Proliferation of Smooth Muscle Cells at Sites Distant from Vascular Injury

Michael A. Reidy

This study investigated the phenomenon that injury at one specific site in blood vessels induces cell replication at distant vascular sites. A polyethylene tube was inserted via the common carotid into rat aortic arch, which caused focal endothelial cell loss and formation of platelet thrombi. In a similar fashion, a polyethylene tube was placed into the lower abdominal aorta via a femoral artery. All animals received $^{3}$H-thymidine continuously for 2 weeks, after which time segments of the aorta distant from the polyethylene tubing were processed for autoradiography. These sites showed no loss of endothelium or adherent platelets, and yet the smooth muscle and endothelial cell replications were significantly elevated as compared to control aortas. There was no significant change in blood pressure during these experiments and no increase in smooth muscle cell ploidy. When the polyethylene tubing was left in situ for 2 months, no increased replication of the smooth muscle cells was observed during the last 2 weeks of the experiment, and at this time the aorta adjacent to the tubing was completely re-endothelialized. Finally, the mitogenic activity of plasma from these animals was tested in vitro. At the time of a significant increase of in vivo cell replication (2 weeks), the mitogenic activity of the plasma from animals with the indwelling tubing was similar to that of the control animals. In summary, these data show that injury at one discrete arterial site leads to general cell proliferation in the same vessel, and the data would support the possibility that cell communication initiates this response. (Arteriosclerosis 10:298-305, March/April 1990)

There is a considerable body of data that supports the concept that injury to vessel walls induces a proliferative response by those vascular smooth muscle cells at the site of injury. For example, direct mechanical injury to a vessel can induce up to a 400-fold increase in the replication rate of smooth muscle cells. A common assumption has always been that this replicative response is limited to the zone of injury and that those uninjured areas in the same animal remain unaltered in terms of their replication state. There have been isolated reports, however, which suggest that injury to one area of the vasculature induces a variety of responses at a distant uninjured site. An example of such a finding comes from the recent study by Hollenberg and Odori where they showed that ischemia to the kidney caused a centripetal spread of endothelial cell replication along the ureteric artery. One interesting explanation for this finding was that injury at one site triggers a proliferative response that is transmitted to other adjacent cells, and hence a gradient of cell replication was found along the length of the ureteric artery. An example of a different form of cell communication was reported by Segal and Duling, who found that a focal mechanically induced vasodilation of microvessels caused a propagated dilation of upstream vessels for some considerable distance. These two reports suggest that pathways of communication exist between vascular cells.

In this study, we observed that focal injury to the aorta caused a significant increase in smooth muscle cell replication at distant aortic sites. These sites could be up to several centimeters away from the original injury, and proliferation occurred regardless of whether the injury was made at upstream or downstream sites. No increased replication was observed in these aortas when the original injury had healed. These data suggest that injury and cellular repair at one vascular site can trigger a proliferative response by the smooth muscle cells throughout the vessel, and in the absence of any detectable humoral factors, these findings would support the concept that vascular cells can communicate with each other over relatively large distances.

Methods

Experimental Design

Male Sprague-Dawley rats (350 to 400 g) used in this study were divided into groups as shown in Table 1. Half of the animals had an indwelling catheter placed into their aortas (see below) via either the left carotid artery or the left femoral artery, and the remainder of the animals were used as unmanipulated controls. Smooth muscle cell replication was determined by continuous infusion of $^{3}$H-thymidine for a 2-week period as shown in Table 1. At the end of each experiment, animals were perfusion-fixed with 4% paraformaldehyde, and the entire aorta was...
Table 1. Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Insertion of catheter (weeks)</th>
<th>Thymidine labelling (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>0-2</td>
<td>0-2</td>
</tr>
<tr>
<td>3 weeks delayed</td>
<td>0-3</td>
<td>1-3</td>
</tr>
<tr>
<td>8 weeks</td>
<td>0-8</td>
<td>6-8</td>
</tr>
<tr>
<td>Femoral, 2 weeks</td>
<td>0-2</td>
<td>0-2</td>
</tr>
</tbody>
</table>

Figure 1. A. The position of the polyethylene tubing inserted into the thoracic aorta in the carotid artery. B. The position of the tubing inserted into the abdominal aorta in the femoral artery.

