The Dimensional Characteristics of Smooth Muscle in Rat Blood Vessels
A Computer-Assisted Analysis

Mary E. Todd, Catherine G. Laye, and David N. Osborne
From the Department of Anatomy, The University of British Columbia, Vancouver, British Columbia

SUMMARY. This investigation was undertaken to provide precise information about the dimensional characteristics of vascular smooth muscle cells as related to their paracellular matrix. The representative types of vessels were fixed at the mean blood pressures of adult male Wistar rats. The shapes, positions of the nucleus, linear dimensions, volumes, and orientation within the vessel wall were determined by a computer-assisted reconstruction of the cells from serial sections. Wall-to-lumen and cellular-to-paracellular ratios also were assessed. The smooth muscle cells were elongate, but whereas some are spindle shaped, most are not, and may be shaped like flattened triangles, paddles, boomerangs, or hourglasses, and in addition, any one of these shapes may be forked. The nucleus tended to be in the largest part of the cell, wherever that region occurred. Thus, the majority of the nuclei (61%) were not centrally located, but overlapped the middle and end thirds of the elongate cells. Of the three arteries investigated, the muscular type tail artery had cells with volumes two to three times larger (P < 0.01) than cells in a musculoelastic (femoral) or elastic (mesenteric) artery, and six times larger (P < 0.01) than those of the portal vein. Therefore, the smooth muscle cells of the vein were significantly smaller than those in any artery (P < 0.01). The smooth muscle cells were aligned at a steeper angle in the vessel wall (15° ± 2°) of the muscular artery than in those with more elastic tissue (9° ± 2°), with a higher percentage of circumferential cells in the latter. The wall-to-lumen ratios decreased as the relative amount of paracellular matrix, particularly elastic tissue, increased in the three arteries. Therefore, irregularly shaped cells, with the nucleus in the thickest region, and having characteristic cell volumes depending on the type of vessel, form the vascular smooth muscle tissue. These factors are relevant if stereology, or measuring from two dimensions, is used to estimate size characteristics in cardiovascular disease such as hypertension. In addition, the optimum angle at which vascular strips are cut would vary, for example, when used in testing pharmacological agents. (Circ Res 53: 319-331, 1983)

SMOOTH muscle tissue is believed to consist of spindle-shaped cells with a central nucleus oriented along the long axis of the cell (see reviews by Rhodin, 1980; Gabella, 1981). The orientation of smooth muscle cells in blood vessels is assumed to be spiral or helical, although the results of experiments are more consistent with a circular orientation in some vessels. Knowledge of the shape and orientation of smooth muscle cells in blood vessels is important in interpreting normal and pathological pressures in blood vessels, as well as tension in strip preparations of vessel wall.

We have developed techniques to provide definitive information about the size, shape, and orientation of smooth muscle cells in blood vessels. Serial sections of vessels are analyzed with the assistance of a computer and a three-dimensional reconstruction of individual smooth muscle cells is created.

In our preliminary studies on the rat tail artery, we have reported (Todd and Green, 1981) that the shape and orientation of the smooth muscle cells and the orientation of the nuclei show considerable diversity, and vary with age. We now present a more complete, comparative analysis of the size, shape, and orientation of smooth muscle cells in adult rat blood vessels, fixed at the animals' mean blood pressures. A muscular artery, a musculoelastic artery, an elastic artery, and a large vein are represented. In this report, we discuss the importance of this information in the interpretation of morphology and pathology, as well as in physiological and pharmacological experimentation.

Methods
Vascular tissue was obtained from inbred male Wistar rats from a breeding colony maintained in the Department of Anatomy. The animals were housed (two per cage) in a controlled environment after weaning at 4 weeks of age and were given water and Purina Laboratory Chow ad libitum. All animals used in this investigation were 16 weeks old.

Fixation and Processing
The method of fixation by perfusion was adapted from earlier studies (Todd and Friedman, 1972). The fixative was 3% glutaraldehyde/2% paraformaldehyde (after
Karnovsky, 1965) in a glucose containing Krebs-Henseleit solution (Palay, 1971) at pH 7.3. The animal was main-
tained on a heated table throughout the procedure under ether anesthesia.

