting to note that the size of the extra Na component extruded in Krebs 0 solution, 5.23 to 5.70 meq Na/100 g dry weight, is about the same size as Palatý's estimate of Na bound to the anionic sites in the paracellular matrix of the rat tail artery (6)--although, of course, their experimental procedure was quite different.

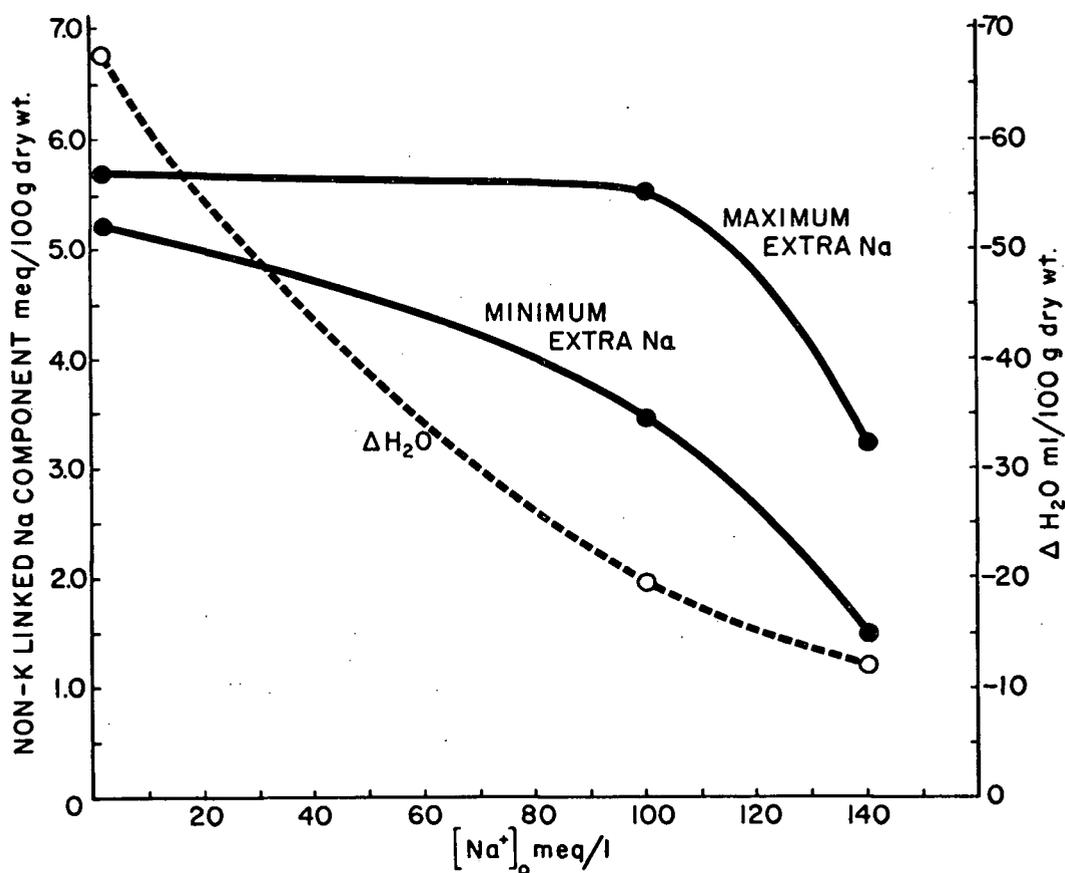


Fig. 22 Effect of external Na concentration, $[Na^+]_o$, on the change in water content, ΔH_2O , and on the size of the extra, non-K linked Na component extruded from the rat tail artery during 15 minute rewarming periods between 2° and 37°C. The minimum extra Na component is the difference between the measured maximum extra Na component and the Na carried out if all the extruded water were from the free extracellular space.

(c) Discussion

The hydration changes during rewarming have to be explained in terms of: (a) the overall water decrease, (b) the peak at 30°C, and (c) the effect of external Na.

The metabolic Na-K pump is not a satisfactory mechanism to explain these changes. Certainly the pump is responsible for removing Na and water from and restoring K to the vascular smooth muscle cells during rewarming. But 15 minute rewarming periods were not long enough for substantial pump activation. Discontinuities have been observed (30,31; also see 32) in the Arrhenius plot of the activity of the ATPase system presumably associated with the Na-K pump (33). But at 30°C, there was not a simple change of the rate of water decrease, but an increase. This would imply that the pump was operating in reverse. But, there was no corresponding decrease in the K content at 30°C. And finally, the external Na concentration did not affect the Na-K pump during the 15 minute rewarming periods, although it drastically affected the water lost from the rewarming artery.

A clue for an explanation of the water changes may lie in a relationship between the water and the Na content of the artery wall. Both the amount of water and the amount of extra non-K lined Na extruded from the rewarming artery varied inversely with the external Na concentration. In addition, there were relative maxima in both the Na content (although not significant) and the water content at 30°C. There are 3 possible explanations of the overall water decrease during the 15 minute rewarming periods which are related to this fast non-K linked Na component:

1. Channels in the smooth muscle membranes, not connected to the Na-K pump, might provide the mechanism of extrusion of this extra Na component, perhaps accompanied by Cl (29,34). The decreased water content of the artery wall could be due to a decrease in the cell water accompanying the loss of cellular NaCl to maintain cellular osmolarity. This NaCl loss might be the result of temperature dependent changes in the state of the contractile proteins.

2. An electrogenic pump which operated between 10° and 20°C and pumped out cellular Na and water without the extrusion of Cl or the entry of K would explain both the Na and water losses during rewarming. This concept is supported by the membrane hyperpolarization observed by Taylor et al. (35) at the start of rewarming of the pregnant rat uterus. Upon immersion into solution at 38°C, precooled rat liver slices showed an immediate loss of Na and Ca, while the gain in K was delayed 10 to 15 minutes (36). It was suggested that the high cell Ca concentration decreased the membrane permeability to K. Ca moving out of the cells could be the source of the hyperpolarization. Presumably there would be a loss of cell water accompanying the Na and Ca losses.

3. It has also been suggested that the extrusion of the fast Na component during rewarming might represent an effect of temperature on ion binding to anionic groups in the paracellular matrix (5). The release of Na from the binding sites might be accompanied by the binding of divalent ions. Such an exchange would be accompanied by conformational changes in the matrix resulting in the expulsion of water from the extracellular space. It should be noted that Palatý et al. (7) found a positive correlation between the water content of the rat tail artery and the amount of Na bound to the protein-polysaccharide complexes.

In each of these cases, lowering the external Na concentration would augment the removal of the extra non-K linked Na from the rewarming artery wall. The presence of the peak in water content at 30°C might be explained by a reversal, for reasons unknown, of these processes.

There is another possible source of the hydration changes during rewarming. There may be anomalies in the temperature dependence of the physical

properties of water (37-42, compare 43). Also, it is known that "orthowater," grown in capillary tubes and found at water-quartz interfaces has different physical properties than normal water (44). At 2°C, the water structure in the immediate vicinity of macromolecules, inside and outside the smooth muscle cells, might be different than the water structure in more open spaces. It is also possible that rewarming affects water next to macromolecules in a different manner than water in the open spaces. It could be speculated that alterations in the amount of water able to act as solvent, due to unequal structural changes in the wall water during rewarming, could result in osmotic imbalances that produce the observed hydration changes. Or, structural changes in the water during rewarming could cause conformational changes in the matrix causing it to shrink and swell at various temperatures.