Surgery

Animals were anesthetized with Innovar (0.08 ml/kg body weight), and the left carotid artery was exposed. A known length of polyethylene tubing (PE10) was inserted in an incision in the external carotid artery to the upper thoracic aorta (Figure 1A), and the incision was closed. In one group of animals the polyethylene tubing (PE10) was inserted into the lower abdominal aorta via the left femoral artery (Figure 1B).

Cell Replication

Aortic cell replication was measured by continuously infusing [3H]-thymidine into the animals by using an indwelling osmotic pump for a 14-day period. The osmotic pump (Alzet Company, Palo Alto, CA) was inserted into the abdominal cavity. Sections cut from the fixed aortas were dipped in autoradiographic emulsion (NTB-2 Kodak) and exposed for a 2-week period, after which time they were developed as previously described. A minimum of five profiles was prepared from each block, and the total number of labeled cells was quantitated. In selected groups, segments of thoracic aorta were prepared for en face autoradiography as has been previously described.

Morphology

Animals were sacrificed by an overdose of sodium pentobarbital (i.v.) according to the recommendations of the American Veterinary Medical Association. One hour before death, each animal was injected with Evans blue to delineate those zones of endothelial damage, and after fixation those areas of the aorta in proximity to the PE10 tubing were taken for scanning electron microscopy. The tissue was prepared in a standard fashion as previously described and viewed in a JEOL 35C scanning electron microscope.

Labeling of Platelets with Indium-111

Nine milliliters of rat blood were drawn from donor rats via cardiac puncture into a syringe containing 1 ml of acid-citrate-dextrose (ACD) solution. After gentle mixing, the blood was centrifuged for 10 minutes at 350 g, and the platelet-rich plasma (PRP) was removed from the red cell pellet. The PRP was then centrifuged at 1000 g for an additional 15 minutes to form a platelet pellet; the supernatant was removed and saved. The platelet pellet was washed with Ringer’s-citrate-dextrose (RCD) and was resuspended in 1.5 ml of RCD. A 3 ml sample of this suspension was taken for Coulter cell counting. In the suspension (500 µCi, Amersham, Arlington Heights, IL) was added to the platelet suspension and was incubated for 20 minutes at room temperature. A further 3 ml sample was then taken for counting radioactivity. The 111In-platelet pellet was re-formed by centrifuging the platelet suspension at 1000 g for 15 minutes, and the supernatant was discarded. The platelet pellet was washed with 2 ml of RCD, and the platelets were resuspended in rat plasma. A 3 ml sample of this suspension was removed to determine labeling efficiency (see below). Each rat was injected with 0.5 ml of the 111In-labeled platelets, and 1 hour later a 100 ml blood sample was obtained. These procedures were carried out under ether anesthesia.

Twenty-four hours later, a catheter was placed into the abdominal aorta, the animal was killed, and the vasculature was washed extensively with Ringer’s lactate. The animal was then perfusion-fixed with 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer. Each animal received an injection of Evans blue 1 hour before death. Both carotid arteries were removed, and equal lengths of denuded and endothelialized arteries were counted for 111In activity.

Platelet labeling efficiency was expressed as the proportion of platelet-bound 111In injected into each rat and calculated as follows:

Labeling efficiency of platelets

$$\frac{111\text{In activity in platelet pellet}}{\text{total } 111\text{In activity of PRP}}$$
Throughout the experiments the calculated efficiency was 92 ± 4%. The 111In activity of each carotid was determined as cpm/1 cm length of aorta and was expressed as the amount of increase above control carotid.