A cannula was inserted into the left common carotid artery. Pang and Scott (1980) found that this method did not cause any alteration in the systolic pressure, as re-
corded by the tail cuff method or by femoral cannulation. The cannula in the carotid artery was connected with a T-
tube to the perfusion pump and to a pressure transducer. The systolic, diastolic, and mean blood pressure values
were recorded. Perfusion then was started. About 30 seconds after the perfusate started to enter the animal, the
tip of the tail was cut and then the skin of the thighs was reflected and both superficial epigastric veins were cut.
After approximately 2 minutes, clear fixative was passing through these vessels. The pressure was carefully moni-
tored throughout the perfusion period to equal that of the
mean systolic blood pressure, using a continuously vari-
able perfusion pump. A continuous recording was made of
the pressure of the perfusion fluid, and the pump rate was
noted. A total of 50 ml of fixative were perfused, which
took approximately 10 minutes.

A check was made on the perfusion pressure by addi-
tional experiments. The femoral artery was cannulated and hooked to a second transducer to confirm that indeed the
pressure was maintained at that of the animal.

The vessels, the superior mesenteric artery, the portal
vein, the tail artery, and the femoral arteries, then were
excised. They were fixed for a total of 1.5 hours at room
and 0.5 hour at 0°C in the 3% glutaralde-
hyde/1% paraformaldehyde solution. After washing,
the tissues were postfixed with 1% osmium tetroxide in
0.1 N cacodylate buffer for 1.5 hours at 0°C. After en
block staining in saturated aqueous uranyl acetate solu-
tion, the tissues were dehydrated in acetone, and then
infiltrated using the Epon-Araldite mixture of Mollen-
hauer (1964).

The tissues were embedded individually in freshly
made pure Epon-Araldite mixture, and were oriented so
that they would be at right angles to the plane of section-
ing. The resin was polymerized at 60°C for 48 hours, and
the blocks were cured further for 2 days at room temper-

Sectioning

The vessels were precisely aligned perpendicular to
the knife edge so that transverse whole vessel thick sections
could be taken to determine wall-to-lumen ratios. These
sections, 0.5 μm thick, were cut on a Reichert Om U3
ultramicrotome with a glass knife. The sections were
placed on a glass slide, heat fixed, and stained with a 1:1
mixture of 1% azur II and 1% toluidine blue made up in
1% borax.

The block face was then retrimmed to form a mesa so
that two of the four corners were at the tunica media-
 adventitial border, to act as a frame of reference in suc-
cessive photographs for tracing the smooth muscle cells.
Up to 150 cross-sections of each vessel, 0.5 μm thick, were
cut serially. It was essential that the section thicknesses be
accurate and consistent throughout the series. This was
confirmed by the method of Small (Weibel, 1979).

Finally, ultrathin sections (60-80 nm) were cut on the
same microtome with a diamond knife. The sections were
placed on formvar/carbon-coated slot grids, post-stained
in Reynolds' lead citrate stain (Reynolds, 1963) for 6
minutes, then examined and photographed using a Philips
300 electron microscope.

Analyses

Three types of quantitative analyses were used. Com-
puter-based reconstruction of whole smooth muscle cells
provided precise cell volume scores (in μm³) as well as
graphic three-dimensional line drawings of the cell and
nucleus at several orientations. Standard morphometric
and stereological methods using photomicrographs of ves-
sels at the light microscopic and ultrastructural level,
yielded wall-to-lumen ratios and measures of cellular-to-
noncellular composition of the vascular wall. Results are
shown as mean ± SEM. Comparisons were made by Student's t-test.

Computer Imaging

The serial sections were photographed, including the
frame of reference, on a Leitz Orthoplan microscope. A
photographic print showing this frame clearly was out-
tlines on clear acetate film. This template was used to
orient all the other prints so that they had the same
alignment. The acetate film was taped to a digitizer inter-
faced with the University computer, and the first print of
the series was oriented with the frame of reference under
the film. This would be the first print in the series in
which a piece of a particular cell appeared. Using a cursor
(an electronic instrument used to register x and y coordi-
nates on the digitizer), the contours of the section of cell,
and the section of nucleus if present, were defined by a
series of points. These points were transferred directly to
a data file. Each point had either the digit 1, 2, or 3
associated with it by pressing a button on the cursor, and
these digits indicated to the computer whether to draw a
line between points, or to move into another plane, or to
move through the same plane without drawing a line. The
distance between planes was equivalent to the distance
between sections, that is, 0.5 μm, and was in the same
scale as the horizontal distance within sections. Provision
was made to outline the nucleus of any cell so that its
morphology and alignment could be visualized.