(d) Summary

1. The incubated rewarmed rat tail artery gained K, and lost Na and water.

2. There was only a slight gain of K during the 15 minute rewarming periods between 2° and 37°C. This gain and the temperature at which it occurred were not affected by the external Na concentration. This suggests that during these 15 minute rewarming periods the cellular metabolic Na-K pump was: (a) not fully operating, and (b) not affected by the external Na concentration.

3. The artery lost more Na than it gained K during rewarming. If a 1:1 exchange is postulated for the Na-K pump, the difference represents an extra Na component extruded at lower temperatures than the metabolic Na-K exchanges. The size of this extra non-K linked Na component increased with decreasing external Na.

4. The amount of water extruded during rewarming was greater and the temperature at which most of the loss occurred was lower when the external Na concentration was lower. There was an increase in the water content at 30°C.

5. The Na-K pump cannot fully explain these changes in the hydration of the rewarming artery. The water changes may be related to the extrusion of the fast non-K linked Na component.

2. On the Intravascular Pressure

In some of the cooling and rewarming experiments previously mentioned, the effects of vascular tension and the response to some vasoactive agents have been examined (25,28). Barr found that spiral strips of the dog carotid artery contracted when they were placed in solutions at 37°C, then progressively relaxed as the intracellular K increased during the rewarming process (25). On the other hand, Friedman *et al.* (28) found that slower rewarming of the rat tail artery resulted in a relaxation which began well before there were significant movements of cellular Na and K. They suggested that the temperature-sensitive tension changes were caused by changes in the membrane permeability for Na or K, or by changes in the elastic properties of the extracellular matrix of the artery wall.

Tobian (16) has suggested that the increased blood pressure in hypertensive arteries could be due to waterlogging of the artery wall. In view of this suggestion and the observed changes in the water content of the rewarming artery, it seemed that a study of tension changes during rewarming might support these observations, and more important, provide some information on the relationship between arterial tension and arterial hydration. There were several questions to be examined experimentally using the intravascular pressure as the measure of arterial tension:

1. What is the effect of temperature on the intravascular pressure of a rewarming artery?
2. What is the effect of the external Na concentration on the intravascular pressure during rewarming?
3. Is there any relationship between the hydration of the artery wall and the intravascular pressure during rewarming?

(a) Methods

This experiment involved simultaneous monitoring of the intravascular pressure and the temperature during rewarming. A 12 cm segment of the rat ventral tail artery was exposed, its collaterals tied, and both ends cannulated. Removed from the tail bed, the artery was tested for leaks, placed in a thin channel in a perspex chamber (the bottom of which was part of a circulating temperature-control system), filled with Krebs 150 solution, and placed in the refrigerator at 2°C overnight (about 18 hours). During rewarming the artery was connected to a rotary pump for open circuit perfusion. By passing the perfusing solution through tubing in the bottom of the artery chamber before it entered the artery, it was always at the same temperature as the chamber and the artery. The temperature was monitored with a thermocouple in the artery channel and the intravascular pressure was monitored proximal to the artery with a Statham transducer. The cooled artery was rewarmed: (a) quickly by allowing water from a bath at 40°C to pass through the chamber, (b) in steps of 1 or 2 degrees every 3 or 4 minutes--about 30 seconds was required for each step, (c) in steps of 10°C every 20 to 90 minutes--from 3 to 15 minutes (depending upon the type of pump) were required for the 10° increase. During rewarming, 9 arteries were perfused with Krebs 100 solution and 4 with Krebs 150 solution.

(b) Results

The patterns of intravascular pressure changes during rewarming with Krebs 100 solution are shown in Figs. 23 to 25. Fig. 23 shows the pressure pattern for a fast rewarming. There are 3 features to notice: (a) the slight dip in pressure near the start of the rewarming, (b) the increase in pressure until about 30°C, and (c) the later decrease in pressure to a level at 37°C below that before rewarming. These 3 changes were observed for almost all the arteries. Since there was probably a lag between the temperature of the artery and that on the record during this fast rewarming, step increases in temperature were required to determine the exact temperatures at which the pressure changes were occurring. Fig. 24 shows the pressure pattern for an artery equilibrated for 1 hour then rewarmed in steps of 1 or 2 degrees every 3 or 4 minutes. The pattern is the same as that in Fig. 23 except that the dip and the increase in pressure occur at lower temperatures. Two separate increases in pressure can be elicited, as shown in Fig. 25, if the artery is kept at 11°C for only about 30 minutes before being raised to 21°C. Fig. 25 is for an artery rewarmed in Krebs 100 solution containing 10^{-3} M iodoacetate. Exactly the same pattern was obtained for an artery (not shown) rewarmed without iodoacetate for the same time periods. This indicates that iodoacetate, which blocks ATP production under these conditions in the smooth muscle cells, has no effect on the pattern of intravascular pressure during rewarming. (Of course, there may have been sufficient ATP stored in the cells to render the action of the iodoacetate inconsequential.) The pressure changes for arteries rewarmed with Krebs 150 solution were much smaller than those for the arteries rewarmed with Krebs 100 solution.

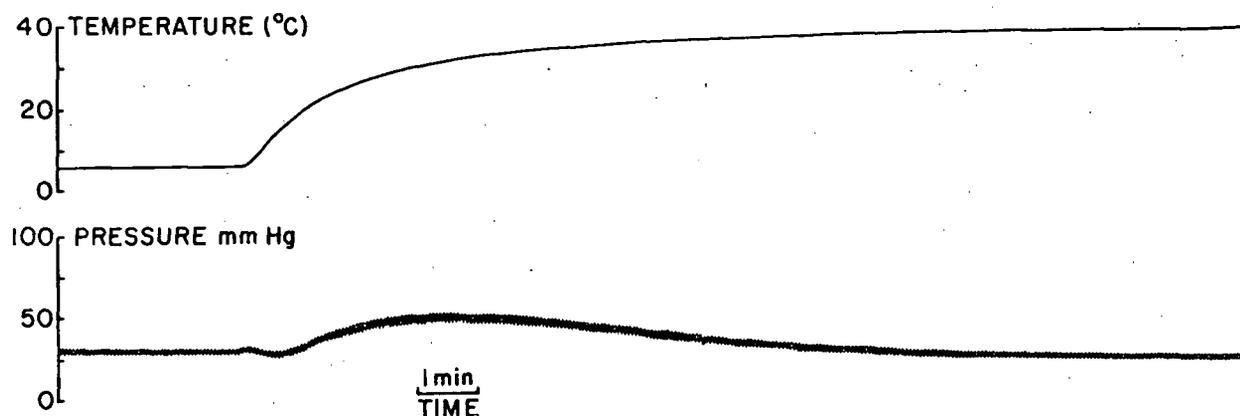


Fig. 23 Temperature and intravascular pressure during rewarming. Rat tail arteries were cooled for 18 hours at 2°C in Krebs 150 solution, then rewarmed during perfusion with Krebs 100 solution at 0.2 ml/min.