Flow Microfluorimetry

Nuclei were prepared for flow cytometry by modification of the procedure of Owens and Schwartz.6 Medial aortic preparations were incubated (37°C) for 1.5 to 2 hours in a collagenase/ elastase solution. The tissue was centrifuged (250 g, 5 minutes) and the cell tissue pellet was suspended in 1 ml of nuclear isolation medium (Tris-buffered isotonic saline [pH 7.0]/0.6% Nonidet P-40/1.0 mM CaCl2/21 mM MgCl2/0.2% bovine serum albumin containing diamidino-phenylindole at 10 g/ml). Nuclei were then syringed three times through a 26-gauge needle to ensure single nuclei. Nuclear yields were approximately 25% as determined by deoxyribonucleic acid (DNA) assay.

Measurement, acquisition, and analysis of DNA content of isolated nuclei were done on an ICP-22 flow cytometer (Ortho Diagnostic, Westwood, MA) interfaced to a PDP 11/03 computer. Diamidino-phenylindole fluorescence was quantitated by using a UGI excitation filter, a TK-400 dichroic mirror, and an LP435 emission filter. Cell cycle compartments were estimated by an adaptation of the method of Dean and Jett,7 including fitting and subtraction of an exponential noise background. Nonlinear least-squares fitting was by the method of Marquardt.6

Microdensitometry

Medial preparations of aortas were minced and digested in a collagenase (170 U/ml) and elastase (15 U/ml) mixture in buffered Waymouth’s media. Individual smooth muscle cells were dissociated from the tissue by incubation at 37°C and periodic pipetting. Suspensions were filtered through 70 m wire mesh centrifuged at 460 g for 7 minutes. The cells were then suspended in 10 ml of phosphate-buffered saline, were centrifuged, and resuspended in 1 ml of phosphate-buffered saline (PBS) to which 3 ml of 100% methanol was slowly added. After centrifugation, the pellet was suspended in 100% methanol, and fixed cells were dropped onto slides for subsequent staining with propidium iodide. The DNA per cell was quantitated by photometric fluorimetry.

3H-Thymidine Incorporation

The mitogenicity of the plasma obtained from animals with an indwelling catheter was quantitated using an in vitro assay. Blood was drawn in ACD (1 ml+9 ml blood) from animals that had an indwelling catheter in place for 2 weeks and was centrifuged at 15 000 g for 15 minutes to obtain platelet-poor plasma (PPP). CaCl2 (1 M added, 1:20) was added; the plasma was allowed to clot and then was recentrifuged at 15 000 g for 30 minutes. The resultant supernatant was filtered through sterile Millipore filters and was stored at −70°C. Similar PPP-derived serum was obtained from control animals.

3T3-D-1 cells were plated in 24-well plates in Dulbecco’s modified Eagle medium (DMEM) + glucose +5% fetal calf serum (FCS). Confluent cells were maintained in carboxy-methyl-Sephadex (CMS) plasma-derived serum (PDS) media, and test substances were added directly to this culture medium. PDS from rats with the indwelling catheter and from control rats was used at 2%, 5%, 10%, and 20%. Bovine serum was used in a similar series of concentrations as a positive standard. CMS-PDS was tested at 2% and 20% as a negative control. All conditions were tested in triplicate wells. Cells were incubated for 20 hours with test substances before 3H-thymidine was added (1 Ci per well in serum-free media). The cells were then left for 2 hours, the tritiated media was aspirated, and the cells were washed twice with cold 5% trichloro-acetic acid (TCA). NaOH (0.8 ml of 0.25 N) was added to each well to dissolve the cells, and plates were left on a rotating shaker for approximately 20 minutes. A 0.6 ml sample from each well was used for quantifying 3H incorporation by using a liquid scintillation counter.

Results

The presence of the catheter in the aortic arch caused endothelial cell loss in areas in contact with the tubing, and 2 weeks after implantation of the catheter, the upper thoracic aorta was still denuded of endothelium, and a large mural thrombus (Figure 2A) consisting mainly of aggregated platelets was present at the distal end of the catheter (Figure 2B). Elsewhere the endothelium appeared normal, and no platelets were found to adhere to the vessel wall. By 8 weeks, the catheter tip was incorporated into the mass of the vessel wall, and the luminal surface was re-endothelialized (Figure 3). No platelets were found in these vessels.