The sections containing a particular cell were digitized
accordingly from end to end, and a three-dimensional
image of a series of contours of the cell was displayed on
a graphics terminal. This image could be rotated about
any of the x, y, or z axes, and plotted on a graph-drawing
machine. From these plots, the length, width, orientation,
shape of the cell, and the position of the nucleus could be
seen and measured. Similarly, the contours could be sepa-
rated, for example, to 5 or 10 times the 0.5 μm distance
between contours to aid in visualizing the cell.

Cell Volumes

A supplementary computer program was developed to
determine the volume of a cell from the digitized repre-
sentations of serial sections. This cross-sectional area of
the cell in each section was calculated, and then this area
was multiplied by the thickness of the section (0.5 μm).
The resulting volumes for each section in which the par-
ticular cell appeared were summed to obtain the total
volume of that cell, including estimated volumes for un-
avoidable missing sections.

All cells were chosen randomly, but the random choice
within any one artery was from the inner layers (one quar-
ter of the cells), middle layers (one half) and outer
layers (one quarter). Cells were chosen randomly from the
separate layers of the portal vein. These cells were evaluated as separate groups, but there were no consistent patterns in the sizes or orientation in the samples described in this report, so the results are the pooled samples.

The fact that the sections were 0.5 μm thick and were differentially stained for cellular and noncellular components, permitted an accurate tracing of the cell outline. In the very few cases in which ends of adjacent cells overlapped, the irregularity of the outline suggested what was happening, and continuing to trace always revealed another cell with another nucleus.

Cellular vs. Noncellular Components

Previously described stereological methods for morphometric analyses have been employed for a number of tissues (Weibel, 1969; Eisenberg et al., 1974; Goldstein et al., 1974; Simionescu et al., 1974; Weibel, 1974; Weibel, 1979), including the rat aorta (Gerrity and Cliff, 1975), where the components of the tunica media were quantitatively measured. These methods, which involve point counting by using a sampling grid, were used in the present investigation. Basically, an acetate sheet with a sampling grid was superimposed over randomly generated micrographs. The electronmicrographs were taken at low power (300X). For each vessel studied, at least three nonoverlapping areas of the complete thickness of the muscle layer were prepared for stereological analysis at a final magnification of 2450X. The number of times a point fell on a specific structure, such as either a smooth muscle cell or components of the paracellular matrix, permitted a relative quantitative analysis of cellular versus noncellular components in the different vessels.

Wall:Lumen Ratio

Each blood vessel had a series of 12 complete cross-sections cut, and light photomicrographs were produced for three sections of each vessel sampled from each animal. The MOP-3 electronic planimeter was used to measure the total areas of the tunica media and the lumen. From these, wall:lumen ratios were calculated and related to the size and type of vessel. The accuracy of the area measurements was confirmed mathematically by measuring lumen perimeter (P) to calculate area (area = P²/4π).

Results

We have obtained results from four vessels representing different types from the 16-week-old male rats. The tail artery is a typical muscular artery, similar in its characteristics to other peripheral muscular arteries, such as those in the thigh (Todd and Tokito, 1981a). The femoral artery is of the musculoelastic type and the superior mesenteric artery is an elastic vessel. The portal vein is representative of large veins with two distinct layers of smooth muscle.

The mean blood pressures for all these animals fell within the normal range with a systolic value of 134 ± 3 mm Hg, and a diastolic value of 86 ± 4 mm Hg. The mean values for each animal are included in Table 1, and represent the perfusion pressure at which the vessels were fixed, and therefore fixation was carried out at each animal’s physiological pressure. A typical recording is illustrated in Figure 1.

Shape of the Smooth Muscle Cells

We have found that vascular cells are elongate cells, some are spindle shaped, but many are of other shapes. For example, they may be shaped like flattened triangles, paddles, boomerangs, hour-glasses, and in addition, any one of these shapes may be forked. Nevertheless, any section through any one of these cells would show a more or less fusiform outline as illustrated in Figure 2, particularly in the tail artery. Samples of typical cells traced from such sections are computer plotted and reproduced in Figures 3–5. An attempt was made to classify the cell shapes, but it proved impossible since virtually every cell was unique. The smooth muscle cells from the femoral and mesenteric arteries had the most irregular outlines in the serial