The average changes in the intravascular pressure were as follows:

Dips: 12/13 arteries had slight dips in the pressure below 10°C. There was no difference between the size or the temperature at which the dip occurred for the Krebs 100 or Krebs 150 solutions. The average pressure change in the dip was -2.5 mm Hg, at an average temperature of 8°C.

Peaks: 11/13 arteries had increases in the intravascular pressure below 30°C. The average pressure changes were: Krebs 100, +44 mm Hg, and Krebs 150, +4 mm Hg.

Overall changes: 12/13 arteries had an overall decrease in the intravascular pressure. The average overall changes were: Krebs 100, -10 mm Hg, and Krebs 150, -4 mm Hg.

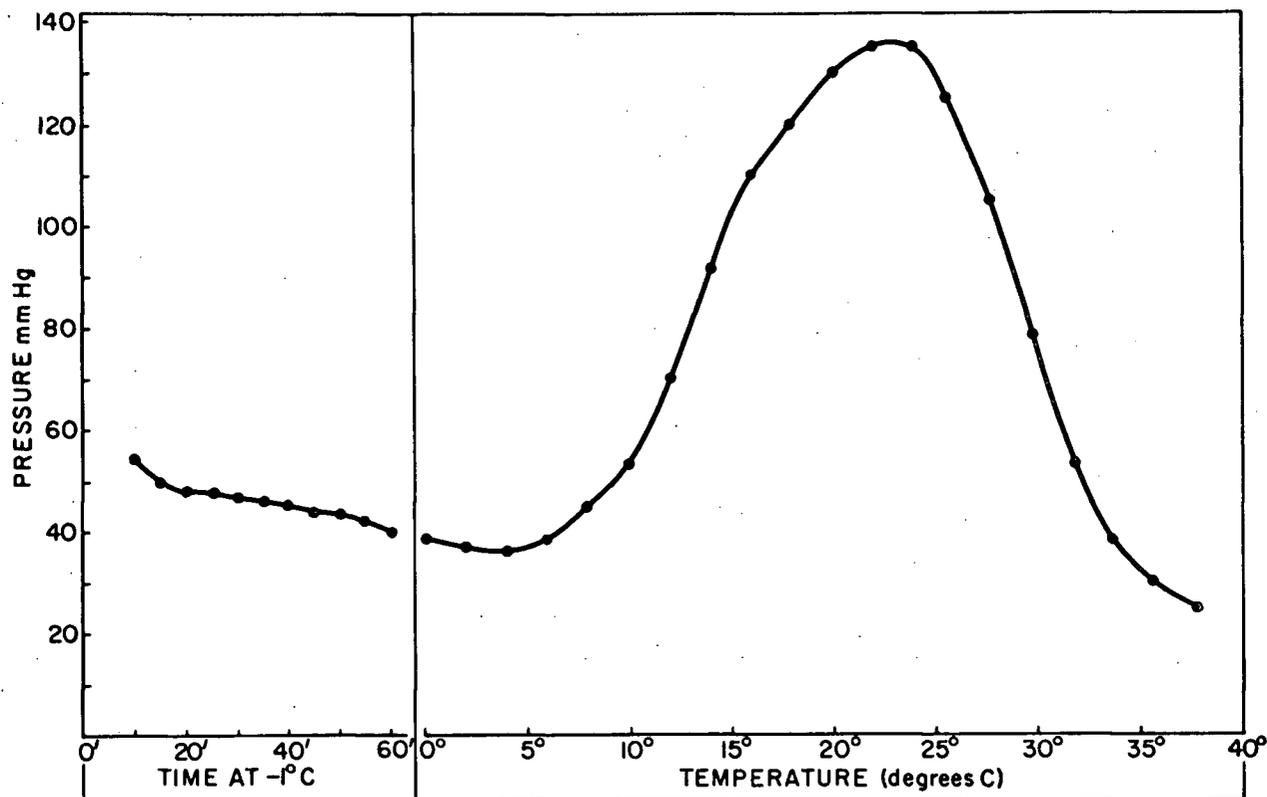


Fig. 24 Effect of time and temperature on intravascular pressure during rewarming. Rat tail arteries were cooled for 18 hours at 2°C in Krebs 150 solution, then rewarmed during perfusion with Krebs 100 solution at 0.2 ml/min.

These results show that the temperature dependence of the intravascular pressure during rewarming was affected by the external Na concentration. The overall decrease in the pressure and the pressure peak at 20° to 25°C were both greater when the external Na concentration was smaller. This was so even though arteries rewarmed in Krebs 100 solution were equilibrated in Krebs 100

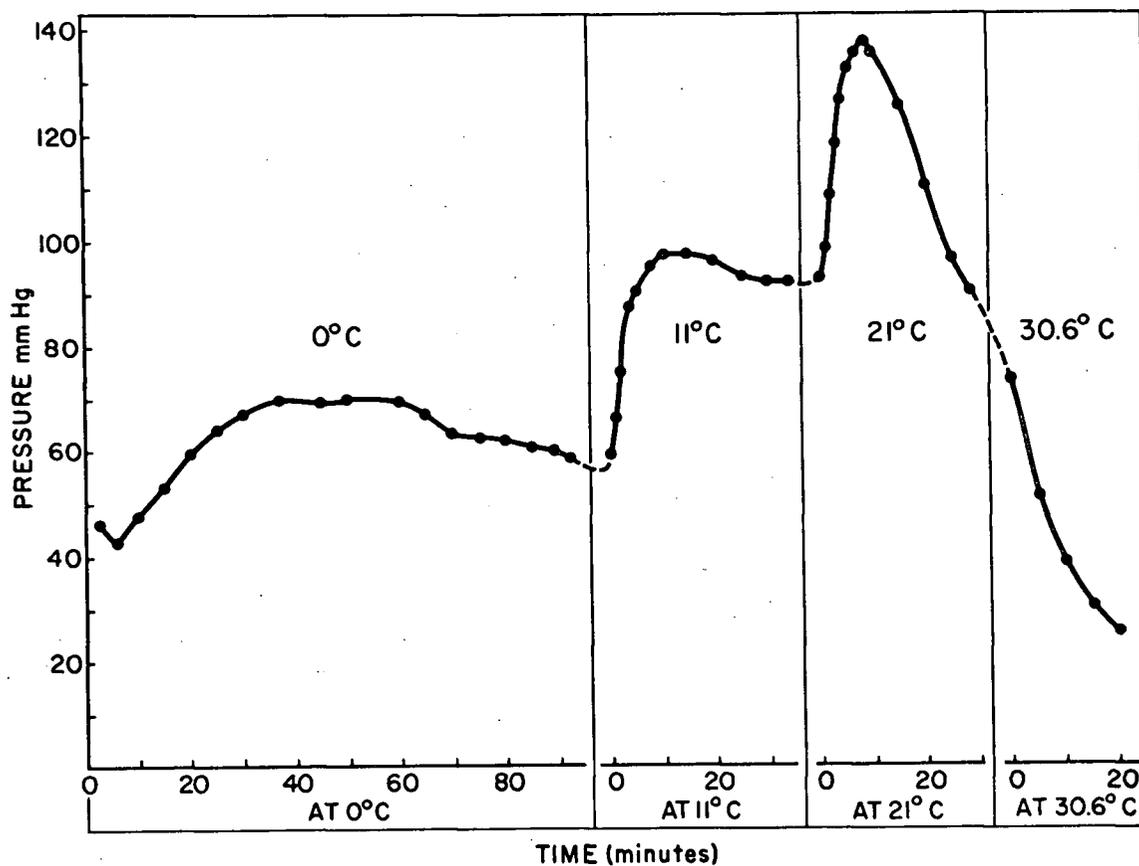


Fig. 25 Intravascular pressure at different temperatures during rewarming. Rat tail arteries were cooled for 18 hours at 2°C in Krebs 150 solution, then rewarmed during perfusion with Krebs 100 solution containing 10^{-3} M iodoacetate, at 0.2 ml/min.

solution for about 1 hour before rewarming. The external Na concentration did not seem to affect the size of the slight dip in the pressure, or the temperature at which it occurred.