In all experiments, smooth muscle cell replication was determined at four sites along the length of the aorta as shown in Figure 1A, and after 2 weeks, the presence of the indwelling catheter caused a significant increase in the replication rate of medial smooth muscle cells in all segments as compared to control tissue (Table 2). (It should be noted that the thymidine indices presented are the cumulative total of all cell replications over a 2-week period. Single or three pulse of 3H-thymidine over a 24-hour period in the same animals gave thymidine indices of <0.05%.) To avoid any nonspecific effects of surgery, the administration of 3H-thymidine was delayed by 7 days and then administered for 2 weeks; these animals still had a significantly higher smooth muscle cell replication rate than did control animals (Table 2). In animals which had the catheter in place 8 weeks and 3H-thymidine given for the last 2 weeks, there was no significant difference in the smooth muscle cell replication as compared to controls. Finally, when the catheter was placed into the abdominal aorta via the femoral artery (see Figure 1B) for a period of 2 weeks, there was no significant difference in the smooth muscle cell replication rate as compared to control animals (Table 2). In animals which had the catheter in place for 8 weeks and 3H-thymidine given for the last 2 weeks, there was no significant difference in the smooth muscle cell replication as compared to controls. Finally, when the catheter was placed into the abdominal aorta via the femoral artery (see Figure 1B) for a period of 2 weeks, the replication rate of the smooth muscle cells of certain proximal aortic segments was still significantly higher than observed in the controls.

Endothelial cell replication was quantitated in a segment of thoracic aorta (between A and B segments) by en face autoradiography (Table 3). The presence of the indwelling catheter caused a significant increase in endothelial replication as compared to control animals. Interestingly, in animals with an indwelling catheter for
2 months, the endothelial replication measured over the last 2 weeks showed no increase in replication. As mentioned above, at this time the catheter and the proximal aorta were totally re-endothelialized.

To explore the possibility that the presence of the catheter caused an increase in blood pressure that was responsible for induced replication and possibly a change in cell ploidy, the blood pressures of animals with and those without the indwelling catheters were measured throughout the 14-day period. The presence of the indwelling catheter and the chronic injury caused no significant increase in blood pressure (Figure 4), and flow cytometry of dispersed cells from the aortas revealed no significant increase in ploidy (Table 4). The DNA content of individual cells was examined by using microdensitometric techniques, and no change in ploidy was observed in cells that were or were not labeled with $^3$H-thymidine (replicating vs. quiescent cells) (Table 5). Thus the presence of the indwelling catheter did not cause any change in smooth muscle cell ploidy values; this suggests that cells labeled with $^3$H-thymidine progressed through the cell cycle to form daughter cells.

In earlier studies we had observed that the presence of a catheter in an artery caused release of platelet contents (as assessed by indium-111 activity), which then bound to downstream endothelialized sites. One possibility, therefore, is that cell replication might be induced by release of platelet factors. In this study a group of animals had a catheter implanted into the aortic arch as detailed above (Figure 1A), and on Day 14 a bolus of indium-111 rat platelets was injected 2 hours before sacrifice. The indium-111 activity of a segment of thoracic aorta distal to the catheter (see Figure 1A) was counted. Table 6 shows that there was no increase in activity in aortas from animals with the indwelling catheter. As a test of released mitogenic activity, plasma-derived serum was obtained from animals that had an indwelling catheter in place for 2 weeks. At all concentrations, this plasma did not cause an increased uptake of $^3$H-thymidine by 3T3 cells as compared to plasma taken from control animals (Figure 5). Finally, the smooth muscle cell replication was determined in sections of the inferior vena cava. The replication rate of these cells was extremely low, and both in animals with an indwelling catheter and in controls only, the occasional labeled cell was detected. These data were not quantitated due to the low replication rate observed (<0.01%), but it was obvious that the presence of the indwelling catheter did not stimulate any increase in cell proliferation.