<table>
<thead>
<tr>
<th>Wt. (g)</th>
<th>Blood pressure (mm Hg)</th>
<th>Tail A†</th>
<th>Femoral A†</th>
<th>Mesenteric A†</th>
<th>Portal V†</th>
</tr>
</thead>
<tbody>
<tr>
<td>466</td>
<td>140/75 = 108</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>502</td>
<td>135/90 = 113</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>525</td>
<td>140/65 = 103</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>409</td>
<td>140/95 = 118</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>365</td>
<td>120/95 = 108</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>378</td>
<td>145/95 = 120</td>
<td>4</td>
<td>4</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>356</td>
<td>125/80 = 103</td>
<td>4</td>
<td>4</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>414</td>
<td>130/90 = 110</td>
<td>4</td>
<td>4</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Total n</td>
<td>18</td>
<td>24</td>
<td>20</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

* Average blood pressures: systolic/diastolic = Mean; mean values for all animals: 134 ± 3/86 ± 4 = 110 ± 2.
† In all tables A = artery, v = vein.

Figure 1. A recording of the blood pressure of a male Wistar rat, 16 weeks of age, and the perfusion pressure during fixation of the vessels via a cannula in the left common carotid artery.
sections, and this characteristic was reflected in the computer plotted images (Figs. 4 and 5).

Location of the Nucleus within the Cell

Accepting the elongate, irregular shape of the smooth muscle cells, the nucleus tended to be within the largest part of the cell, whether that be in the middle or at one of the ends (Figs. 4 and 5). The overall longest straight dimension of each cell (Table 2) was divided into thirds, and the nucleus was classified as occupying the end third or the middle third, or overlapping the two. Figure 6 summarizes these results. The trend was similar in each vessel. Therefore, with all the results pooled (n = 74), approximately a third (31%) of the nuclei were centrally located, the majority overlapped between the end and middle regions (61%), and the remainder were wholly within the end third (8%).
When we tried to define the orientation of the nucleus with respect to the long axis of the cell, the same problem arose as when we attempted to classify cell shapes. There was no consistent pattern. The long axis of the nucleus was randomly oriented in relation to the long axis of the cell.

Cell Measurements

The mean dimensions of cells in the various vessels are shown in Table 2, and Table 3 summarizes the statistical comparisons. The cells from the tail and femoral arteries were similar in maximum length and cross-sectional dimensions. Cells from the mesenteric artery were significantly shorter than those from tail and femoral vessels, but similar in cross-sectional dimensions. Cells from the portal vein were significantly less in both linear dimensions than cells from the three arteries.

However, the cell volumes probably provide the most accurate size measurement of any that we have included in this report. Volume estimates presented in Table 2 indicate that cells from the tail artery were on the average two to three times larger than cells from the femoral and mesenteric arteries, and six times larger than cells from the portal vein. Mean volumes of cells from the mesenteric and femoral arteries did not differ significantly, and the cells
from the portal vein were smaller in volume than all the others.

**Orientation of Smooth Muscle Cells**

Aligning vessels at right angles to the plane of sectioning meant that the internal and external elastic laminae were each virtually superimposed in the first and last sections of the series. This fact allowed the orientation of the long axis of the smooth muscle cells to be assessed. It was possible to determine the degree to which cells were circumferential to longitudinal in orientation and whether they had a spiral or helical arrangement in the vascular wall. The measured angles of the cells within the tunica media represent the angle difference from a truly circumferential orientation, that is, zero degrees. The mean values for the angles of the cells are indicated in Table 4.
The pattern was different in the muscular type of artery, the tail artery, than in those with more elastic tissue. Only 6% of the cells in the tail artery were circumferentially oriented, and the mean angle for all cells was $15^\circ \pm 2^\circ$. In contrast, there were 21% and 30% circumferential cells in the femoral and mesenteric arteries, with mean angles in both being $9^\circ \pm 2^\circ$ (Table 4). The only significant difference in these groups was between the angles of the tail and mesenteric artery cells at the 5% level, due to the
TABLE 2
Summary of Results from Computer-Reconstructed Cells

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Tail A</th>
<th>Femoral A</th>
<th>Mesenteric A</th>
<th>Portal V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (μm)</td>
<td>89 ± 7</td>
<td>93 ± 6</td>
<td>61 ± 5</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>X-Sectional Dimension (μm)</td>
<td>21 ± 2</td>
<td>22 ± 3</td>
<td>20 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Volume (μm³)</td>
<td>2505 ± 238</td>
<td>919 ± 76</td>
<td>788 ± 87</td>
<td>399 ± 79</td>
</tr>
<tr>
<td>No. of cells, n</td>
<td>18</td>
<td>24</td>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

* Longest straight dimension
† Maximum length and width of the middle x-section averaged.