(c) Discussion

During rewarming of the artery, the cellular Na-K pump restores the Na and K gradients and the artery relaxes. It was thus expected that the intravascular pressure of the perfused artery would decrease between 2° and 37°C. However, since the arteries were never at temperatures close to 37°C for very long, the pump was never fully operating. In addition, the presence of iodoacetate did not affect the overall decrease. Friedman et al. (28) also noticed that the overall relaxation of the artery began well before there were movements of cellular Na or K. For these perfused arteries, no ion measurements were made so the activity of the Na-K pump was not known. However, it seems that the metabolic Na-K pump does not fully explain the overall decrease in the intravascular pressure. The peak in the pressure at 20°C to 25°C cannot be explained at all in terms of the Na-K pump. The size and shape of the peak pressure were quite dependent upon the length of time the artery remained at a given temperature. This might suggest the involvement of the Na-K pump since its activity is less at lower temperatures so it requires longer times to restore the ionic gradients. But: (a) the restoration of the Na and K gradients relaxes the artery, i.e. decreases the intravascular pressure, not increases it, and (b) iodoacetate did not affect the size and shape of the pressure peak. It is possible that the extrusion or storage of cellular Ca during rewarming might play a role in the overall relaxation and/or the pressure peak of the rewarming artery.

There seems to be quite a close link between the pressure changes for the perfused artery and the hydration changes of the incubated artery during rewarming: (a) Both the pressure and the water content decreased overall

between 2° and 37°C, and both increased between 20° and 30°C, (b) Both these changes in the pressure and hydration were increased by lowering the external Na concentration. Tobian (16) suggested that the increased intravascular pressure associated with hypertension might be explained by an increase in the hydration of the artery wall and a resultant thicker wall and smaller lumen. The same phenomenon might partly explain the overall decrease and peak in the intravascular pressure during rewarming. Rough calculations support this suggestion:

From Poiseuille's equation, with constant viscosity, length and flow:

$P_1/P_2 = (V_2/V_1)^2$ where: P = pressure gradient down the artery segment, V = lumen volume, and states 1 and 2 refer to the artery before and after the changes in wall water content respectively.

Knowing P_1 from the pressure recordings during rewarming and estimating V_1 and V_2 enables P_2 , the pressure gradient after the wall water change, to be calculated. V_1 was calculated from either:

(a) Poiseuille's equation (knowing P_1 and the flow, and estimating the artery length and viscosity of the Krebs solution), or

(b) the inner radius values in Table I in Chapter II. V_2 was determined from $V_2 - V_1$, the change in lumen volume associated with the changes in artery wall volume resulting from the wall water changes. It was assumed for the calculations that the changes in water content of the rewarmed perfused artery were the same as those determined in the above section for the rewarmed incubated artery. The lumen volume changes were calculated from the wall volume changes by assuming either: (a) the lumen volume increase was 1/2 the size of the wall volume decrease, or (b) the increase in inner radius was the same size as the outer radius decrease.

The overall decrease in the hydration of the rewarming artery could account for 80% of the overall decrease in the intravascular pressure. The increase in the water content at 30°C could account for about 30% of the observed increase in the pressure at 20°C to 25°C. These estimates, as well as the correlation between their temperature dependencies and external Na concentration dependencies, indicate that the intravascular pressure changes can be at least partially explained in terms of the hydration changes of the rewarming artery.

There is no comparable correlation between the dip in pressure at 8°C and the water content. The dip may have been part of the overall pressure decrease and only appeared because of the subsequent peak in the intravascular pressure. It is also possible that the pressure dip was simply due to the artery wall volume being smallest at 4°C when the density of water is greatest.

(d) Summary

1. The perfused rewarmed rat tail artery showed: (a) a slight dip in the intravascular pressure at 8°C, (b) a peak in the pressure at 20° to 25°C, and (c) an overall decrease in the pressure between 2° and 37°C.
2. When the external Na concentration was lower: (a) the dip in the pressure was not affected, (b) the peak in the pressure was greater, and (c) the overall decrease in the intravascular pressure was greater.
3. The shape and size of the pressure peak at 20° to 25°C was strongly affected by the length of time the artery was kept at a given temperature.
4. The cellular metabolic Na-K pump cannot explain the increase in pressure at 20°C to 25°C, and can only partly explain the overall decrease in the intravascular pressure.

5. There seems to be a link between the intravascular pressure changes and the hydration changes during rewarming. Both have the same temperature and external Na concentration dependencies.

6. On the basis of a smaller artery wall and a larger lumen, the overall decrease of wall water could account for most of the overall decrease in the intravascular pressure during rewarming. The increased water content at 30°C could account for only part of the increased intravascular pressure at 20° to 25°C.

CHAPTER VI

SUMMARY AND DISCUSSION

1. Extracellular fluid is expelled from the constricting artery wall.

The following points support this conclusion:

- (a) Constricted arteries lost 16% of their water, 15% of their Na, and 17% of their Cl.
- (b) Constricted arteries had smaller inulin spaces.
- (c) Constricted arteries had a smaller wall cross-sectional area than non-constricted arteries. Constant length meant the volume of the constricting artery wall decreased by 14%-- compared to 13% calculated from the wall water loss.
- (d) The flow pattern of a constricted artery suggested that fluid was added to the lumen from the constricting wall.

2. Contracting smooth muscle cells increase their volume due to the entry of water.

3. The artery wall is permeable to fluids. The permeability is increased by an increase in intravascular pressure. Vasoconstriction also increases the permeability with or without a short lived initial decrease.

4. Isosmotic alterations in external ion composition alter the hydration of the artery wall presumably by ion exchange and/or vasoconstriction.

5. The hydration of rewarming arteries depends on the temperature and the external Na concentration. The extrusion of wall water during rewarming:

- (a) may be related to the extrusion of a fast non-K linked Na component, and
- (b) partly explains the intravascular pressure changes during rewarming.

These studies have demonstrated that constriction of the rat tail artery is associated with a loss of water, a change in wall permeability, and an increase in smooth muscle cell volume. How do these changes occur? Are there causal relationships between these 3 effects of vasoconstriction? What are the consequences of these alterations? Although no definite answers can be given, some possible explanations will be examined.