**Discussion**

The data from this study show that injury to one focal site of an artery leads to widespread proliferation in the same artery of smooth muscle cells that are distant from the site of injury. Our first concern with this result was that placement of the catheter had somehow inadvertently caused loss of endothelium at these distant sites, initating both endothelial and smooth muscle cell proliferation. This is extremely unlikely because the sites used for this study were deliberately chosen to be at least 1 cm away from the distal tip of the catheter (see Figure 1), and examination by scanning electron microscopy revealed...
Table 2. Cumulative Smooth Muscle Cell Proliferation over a 2-Week Period

<table>
<thead>
<tr>
<th>Experiment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indwelling catheter (2 weeks)</td>
<td>0.79±0.51*</td>
<td>1.9±1.32*</td>
<td>0.78±1.0*</td>
<td>0.51±0.35*</td>
</tr>
<tr>
<td>Control</td>
<td>0.11±0.11</td>
<td>0.18±0.08</td>
<td>0.10±0.12</td>
<td>0.06±0.05</td>
</tr>
<tr>
<td>Indwelling catheter (3 weeks delayed)</td>
<td>0.37±0.15*</td>
<td>1.04±0.48*</td>
<td>0.69±0.55*</td>
<td>0.64±0.43*</td>
</tr>
<tr>
<td>Indwelling catheter (2 months)</td>
<td>0.11±0.12</td>
<td>0.14±0.17</td>
<td>0.08±0.10</td>
<td>0.14±0.06</td>
</tr>
<tr>
<td>Indwelling catheter (femoral, 2 weeks)</td>
<td>0.23±0.21</td>
<td>0.72±0.41*</td>
<td>0.50±0.20*</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are the means of three animals per group and a total of five cross-sections for each site. As described in the Methods section, each animal had continuous ^3H-thymidine delivered by an aortic pump for the last 2 weeks of the experiment. Thymidine indices are the total number of labeled cells expressed as a percentage of the total cell population counted. *p<0.01 vs. control.

Table 3. Endothelial Cell Replication

<table>
<thead>
<tr>
<th>Indwelling catheter</th>
<th>Thymidine index</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>17.3±7.09*</td>
</tr>
<tr>
<td>Control</td>
<td>4.7±1.36</td>
</tr>
<tr>
<td>2 months</td>
<td>6.8±4.63</td>
</tr>
</tbody>
</table>

The thymidine index of endothelial cells is the number of labeled cells/total number of cells counted expressed as a percentage. n=3 for each group, and >150 000 endothelial cells were counted in each preparation. *p<0.01.

Figure 4. Blood pressure of an animal before and after insertion of tubing into the thoracic aorta.

no loss of endothelium nor any adherent platelets at these sites. A further possibility is that surgery and placement of the catheter caused a nondenuding injury to the artery, which triggered cell replication throughout the vessel. For that reason, the administration of ^3H-thymidine was delayed for 7 days, and the animals were killed after a further 14 days. In this way, cell replication possibly induced by the surgery and for the following 7 days would not be detected, and only those cell divisions that took place between Days 7 to 21 would be labeled with ^3H-thymidine. Animals treated in this way showed a similar increase in the smooth muscle cell replication as found in animals where the ^3H-thymidine was administered concurrently with placement of the catheter into the aorta. Likewise, sham-operated animals showed no increase in proliferation (data not shown). Thus we feel that the trauma induced by the surgery was not responsible for the induction of smooth muscle cell replication seen throughout the entire aorta.

There are several features that are somewhat unique to this experiment. The first is that medial smooth muscle proliferation occurred without the subsequent formation of an intimal lesion and that replication occurred in the presence of an intact endothelium. In most models of

Table 4. Flow Cytometric Analysis of Frequency of Polyploid Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>2N (%)</th>
<th>4N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals with indwelling catheter</td>
<td>91.3±2.1</td>
<td>8.7±2.1</td>
</tr>
<tr>
<td>Control animals</td>
<td>93.3±1.5</td>
<td>6.7±1.5</td>
</tr>
</tbody>
</table>

The values are the total number of cells found to be 2N or 4N expressed as a percentage of the total number of cells counted. There were three animals in each group.