See table 1 for abbreviations.

greater proportion of circumferential cells in the latter.

The portal vein cells were sampled primarily to compare their dimensions with those from arteries. In the rat, there are two distinct layers of muscle comprising the tunica media, and cells were sampled from both layers (inner-8, outer-4). The mean value for the angles of inner layer cells was 36° and was 79° for cells from the outer layer. The values from both layers were pooled to give the results recorded in Table 4, which indicates the more longitudinal orientation of the portal vein cells compared with arterial cells.

In those cells positioned at an angle within the vessel wall, the computer-reconstructed image indicated whether the cell was oriented in a clockwise or counterclockwise arrangement. Both of these were found in all vessels, with no consistent pattern emerging with respect to any one vessel. When all cells from all the vessels were compared (n = 74), 45% were in a clockwise orientation, 38% were counterclockwise, and the rest were circumferentially or longitudinally (in the portal vein) oriented (Table 4).

The smooth muscle cells could also be classified according to whether they maintained their relative position within the wall or whether they verged toward either the lumen or adventitia. In other words, the arrangement could be that of a helix, where each turn is equidistant from the center, or that of a spiral, moving either inward or outward, such as in a watch spring. The cells were almost equally divided between these two groups, 53% in spirals and 47% in helices. However, the helical arrangement was weighted by the greater number of circumferential cells in arteries with elastic laminae.

Cellular vs. Intercellular Components

The point-counting method outlined by Weibel (1979) using electronmicrographs was carried out on the three arteries. Representative examples of electronmicrographs from which the counts were made are illustrated in Figure 7. This gave a comparison between a muscular artery with only internal and external elastic laminae, an artery with discontinuous elastic laminae in the tunica media (the femoral), and an artery with a prominent elastic laminar component (the mesenteric). The muscle layers of the vein also were analyzed. The results summarized in Table 5 indicate that the proportion of intercellular material is smallest in the muscular artery (tail) and greatest in the elastic artery (mesenteric). It should be noted that smaller cells, as measured by the cell volume (Table 2), were found in the arteries with the increased paracellular matrix. The muscle layers of the vein were most similar in composition to that of the muscular artery.

TABLE 3
Summary of the Comparisons between Cells from the Different Vessels

<table>
<thead>
<tr>
<th>Vessels</th>
<th>Length (μm)</th>
<th>X-Sectional Dimension (μm)</th>
<th>Volume (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail AVS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Femoral A</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Mesenteric A</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Portal V</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Femoral AVS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mesenteric AVS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Portal</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

NS = not significant.
* Significant P < 0.01.

See table 1 for abbreviations.
Table 4

Summary of Cell Orientation within the Tunica Media

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Tail A</th>
<th>Femoral A</th>
<th>Mesenteric A</th>
<th>Portal V</th>
<th>Mean values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clockwise</td>
<td>61%</td>
<td>17%</td>
<td>50%</td>
<td>67%</td>
<td>45%</td>
</tr>
<tr>
<td>Counterclockwise</td>
<td>33%</td>
<td>62%</td>
<td>20%</td>
<td>25%</td>
<td>38%</td>
</tr>
<tr>
<td>Circumferential</td>
<td>6%</td>
<td>21%</td>
<td>30%</td>
<td></td>
<td>16%</td>
</tr>
<tr>
<td>Longitudinal</td>
<td></td>
<td></td>
<td></td>
<td>8%</td>
<td>1%</td>
</tr>
<tr>
<td>Mean angle in degrees</td>
<td>15 ± 2</td>
<td>9 ± 2</td>
<td>9 ± 2</td>
<td>50 ± 8</td>
<td></td>
</tr>
<tr>
<td>No. of cells, n</td>
<td>18</td>
<td>24</td>
<td>20</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

See table 1 for abbreviations.

Wall-to-Lumen Ratios

Table 6 summarizes the mean values (±SE) of cross-sectional areas of tunica media vs. the lumenal area in the vessels. The figures indicate the greatest tunica media-to-lumen ratio in the tail artery, and the least area of tunica media to lumen in the two arteries with an elastic tissue component. The vein, as would be expected, has relatively the thinnest wall.