A. LOSS OF WALL WATER DURING VASOCONSTRICTION

The loss of water from the constricting rat tail artery was due to the expulsion of extracellular fluid. Similar losses were observed for contracting gels (1,2), muscle homogenates (3), frog stomach muscle (4,5), uterine muscle (6,7), rat aorta (8), and carotid arteries (9). To understand why this water loss occurred, it may be useful to extend Bozler's explanation of frog stomach muscle behavior to the artery wall: it behaves "like a cross-linked gel in which osmotic balance is determined in part by hydrostatic pressure arising from elastic forces within the fibers" (4).

Water moves down its chemical potential gradient. A movement of water in the absence of gravitational forces, indicates the presence of osmotic or hydrostatic pressure gradients. It is difficult to imagine why ions or other molecules would leave the artery wall first, causing a subsequent water shift to balance osmolarity. In addition, the fact that the fluid which left the constricting artery was not too hypotonic suggests that osmotic forces were probably not involved. If this were so, then how could constriction be associated with a hydrostatic pressure difference forcing water from the artery wall? To attempt to answer this question, the forces that normally determine the arterial water content will first be considered; then how vasoconstriction could alter these forces.

When the arterial hydration is in equilibrium, the osmotic and hydrostatic forces determining the hydration will be balanced. The forces involved are essentially those for capillary filtration. There must be a balance between the osmotic pressure of the blood and the hydrostatic pressure of the artery wall fluid on one hand, and the osmotic pressure of the wall fluid and the hydrostatic pressure of the perfusing blood on the other. There must be a similar equilibrium between the wall and the surrounding tissues: the tissue osmotic and the wall hydrostatic pressures must balance the wall osmotic and the tissue hydrostatic pressures. The consideration of these 2 balance sheets leads to some interesting conclusions.

The only complex factor is the hydrostatic pressure in the artery wall. This pressure is a consequence of the tension in the wall, active and elastic, which balances the transmural pressure (10). For a gel or an ion exchange resin this relationship can be easily seen. As the resin swells, the elastic forces between the components of its matrix increase until the resultant hydrostatic pressure in the resin balances the pressure in the surroundings and equilibrium is attained (11,12). In the artery wall the hydrostatic pressure is a complex function of the wall tension (13). Essentially, the tissue pressure decreases from the level of the intravascular pressure to that in the tissues surrounding the artery. [The claim that the interstitial fluid pressure is negative (14,15) has been fairly well disputed (16-19).] An increase in tissue pressure from the adventitial to the intimal layers of the aorta was observed by Brinkman et al. (20). This tissue pressure gradient apparently determines the extent of penetration of vasa vasorum (21) and the direction of net transport of ions across the vascular wall (21,22).

To prevent water movements in the presence of the tissue pressure gradient across the artery wall, there must also be an osmotic gradient across the wall. These gradients are shown in Fig. 26. For a steady state of the water in the wall, lumen and surrounding tissues, the situation might resemble the following. If it is assumed that the endothelial layer is freely permeable to ions and small molecules but not to the plasma proteins, then the osmotic pressure in the lumen is about 25 mm Hg higher than that in the wall. (The vascular smooth muscle cells will be temporarily ignored.) To prevent water leaving the artery wall there must be a lower tissue pressure in the innermost layer of the wall. For an intravascular pressure of 90 mm Hg, the tissue pressure need only be 65 mm Hg just inside the wall. [This point was ignored in Burton's consideration of wall tissue pressure (13).] If, in the artery wall fluid there are soluble molecules which cannot enter the lumen, then the discontinuities in hydrostatic pressure and osmolarity at the lumen-wall interface would be smaller. In the wall layer adjacent to the innermost layer, the tissue pressure must be even lower (see Fig. 26). If the chemical potential of water in these 2 layers were equal, the osmolarity of this adjacent layer must also be lower. This decrease in tissue pressure and in osmolarity continues to the adventitial surface where the tissue pressure equals that of the surrounding fluid. Since the manner in which the tissue pressure decreases across the wall depends on the tension in the different wall layers (13), the same will be true for the osmotic decrease. It should be noted that the exact shape of the transmural pressure and osmolarity gradients are not known and apparently are quite complex (see 13). If the innermost layer had a tissue pressure of 65 mm Hg and the outer layer was at 5 mm Hg, the overall decrease in concentration of the wall fluid across the wall will be about 3 mM. This

means that the wall fluid is more dilute in the loose adventitial layers than in the inner layers of the artery wall.

How is this situation altered when, in response to nervous or humoral influences, the artery constricts? The active tension in the wall increases and the elastic tension is decreased so that the total tangential wall tension, T , balances the transmural pressure, P , according to the law of Laplace: $T = P r$ (see 10,13,23,24,25). The changes during vasoconstriction with a constant transmural pressure are straight forward and have been described by Burton (10). However, when the intravascular pressure increases, the elastic tension must adjust to both the decreasing radius and the increasing transmural pressure in order that the sum of the active and elastic tensions balances the pressure. It is possible that during constriction, the actions of the increasing active tension and the decreasing elastic tension might cause an increased hydrostatic pressure in the wall, upsetting the pressure balance with the lumen. Such an increase could result in a movement of fluid out of the constricting artery wall. Some of the factors which might produce a pressure imbalance are: different rates of development of wall tension changes and intravascular pressure changes, delays in translating active tension into a decreasing radius, or delays in adjusting the elastic tension to the active tension changes. Since a water loss would affect the size of the artery wall, the volume of the lumen and thus the intravascular pressure, the movement of the water itself might act to correct the pressure imbalance. A reversal of the imbalance would cause fluid to return to the wall. Although this discussion is totally conjecture, some changes of this sort are required to explain the loss of wall water during vasoconstriction.