Table 5. Microdensitometric Analysis of Frequency of ^3H-Thymidine-labeled Polyploid Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>2N (%)</th>
<th>4N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals with indwelling catheter</td>
<td>74±4.7*</td>
<td>26±4.7*</td>
</tr>
<tr>
<td>Cells not labeled</td>
<td>95±5.2</td>
<td>5±5.1</td>
</tr>
<tr>
<td>Control animals</td>
<td>70±8.0</td>
<td>30±8.0</td>
</tr>
<tr>
<td>Cells labeled</td>
<td>92±3.0</td>
<td>8±3.0</td>
</tr>
</tbody>
</table>

*The values are the total number of cells found to be 2N or 4N expressed as a percentage of the total number of cells counted. Cells were labeled with ^3H-thymidine.

Table 6. Indium Activity of Aorta at Site Distal to Injury

<table>
<thead>
<tr>
<th>Group</th>
<th>Activity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals with indwelling catheter</td>
<td>265.5±90</td>
</tr>
<tr>
<td>Control animals</td>
<td>171.0±43</td>
</tr>
</tbody>
</table>

The values are given as cpm (means±SD). The activity was measured on a 1-cm segment of aorta located between segments A and B. N=6 animals in each group.
induced proliferation, vessel wall injury plus loss of endothelium leads to an increase in cell replication followed approximately 4 days later by a migration of smooth muscle cells into the intima.\(^9,10\) In fact, regardless of the initial rate of this replication, the development of an intimal lesion is a hallmark of vascular injury. Why in this study smooth muscle cell replication occurred without intimal thickening is not clear, but one explanation could be because the integrity of the endothelium was not disturbed. In other studies where the vessel wall has been injured, the continued presence of endothelium or the rapid regrowth of endothelium was thought to inhibit the migration of cells into the intima, despite in some cases a significantly increased medial proliferation.\(^11,12,13\) The data from this experiment would support the hypothesis that loss of endothelium is required for cell migration, but is not necessary for smooth muscle cell replication to occur.

The presence of endothelium also might be important in influencing how the smooth muscle cells replicate. In hypertension, cells incorporate \(^{3}\)H-thymidine but do not divide and instead form polyploid cells.\(^6\) A possibility, therefore, was that the presence of an indwelling catheter caused an increase in blood pressure and that the \(^{3}\)H-thymidine-labeled cells had not divided but were tetraploid. We could detect no change in blood pressure, however, and flow microfluorimetry on cells isolated from the aortas showed no increase in ploidy. The data on ploidy might be inconclusive because in this experiment only a small number of cells replicated in the 2-week period and might not be detected by flow. We therefore measured the DNA content of individual cells from animals with a carboxy-methyl-Sephadex column were used as controls.

\(^{3}\)H-thymidine divided to form daughter cells and were not tetraploid.

One concern raised by this experiment is why, despite numerous studies on the kinetics of vascular cells subjected to injury, we and others have not observed this increase in the proliferation of cells from untraumatized zones of injured animals. Our explanation is because of the increased sensitivity of the method used in this study to detect cell replication. This was achieved by administering \(^{3}\)H-thymidine continuously for the duration of the experiment. In a recent paper we showed that significant increases in the proliferation of vascular smooth muscle cells were detected after a very limited injury when \(^{3}\)H-thymidine was used for labeling during 7 days—increases which we were not able to detect if \(^{3}\)H-thymidine was administered by bolus injection.\(^14\) Thus many past experiments may not have detected these small changes in vascular cell proliferation rates because of the insensitivity of the protocols for \(^{3}\)H-thymidine labeling. It should be noted, however, that Weigensberg\(^15\) did detect an increased aortic DNA synthesis at sites distant from an injury when he used a \(^{3}\)H-thymidine technique different from the one used in this study.\(^16\)