Discussion

The present investigation has demonstrated that vascular smooth muscle cells are more heterogeneous than has been previously thought. They are elongate but irregularly shaped cells, varying considerably in size depending on the type of vessel, and with the nucleus of the cell not necessarily centrally located.

The smooth muscle cells often have a spindle-shaped overall appearance in sections. The outline may be more or less irregular, depending on the type of vessel, since elastic arteries have cells with processes that are attached to the matrix (Gabella, 1981). However, spindle- or fusiform-shaped sections do not necessarily add up to a spindle-shaped smooth muscle cell when it is reconstructed using serial sections. Evidence from reconstruction of arteriolar smooth muscle cells supports the irregular shape (Carlson et al., 1982; Komuro et al., 1982).

When the smooth muscle cells are reconstructed by computer imaging, the nucleus is found to be in
the thickest part of the cell, wherever that part is, including at one end. Thus, previously, the supposition had been made that the nucleus was in the center of the cell, based on the assumption that the thickest part of the cell was there. Measurements of cell dimensions based on such criteria (Friedman et al., 1971; Osborne-Pellegrin, 1978) are not supported by our evidence from reconstructed cells.

In addition, the cells vary considerably in size as measured by their volumes. It is unlikely that the nature of this variable in volume could be determined accurately by means of two-dimensional measurements alone. Morphometric techniques which estimate the volume of three-dimensional objects of irregular shape using two-dimensional measurements involve a significant potential for error. Volume estimates on structures that can readily be classified as standard mathematical shapes, such as spheres or cones, can be adequately dealt with using standard morphometric techniques (Weibel, 1979); however, "Mixtures of spheroids, triaxial ellipsoids and bodies of more complex shape are not yet treatable and the prospects are poor..." (Williams, 1977). It should be emphasized that these problems arise because of the irregular shapes of the smooth muscle cells.

For example, Osborne-Pellegrin (1978) measured the cross-sectional areas of nucleus-containing portions of smooth muscle cells from the aorta and renal arteries of the rat. The cells were assessed as being the same size, on the basis of these measurements. In the present investigation using serial sections, if the cells from the tail and femoral arteries are compared, there is no significant difference in the lengths or cross-sectional average dimensions, suggesting cells of the same size. However, with the calculated cell volumes, the average volume for the femoral artery cells was only a third of that for the cells from the tail artery, and therefore they were significantly smaller due to their more irregular outline. This reemphasizes the usefulness of accurate volume determinations in assessing cell size.

In comparing the dimensions of vascular smooth muscle with that in the Taenia coli (Gabella, 1976), the cells in that tissue (under load) were estimated to be 515 μm long with volumes of 3500 μm³. The assumption was made that the cells were of similar lengths and did not fork, which, in fact, was not found to be the situation in the vascular tissue described here. Shorter fiber lengths of 319 to 435 μm were reported by Cooke and Fay (1972), measured in cells dissociated from the rabbit deferens (Merrillees, 1968), the cells in that tissue (under load) were estimated to be 515 μm long with volumes of 3500 μm³. This reemphasizes the usefulness of accurate volume determinations in assessing cell size.

### Table 5

<table>
<thead>
<tr>
<th>Component in volume %</th>
<th>Tail A</th>
<th>Femoral A</th>
<th>Mesenteric A</th>
<th>Portal V</th>
</tr>
</thead>
<tbody>
<tr>
<td>All noncellular</td>
<td>35 ± 1</td>
<td>48 ± 2</td>
<td>69 ± 1</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Elastic</td>
<td>4 ± 1</td>
<td>18 ± 1</td>
<td></td>
<td>66 ± 2</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>65 ± 1</td>
<td>52 ± 2</td>
<td>31 ± 1</td>
<td></td>
</tr>
<tr>
<td>Percentage cell/noncell</td>
<td>186</td>
<td>108</td>
<td>45</td>
<td>257</td>
</tr>
<tr>
<td>Percentage elastic/cell</td>
<td>8</td>
<td>38</td>
<td></td>
<td>197</td>
</tr>
<tr>
<td>No of samples, n</td>
<td>21</td>
<td>23</td>
<td>22</td>
<td>27</td>
</tr>
</tbody>
</table>

Each artery is significantly different from the other two (with respect to the noncellular component, the elastic component and the muscle component) at P < 0.01 level. The proportion of muscle in the layers of the portal vein is not significantly different at the 0.01 level from that of the tail artery, but is when compared with the other two arteries. There were no significant differences between the two layers of the portal vein.