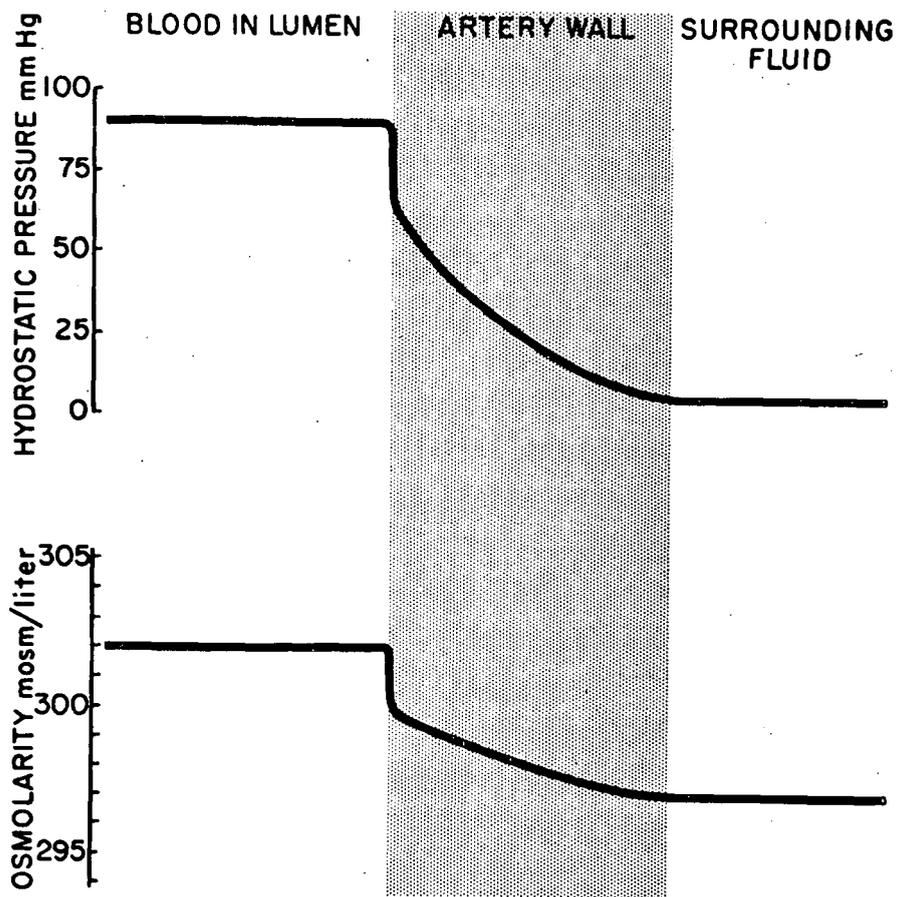


Fig. 26 Schematic representation of the hydrostatic pressure and the osmolarity across the wall of a distributing artery. The numerical values chosen are only for demonstration of the profiles.

Imbalances between the artery wall and the surrounding tissues are probably not too important. Van Citters et al. noted that in the constricted femoral artery, the "smooth muscle cells adjacent to the elastic membrane were most severely deformed, and there was a diminution in cytoarchitectural alterations as the adventitia was reached" (26). The same phenomenon was noted in the rat tail artery. This suggests that not only the tissue pressure but also the active tension changes are greatest in the inner layers of the wall.

The law of Laplace was used in the above discussion of pressure-tension relations and not the equation suggested by Peterson (27) which includes the wall thickness: $T = P r / \delta$. This latter equation really describes the stress on the vessel wall, not its tangential tension, and was put forward by Frank in 1920 (21,28). Many authors ignore both this difference and the fact that tension is in dynes/cm while stress, Peterson's "tension", is in dynes/cm².

The important role played by the law of Laplace in arterial behavior can be seen in the effects of altering the pressure in tissues surrounding arteries. This alters the transmural pressure and thus the tension in the artery wall. Altered hydrodynamics in vascular beds have been observed during elevated ureteral pressure (29), altered cerebrospinal fluid pressure (30), and striated muscle contraction (31-34).

Studies to test the effect of the arterial pulse pressure have established that the artery is incompressible when subjected to small strains (35,36,37, compare 38). This means that the ratio of transverse to longitudinal strain, Poisson's ratio, is 0.5 (36). [This may not be strictly

true for large strains (39).] It is incorrectly assumed that the same isovolumetric situation applies during vasoconstriction (25). However, passive stretching, by applying a load or pressure, occurs without the active tension and expenditure of energy of vasoconstriction (23). External forces are required to stretch the artery, while the forces involved in constriction arise within the artery itself.

Could the loss of water from the constricting artery wall have an effect on the visco-elastic properties of the constricted artery? Stretching arteries decreases their distensibility (23,40). On the other hand, it has been reported that constriction increases (23,25,41-44), decreases (27,45-48), or does not change (49) arterial distensibility. Apparently, the findings depend on the degree to which the arteries are stretched (49). [The elastic modulus used for these studies may be a deceptive index of wall extensibility for large strains (50) as are involved in vasoconstriction.] The viscosity of the constricted artery is apparently increased (27,51). None of these studies considered the water content of the arteries. It is possible that the loss of extracellular fluid during vasoconstriction may play a role in these physical changes.

There is some indication that in states of the artery other than constriction, hydration changes are associated with physical changes. Aortic strips immersed in hypertonic saline undergo a great increase in arterial viscosity (52,53). It is known that age (54), hypertension (55), and increased distance from the heart (56,57) result in stiffer arteries. In addition to changes in content and composition of the wall solids (58,59, 60), there are hydration changes associated with these arteries (55,61,62).

It is possible that interrelationships exist between the structural and hydration changes and the observed physical changes of these arteries.

One interesting aspect of extracellular vascular water changes is the presence of negatively charged mucopolysaccharides in the paracellular matrix of extracellular solids (see 63). These mucopolysaccharides bind water (21,58,64), and ions (63,65-68), and form complexes with extracellular proteins (58,63). The present study suggested that exchanging Ca^{++} for Na^+ as the main counter-ions to the anionic groups of the protein-polysaccharides resulted in conformational changes in the matrix, an increased resistance to perfusion, and a loss of water from the artery wall (see 63,68). Similarly, the rewarming of a cooled artery resulted in a loss of wall water--possibly related to the extrusion of a fast, non-K linked Na component, which may be released during rewarming because of a temperature effect on the binding of Na to the paracellular matrix (67,69,70). These experiments suggest a possible relationship between conformational changes of the paracellular matrix and loss of water from the artery wall. The imbalance of hydrostatic forces, suggested as causing the wall water loss during vasoconstriction, may involve tension developed in the paracellular matrix. The ion exchange and rewarming processes may simply have artificially produced changes in the matrix which normally occur during vasoconstriction. There are 2 possible ways the paracellular matrix could affect the hydration of the artery during constriction:

- (a) The contraction of the vascular smooth muscle cells might compress the matrix, resulting in an expulsion of extracellular fluid from the artery wall.
- (b) The contraction of the smooth muscle cells might be accompanied by changes in the matrix counter-ions. This would alter the charge density of the

matrix, perhaps resulting in shrinkage of the matrix and expulsion of extracellular fluid.

These 2 explanations could be combined if conformational changes in the matrix during vasoconstriction altered the charge density or counter-ion selectivity of the paracellular matrix, causing shrinkage of the matrix and fluid expulsion.

Other authors have mentioned the possible role of the mucopolysaccharides in arterial hydration: Bader noted that "the ground substance has the properties of a colloid--it is water insoluble, but can bind water. It consists of mucopolysaccharides ... is a very viscous material and it probably contributes to the typical visco-elastic behavior of distensible vessels" (21). In his study of a combined structure of hyaluronic acid, water and collagen fibers which had a definite resistance to compression, Fessler suggested that mucopolysaccharides have a mechanical function (71). Frasher commented that "it seems reasonable to assume that [the ground substance] is involved in changes in water content of the wall and also that its physical state may determine the mechanical resultant of the linkages of the other components" (52). Zugibe and Brown demonstrated a tight band of acid mucopolysaccharides in the subendothelial layer of the aorta (72). It is interesting that in this layer the wall hydrostatic pressure is largest (13) and most of the tension is developed during vasoconstriction (26).

Further studies which could be performed to demonstrate and explain the loss of wall water during vasoconstriction include:

1. measuring the density of relaxed and constricted arteries by dropping arterial segments into a series of solutions with densities between 1.05 and 1.08 g/cc (see 73).
2. analysis of the water content of incubated arteries every 30 seconds throughout constriction and relaxation.
3. examination of a relationship between the wall water loss and the extent of vasoconstriction by varying the doses of vasoconstrictive agents over wide ranges.
4. determination of inner and outer wall radii, transmural pressure and water content during vasoconstriction in order that the average wall tension (see 25) can be related to the water lost from the constricting artery wall.
5. isolation of arterial protein-polysaccharides for a study of the relation between their hydration and visco-elastic properties (see 74).