There are several possible mechanisms that might account for the cellular proliferation observed in this experiment. One is that humoral factors might be released from the original injury site both from platelets and from damaged cells\(^17\) to act on the downstream cells. In such a situation, one might have expected the proximal aorta to have a significantly higher proliferation rate than the more distal aortic segments. This was not the case. This explanation is also unlikely because, when the catheter was placed in the abdominal aorta, the upstream cells still showed an increase in proliferation, and no increase in smooth muscle cell proliferation was detected in the vena cava (data not shown). Furthermore, given the size of the original injury and the limited platelet response, it would be difficult to envisage growth factors released in sufficient concentrations to act on arterial smooth muscle cells after passing through the venous and pulmonary system, since it is known that certain platelet mitogens are cleared from plasma in seconds.\(^18\) Also when indium-111-labeled platelets were infused into these animals, no increase in indium-111 activity was detected at sites downstream from the catheter, which suggests that neither platelets nor their released products were present. Perhaps most convincing is the lack of any increased mitogenic activity found in plasma observed from animals with the indwelling catheter. Taken together, these facts would suggest that no mitogenic signal was transmitted via the blood. It is possible, however, that the sensitivity of the techniques used were inadequate to detect the presence of low concentrations of a mitogenic agent, which still could influence arterial cells in vivo.

An attractive explanation for this induction of proliferation is found from the work of Hollenberg and Odori,\(^2\) who observed a centripetal spread of endothelial cell replication along the periureteric artery after renal artery occlusion. The pertinent facts of this study were that an injury appeared to cause proliferation of vascular cells at adja-
cent, but uninjured, sites on the same vessel. Using another in vivo model, Schwartz et al.\textsuperscript{19} noted that injury to rat endothelium resulted in increased replication in cells up to 7 mm away from the original wound edge. A suggestion put forward by these authors was that a signal for replication generated in the injured tissue was communicated to adjacent cells and induced replication along the entire artery. At present, we are not able to validate the presence of this pathway, but we also found an increase in endothelial cell replication at sites distant from injury. Segal and Duling\textsuperscript{20,21} also noted an increase in endothelial cell replication at rates distant from the injury and have recently presented evidence of communication between smooth muscle cells in blood vessels. Furthermore, Larson and Haudenschild\textsuperscript{22} have shown that vascular smooth muscle and endothelial cells have the ability to transfer certain molecules between each other, presumably via gap junctions. Arterial smooth muscle cells are connected to each other via junctional complexes, and transmission of a mitogenic stimulus could possibly occur via this route. Other laboratories and our own have shown that injury can induce smooth muscle cells to express the message for platelet-derived growth factor (PDGF) and even secrete a PDGF-like molecule.\textsuperscript{22-25} These cells also possess the PDGF receptor.\textsuperscript{24} Therefore, smooth muscle cells might be capable of stimulating their own growth via a paracrine or autocrine pathway that involves synthesis and release of this mitogen. Thus, in our experiment the direct injury of the catheter induced focal proliferation, and a signal for replication might then be transmitted to other aortic cells. Such a scheme of events would not only be independent of the direction of blood flow, which would also explain why, when the original wound was healed (2 months), no increased replication was observed at the distant aortic sites. In this situation, the signal for cell replication would be shut down and, therefore, no distant replication would occur.

In summary, this study demonstrates that a focal injury at one vascular site stimulates a proliferation of smooth muscle cells in the same artery at distant sites and that the proliferating cells were not polytrophic. The stimulus for this induced proliferation was not related to any surgical trauma and occurred independent of whether the injury was made upstream or downstream to proliferating cells. Furthermore, proliferation ceased when the initial injury had healed. These data suggest that some form of cellular communication generated by the focal mechanical injury might be responsible for this increased proliferation to the rest of the untraumatized vessel.

Acknowledgment

The authors thank Colleen Irvin for the excellent work on this project.

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growth factor (PDGF) A chain mRNA, secrete a PDGF-
like mitogen and bind exogenous PDGF in a phenotype
106:403

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tured arterial smooth muscle cells accompanies proliferation
after arterial injury. Proc Natl Acad Sci USA 1986;83:7311

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