* A minimum of three nonoverlapping areas of the muscle layer were analyzed for each vessel.

See table 1 for abbreviations.

### Table 6

<table>
<thead>
<tr>
<th>Area in μm² × 10⁴</th>
<th>Tail A</th>
<th>Femoral A</th>
<th>Mesenteric A</th>
<th>Portal V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunica media</td>
<td>6.8 ± 0.6</td>
<td>6.2 ± 0.2</td>
<td>15.8 ± 0.8</td>
<td>20.9 ± 1.6</td>
</tr>
<tr>
<td>Lumen</td>
<td>11.3 ± 1.4</td>
<td>53.4 ± 2.7</td>
<td>85.1 ± 1.9</td>
<td>236.9 ± 39.5</td>
</tr>
<tr>
<td>Mean W:L ratios</td>
<td>66.8 ± 8.9</td>
<td>12.4 ± 0.9</td>
<td>18.6 ± 0.9</td>
<td>9.5 ± 1.0</td>
</tr>
<tr>
<td>x-sections of vessels, n</td>
<td>7</td>
<td>14 (Left and right) 9</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Each of the arteries is significantly different from the other two at P < 0.01 level.

* Each value was the average of three separate sections from each vessel.

See table 1 for abbreviations.
was stretched to approximately 1.75 times, or there may also be a species difference. Estimated cell lengths of 92 to 161 \( \mu m \) in a muscular artery (Friedman et al., 1971) also support the smaller size of vascular cells compared with those of the Taenia coli and vas deferens. Two reconstructed arteriolar smooth muscle cells were 22 and 70 \( \mu m \) long with volumes of 139 and 750 \( \mu m^3 \), respectively (Komuro et al., 1982). Another study (Carlson et al., 1982) estimated arteriolar muscle cells to reach 50–60 \( \mu m \) in length.

The results obtained from our computer-reconstructed images of smooth muscle cells indicate that the determination of the orientation of the cell within the vessel wall requires more accurate methods than have been used previously. For example, it cannot be adequately assessed on the basis of the cross-sectional vs. longitudinal appearance of cells in arteries (Keech, 1960; Rhodin, 1962, 1967; Osborne-Pellegrin, 1978). Other methods which have been used are scanning electron micrographs (Cope and Roach, 1975) and microdissection techniques (Strong, 1938; Bohr et al., 1962). More recently, using a mathematical modeling technique based on the assumption that the long axis of the nucleus is that of the cell, a circumferential orientation of smooth muscle cells within the vessel wall has been proposed (Canham, 1977; Canham and Mullin, 1978; Walmsley and Canham, 1979; Peters and Canham, 1980; Canham et al., 1982a). We did not find that the nuclear alignment corresponded to the cellular long axis. However, recent work on brain arteries by this group, using a polarizing microscope, does provide evidence for circumferential orientation in those particular vessels (Canham et al., 1982b).

From the multitude of reports, recent reviews (Rhodin, 1980; Gabella, 1981) would suggest that elastic arteries tend to have cells that are circumferentially arranged, and the arrangement tends toward the helical or spiral in muscular arteries. Our results would support this, since the greater the proportion of elastic tissue in the tunica media, the greater the number of circumferentially arranged cells.

The orientation also may be tested by determining the tension-generating capacity of strips cut at various angles (Herlihy, 1980). In these experiments, the circumferential orientation of the aortic cells is convincing. The vascular strip preparation is widely used by physiologists and pharmacologists since it was introduced by Furchgott and Bhadrakom (1953). The orientation of the vascular smooth muscle cells could well be significant if the maximum tension is developed along the long axis of the vascular cells, particularly in view of Herlihy’s (1980) results. It should be stressed that each type of vessel, or even the same type from different locations, must be individually examined using proven techniques.