B. ALTERED VASCULAR PERMEABILITY DURING VASOCONSTRICTION

The wall of the rat tail artery was quite permeable to fluid. Increased intravascular pressure increased the permeability as did vasoconstriction with or without an initial short-lived decrease. The permeability of the artery wall will first be considered, then the effects of intravascular pressure and vasoconstriction.

It is usually assumed that all fluid exchange between blood and the tissues occurs across the capillaries. However, there are some indications that larger vessels are permeable. Zweifach has commented that "there is good evidence that movement of gases, water and small water-soluble

molecules occurs even across the walls of terminal arterioles and pre-capillaries" (75). Jennings noted that "even particles seem to be able to leave normal blood vessels" (76). Wilens and McClusky observed the passage of blood serum through excised iliac arteries and veins (77), although the vessels may not have been viable. Sawyer and Valmont observed a net transport of Na and Cl from the inside to the outside of the aorta and in the opposite direction for the vena cava (22). Water can be transported across the artery wall by electro-osmosis (78). It was suggested that "both ion and water flow take place largely through [the] extracellular spaces (pores), which are the source of least resistance to water and ion movements" (78).

While it is possible that the large transmural fluid movements observed for the rat tail artery occur in vivo, this seems unlikely. The permeability of arterial and capillary endothelium are probably not the same. Duff found that dyes entered large arteries because the wall of the vasa vasorum was more permeable than the intima of the large blood vessels (79). In addition, the removal or separation of the rat tail arteries from their surroundings meant that the physical equilibrium of the in vivo situation was no longer present. Large fluid movements across the artery wall would certainly affect the hydrostatic and osmotic pressure balance described above. It is also possible that the large vascular permeability was due to anoxia, which is known to increase capillary permeability (80). However, the presence of the normal pattern of norepinephrine induced constrictions in the arteries argues against this (see 81). In any case, the significant finding was that fluid could pass freely through the rat tail artery wall--the direction of flow depending on the experimental conditions.

Arterial permeability is increased in hypertension (82,83), inflammation (84), and treatment with histamine or serotonin (85,86). It has been suggested that the areas of blood vessels which readily become sites of arteriosclerosis have, even under normal conditions, a higher permeability than other parts of the vessel (87).

It is to be expected that increased intravascular pressure caused an increase in flow out of the lumen through the wall of the perfused rat tail artery. Landis showed that an increase in capillary pressure increased filtration (88,89) as suggested by Starling (90). This increased permeability may be related to openings in the capillary wall which close when the pressure decreases (88). Increased intravascular pressure increased the passage of blood serum through the vascular wall, although the pressure had no effect when the vessel was not allowed to dilate (77). This means that strain, not pressure, determines the permeability of the vascular wall. Stretch alone has been shown to increase the permeability of gelatin films to hemoglobin (91) and the absorption of dyes by elastic membranes (92).

The effect of constriction on arterial permeability has not been considered. There is, however, some information on the effect of vasoconstriction upon capillary filtration and upon the whole circulatory system where the effects were assumed to have occurred at the capillaries. Renkin observed that for a given blood flow, the clearance of test molecules was much smaller during vasoconstriction (93). It is also known that prolonged intravenous administration of catecholamines can deplete the blood volume (94-97, compare 98). In their studies on shock and reduced blood volume, Freeman et al. commented that "capillary permeability is probably increased during vasoconstriction produced by adrenaline" (95). However, Haddy et al.

suggested that the depletion of blood was instead due to the different effects of adrenaline on veins and arteries so the ratio of these 2 pressures, hence the capillary hydrostatic pressure, is altered, so the tissues gain weight (97). This suggestion may well apply to Renkin's findings. Also, the usefulness of epinephrine in treating edema is not due to the effect of constriction on permeability, but to the reduced blood flow during vasoconstriction (89). This action of vasoactive agents on the capillary pressure thus makes it very difficult to assess any direct effects of constriction on vascular permeability.

However, there is indirect evidence to suggest a relationship between vasoconstriction and altered vascular permeability:

1. Vasopressin (pitressin) is a vasoconstrictor (99) and increases intestinal water absorption (100), the permeability of frog skin (101), and active transport of Na by frog skin or bladder (102).

2. Serotonin is a vasoconstrictor (103) and increases capillary permeability (104).

3. Histamine is a vasodilator of small arterioles and a vasoconstrictor of larger arterioles and small arteries (105) and increases capillary permeability (104).

4. Norepinephrine is a vasoconstrictor (106) and increases liver membrane permeability to K (107).

5. Epinephrine is a constrictor of most vascular smooth muscle (106) and increases the permeability of frog skin to Na (108) and gut to sugars (109).

6. Bradykinin is a vasodilator (110) and increases the permeability of skin blood vessels (111).

In short, many vasoactive agents increase permeability. In light of the findings of the present study, the whole relationship between these two properties should be investigated.

The fact that the changes in permeability during vasoconstriction depended upon the intravascular pressure, suggests that the above analysis for the wall water loss may also apply to this altered permeability situation. Transmural fluid movements were observed. Altered hydrostatic pressures in the wall and lumen of the constricting artery could result in the lumen and extravascular fluid being essentially "connected" so that the osmotic and hydrostatic pressure differences between them would cause fluid to move through the wall. In addition, the extracellular solids undergo conformational changes during vasoconstriction. These changes would alter the path of flow (i.e. the extracellular space) across the constricting artery wall and would affect vascular permeability. It was pointed out above that pressure alterations during constriction could cause the wall water loss which in turn would decrease the intravascular pressure. There may be a similar system operating here. Alterations in the transmural balance of osmotic and hydrostatic pressures result in altered wall permeability, which in turn affects the osmotic and hydrostatic pressures developed within the artery wall. That is, an increased permeability would decrease the pressure gradients, the alterations in which were the cause of the increased permeability. The explanations for the altered permeability could consequently be turned around: an increase in the path for flow after configurational changes could cause transmural fluid movements which would upset the osmotic and hydrostatic pressures across the artery wall

resulting in an expulsion of extracellular fluid. It is interesting that Brinkman et al. found that X-irradiation caused a decrease in injection pressure i.e. an increase in water permeability, in the wall of the aorta due to "a slight depolymerization of the mucopolysaccharide connective-tissue matrix" (20,112). Measurements of D₂O flux across the constricting artery wall (see 113) with controlled intra- and extravascular pressures could supply some data to test these speculations.

C. INCREASED SMOOTH MUSCLE CELL VOLUME DURING VASOCONSTRICTION

The geometrical and histological studies of Chapter II showed that the arterial smooth muscle cell can be represented as a double cone which had a constant surface area and an increasing volume, due to the entry of water, during vasoconstriction. This finding agrees with the cell volume increases observed during vasoconstriction (114,115,116) and with the effects of anisotonic solutions: vascular smooth muscle cells swell and contract in hypotonic solutions (116) and shrink and relax in hypertonic solutions (117).

It is generally assumed that cells have the same osmolarity as the fluid which surrounds them (see 118 for a full discussion). However, this might not be so in the artery wall if the intracellular tension caused a hydrostatic pressure difference across the smooth muscle cell membrane. In addition, the pressure and osmotic gradient across the artery wall might mean that the smooth muscle cells in the different layers of the media have different osmolarities and hydrostatic pressures, and hence different tensions. This might be related to the fact that the inner smooth muscle cells undergo the most drastic contractions during vasoconstriction (26).

Movement of water into the arterial smooth muscle cells means that one or more of the following occurred during constriction: (a) cellular osmolarity increased, (b) extracellular space (ECS) osmolarity decreased, (c) cellular hydrostatic pressure decreased or (d) ECS hydrostatic pressure increased. The explanation chosen must not conflict with the fact that extracellular fluid was expelled from the constricting artery wall, although of course, the cellular swelling may not have occurred at the same time as the ECS fluid loss. Ion movements, tension changes and permeability changes could all have caused these proposed osmotic and hydrostatic pressure changes.

The only explanation which can be ruled out is (c): a decrease in cell hydrostatic pressure is unlikely since the tension within the contracting cell would increase. Jonsson suggested that the increase in active tension in the smooth muscle cells of the constricting rat portal vein does not generate an intracellular hydrostatic pressure large enough to filter fluid out of the cells (116). While this may be true, the increase in extracellular hydrostatic pressure may have balanced some of the cellular increase. Of the other 3 explanations, there is little or no data available either to refute them or enable a choice to be made between them:

(a) An increase in cellular osmolarity could be caused by a net gain in cellular ions, by bound cellular ions becoming free, or by free cell water becoming bound during contraction. Nothing is known about changing amount of bound and free ions and water in contracting arterial smooth muscle cells. During contraction, K leaves (119), while Na (73) and Ca (120) enter the smooth muscle cells. These ion shifts may not be balanced osmotically, Na may be accompanied by Cl, and an unknown amount of Ca may be

free or bound in the cell. If much of the smooth muscle cell ATP were involved in active muscle contraction, it is possible that the membrane ion pumps could be affected, increasing the permeability of the smooth muscle cell to ions and perhaps water. An increase in cellular osmolarity alone, however, could not explain the expulsion of extracellular fluid from the constricting artery wall.

(b) A decrease in ECS osmolarity could occur if the endothelial permeability were altered, if ECS ions became bound, or if water bound to extracellular macromolecules became free. Alterations in the distance between the fixed charges on the extracellular mucopolysaccharides (63) during constriction might result in an increase in bound extracellular ions. A decrease in ECS osmolarity would cause water to move from the ECS into the cells, lumen and surrounding tissues to restore osmotic equilibrium. However, it seems unlikely that the expelled ECS fluid would have contained 70 to 100 meq Na/liter if this were the only explanation of the wall water loss.

(d) An increase in extracellular hydrostatic pressure would result when the network of cells and extracellular macromolecules acts to constrict the artery wall. The existence of the transmural pressure gradient (13) means that the increasing intravascular pressure would be accompanied by an increasing wall hydrostatic pressure. Decreasing the endothelial permeability to water would also increase the wall hydrostatic pressure. As with a decreased ECS osmolarity, an increased ECS hydrostatic pressure, resulting in an increased extracellular chemical potential of water, would drive fluid into the smooth muscle cells and into the lumen and surrounding tissues.

No matter what the cause, the increase in cell water during vasoconstriction would decrease cellular ion concentrations. Associated with a loss of cell K and a gain of cell Na, this means the $[K^+]_i/[K^+]_o$ gradient would decrease resulting in depolarization of the vascular smooth muscle cell membrane. The change in Na gradient would depend on the relative sizes of the cell Na and water gains. It is not known whether the water entry is associated with altered cell membrane permeability and thus aids in membrane depolarization or whether the water entry occurs after the initiation of contraction.

The most important conclusion to be drawn from this study is that the hydration of the artery wall cannot be treated as a static component of the wall. In particular, during vasoconstriction, water moves through the artery wall, water enters the smooth muscle cells and water is expelled from the extracellular space.

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APPENDIX I

DIRECT MEASUREMENT OF ARTERY WALL RADII

Determination of the inner and outer radii of the artery wall enables the wall cross-sectional area to be calculated (1,2). To test the assumption of isovolumetric constriction, Wiederhielm's TV scan method (3) was used with a considerably less sophisticated means for displaying the video signal information onto the cathode ray oscilloscope (CRO). A 3 cm length of the rat tail artery was enclosed in a thin plastic chamber while being bathed and perfused with Krebs solution. Using a TV camera - microscope assembly, the artery was displayed on a TV monitor. The voltage from 1 line on the screen was then displayed on the CRO. This voltage display represents the artery dimensions. This experiment was terminated when accurate measurements of the relaxed and constricted artery dimensions could not be made. The essential problem was magnification vs field of view. The necessity of including the whole wall meant that the magnifications used were too low for determination of small dimensional changes.

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APPENDIX II

SPIRAL ANGLE OF ARTERIAL SMOOTH MUSCLE CELLS

Although the smooth muscle spiral angle has been measured in various arteries (1-6), very little is known about the spiral angle during vasoconstriction. Longitudinal sections of the rat tail artery were used:

(a) Epon sections, cut 1.5 μ thick, were stained with toluidine blue,
 (b) freeze-substituted arteries with known state of constriction (see Chapter II), were embedded in paraffin, sectioned and stained with Mallory trichrome. The smooth muscle cells in the relaxed arteries were at 90° to the artery axis, except cells near the adventitia which were at 70° to 80° to the axis. The cells in the constricted arteries were similar except the outer cells deviated even further from 90°. Also, some groups of cells near the lumen were at 30° and 160° to the axis. In short, no one spiral angle could be assigned to these vascular smooth muscle cells and the cell arrangement was more random in constricted than in relaxed arteries.

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APPENDIX III

WEIGHING AN ARTERY WITH AN ELECTROBALANCE

The rat tail artery was suspended in Krebs solution from a Cahn electrobalance (see 1). The change in artery wall volume, ΔV_w , during constriction is:

$$\Delta V_w = \Delta W_s / \rho_f - \rho_s \dots\dots\dots(1)$$

where: ΔW_s = change during constriction of the recorded weight of the system, ρ_f = density of the fluid leaving the artery, and ρ_s = density of the solution in which the system is suspended. Although weights were recorded for the rat tail artery, it never proved possible to add a vasoconstrictive agent such as norepinephrine to the solution without considerably disturbing the weighing. Dripping solution down the artery suspended in air proved equally unsatisfactory. Consequently, this experiment was terminated.

Derivation of equation (1):

The system is the artery, the suspending wire and a small weight. For W_a = weight of system in air, and W_s = weight of system in solution, the buoyancy, B , is the weight of solution displaced by the system: $B = W_a - W_s$. So, $\Delta B = \Delta W_a - \Delta W_s$ during constriction. Let V = volume of displaced solution, i.e. $V = B/\rho_s$, so $\Delta B = \rho_s \Delta V$. Thus, $\Delta W_s = \Delta W_a - \rho_s \Delta V$. Since only the artery changes volume during constriction, $\Delta V = \Delta V_w$. The movement of fluid from the wall means $\Delta W_a = \rho_f \Delta V_w$. Substituting these last 2 equations into that for ΔW_s gives equation (1).

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