The amount of extracellular material is relatively sparse in muscular arteries and increases to a maximum in elastic arteries, whereas the wall-to-lumen proportions decrease. However, the change in smooth muscle cell size that also occurs has not been documented previously. Does the increase in extracellular material in prolonged hypertension parallel a decrease in smooth muscle cell size? There is some evidence for this, since Friedman et al. (1971) reported that cells that were hypertrophied in a muscular artery in the rat during acute deoxycorticosterone acetate hypertension, were no longer hypertrophied at a later stage, although the blood pressure was still elevated. The results would suggest that other structural alterations had occurred to maintain the elevated pressure.

The difference in cell size may also be associated with the degree of vasoconstriction in the arteries. The density of the adrenergic endings is greater in the muscular tail artery, and other similar muscular arteries, than in the femoral (Todd, 1980; Todd and Tokito, 1981a). Thus, the design appears to associate a larger cell at a steeper angle with denser innervation. The densely innervated portal vein (Ljung and Stage, 1975), with little paracellular matrix within the muscle layers, has the smallest smooth muscle cells. In this case, the size may be related to the very low pressure within that vessel. In preliminary studies (Todd and Green, 1981), the cells in the tail artery of 3-day-old rats were more circumferential, and smaller than in adult animals, and the subsequent development may parallel the postnatal increase in blood pressure (Gerrity and Cliff, 1975).

The perfusion pressure was not measured on the venous side of the circulatory pathway, but perfusate was allowed to escape to prevent buildup of pressure. For the purposes of this report, we were interested in evaluating any major differences in the dimensional characteristics of smooth muscle cells from veins vs. those from arteries. Unpublished data from this laboratory have compared the dimensional characteristics of both arterial and portal vein smooth muscle cells either after the glutaraldehyde/paraformaldehyde fixation, or by means of an improved permaganate technique (Todd and Tokito, 1981b). The immersion fixation, with the permanganate, preserved cells with characteristics that were essentially similar to those described here.

Therefore, vascular smooth muscle cell size and orientation may be interacting with innervation, blood pressure, paracellular matrix, or a combination of these. Except for orientation, these factors are well known to be altered as the blood pressure is elevated during developing hypertension. However, there is no valid reason at this stage to assume that cell orientation is not changed, nor has this aspect been investigated in hypertension.

The results presented here provide a baseline for further experimental investigation, as for example with volume changes in smooth muscle cells during contraction. The evidence at the moment is controversial, with results indicating an increase (Friedman...
et al., 1971), a decrease (Fay and Delise, 1973; Kominz and Groschel-Stewart, 1973), and no change (Gabella, 1981). Further investigation using our experimental techniques would solve this contradiction.

In terms of physiological and pharmacological experimentation with vascular tissues, perhaps certain results previously obtained and involving the comparison of different vessels may need to be reexamined in view of some of the information presented here. For example, the reactivity to pharmacological agents may be fundamentally influenced by the sizes of smooth muscle cells themselves, in addition to other variables like the density of innervation and the proportion of paracellular matrix.

Therefore, the results of the present investigation have demonstrated some unexpected findings with respect to the vascular smooth muscle cell. The methods described here will permit more accurate estimations of the dimensional characteristics, and, in particular, the morphological changes that develop in vascular smooth muscle cells in cardiovascular diseases such as hypertension.

Supported by the British Columbia Heart Foundation and the Medical Research Council of Canada.

Address for reprints: Dr. Mary E. Todd, Department of Anatomy, University of British Columbia, 2177 Wesbrook Mall, Vancouver, B.C., V6T 1W5, Canada

Received February 28, 1983; accepted for publication June 29, 1983.

References


Furchgott RF, Shadakrom S (1953) Reactions of strips of rabbit aorta to epinephrine, isopropylterenol, sodium nitrite and other drugs. J Pharmacol Exp Ther 108: 129–143


Todd ME, Friedman SM (1972) The ultrastructure of peripheral arteries during the development of DOCA hypertension in the rat. Z Zellforsch 128: 538–554


Circulation Research/Vol. 53, No. 3, September 1983

Downloaded from http://circ.ahajournals.org/ by guest on July 9, 2017
Todd ME, Tokito MK (1981b) Improved ultrastructural detail in tissues fixed with potassium permanganate. Stain Technol 56: 335–342

INDEX TERMS: Blood vessel • Artery • Vein • Smooth muscle • Morphometry
The dimensional characteristics of smooth muscle in rat blood vessels. A computer-assisted analysis.

M E Todd, C G Laye and D N Osborne

Circ Res. 1983;53:319-331
doi: 10.1161/01.RES.53.3.319

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/53/3/319

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/