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# Differential proliferation of rat aortic and mesenteric smooth muscle cells in culture

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**Summary.** Smooth muscle cells (SMC) from various arterial origins have been successfully maintained in culture. The present study evaluates the proliferative activity of aortic and mesenteric SMC in culture. Aortic and mesenteric SMC were obtained from male Wistar rats by explant and enzyme digestion techniques, respectively. Vascular SMC obtained by either method exhibited a characteristic hill-and-valley growth pattern in culture after confluence and were positively labelled with either anti-smooth muscle actin or myosin by an indirect immunofluorescent method. The rate of incorporation of thymidine into DNA and cell number counting were used as indices of proliferation *in vitro*. Vascular SMC from passages 4-33 were first synchronized with either Dullbecco's Modified Eagle's Medium (DME) or Ham's F-12 medium, supplemented with insulin-transferring-selenium (ITS), for 72 hours. SMC were then stimulated with 10% bovine serum for either 24 or 72 hours with the former processed for scintillation counting, the latter for cell number determination. The incorporation of tritiated thymidine into DNA following a 2 hour incubation was determined by scintillation counting after perchloric acid extraction. In terms of cell numbers, proliferative responses to bovine serum were determined by Coulter counting. Autoradiography was also carried out in some cultures to determine both thymidine and mitotic labelling indices. The rate of thymidine incorporation in aortic cells was 2-3 fold higher than in mesenteric cells. Aortic and mesenteric SMC lines exhibited similar cell cycle intervals in terms of total duration and individuals cycle parameters. However, the total thymidine index was higher in the aortic than mesenteric SMC. These results suggest that SMC from different arterial origins possess different rates of proliferation. Differences in the rate of *in vitro* proliferation in these cell lines are due to differences in growth fraction, the number of cells traversing the cell cycle. The mechanisms underlying these differential proliferative potentials remain to be determined.

**Key words:** Vascular smooth muscle, Aorta, Mesenteric arteries, Cell culture, Proliferation, Autoradiography, Cell cycle, Wistar albino rat

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## Introduction

Cultured vascular smooth muscle cells (SMC) have been used as *in vitro* models for investigation of various disease process such as atherosclerosis (Campbell and Campbell, 1985; Ross et al., 1986) and hypertension (Yamori et al., 1981; Grünwald and Mey, 1983; Schwartz, 1984; Haundenschild et al., 1985; Pang, 1989). Methods of isolating and cultivating vascular SMC, and their *in vitro* characteristics have been considered in detail (Chamley-Campbell et al., 1979). In general, vascular SMC are anchorage-dependent in culture forming a hill-and-valley growth pattern after reaching confluence. Cultured vascular SMC are capable of producing collagen, elastin and other matrix components (Ross, 1971). As well, these cell express myofilamentous proteins such as actin and myosin (Chamley-Campbell et al., 1979; Owens et al., 1987; Pang, 1989).

Although SMC have been isolated from various vascular sources and successfully maintained in culture, comparison of their *in vitro* proliferative characteristics have not been documented in detail. The present study examines the proliferative activity of cultured aortic and mesenteric SMC of the rat.

## Materials and methods

### *Experimental animals*

Six male, 12-week old Wistar albino rats (Charles Rivers Breeding laboratories, Quebec) were used in each preparation. A total of three different preparations were compared and results obtained were similar. Rats were allowed a minimum of three days of acclimatization preceeding experiments. They were sacrificed by cervical dislocation following ether inhalation. Vascular SMC were isolated from both the thoracic aorta and mesenteric arterial bed.

### *Source of materials*

Dulbecco's modified Eagle's medium (DME), Ham's F-12 medium, bovine serum (BS), fetal bovine serum, antibiotics (penicillin and streptomycin, P & S), Hank's Balance Salt Solution (HBSS), and plastic culture dishes and flasks were obtained from Gibco Laboratories (Grant Island, NY 14072, U.S.A.). Insulin-transferring-selenium (ITS) was purchased from Collaborative Research, Inc. (Bedford, MA 01730, U.S.A.). Polyclonal antibodies to smooth muscle actin or myosin were products of Biomedical Technologies Inc. (Stoughton, MA 02072, U.S.A.). $H^3$-thymidine (in the form of [$^3$H-methyl]thymidine; specific activity 20 Ci/mmol) was bought from New England Nuclear Research Products (Boston, MA 02118, U.S.A.) and other chemicals from Sigma Chemical Company (St. Louis, MO 63178, U.S.A.).

### *Cell isolation and culture techniques*

Aortic SMC were obtained by an explant technique modified from that of Ross (1971) as previously described (Pang, 1989). We have also isolated aortic SMC by the enzyme digestion method as described below for mesenteric SMC. The proliferative activity of cultured aortic SMC from either method was similar.

Mesenteric SMC were isolated by procedures slightly modified from those previously described by Gunther et al. (1982) and Hamet et al. (1989). The entire mesenteric arcade including the superior mesenteric artery was excised for further dissection. The excised vessels were placed in a series of three petri dishes containing chilled Ham's F-12 medium supplemented with 300 units/ml penicillin and 300 μg/ml streptomycin. Two to three mesenteric arcades cleaned of connective tissue and fat by fine jeweller forceps were transferred to a 25 $cm^2$ Nunclon cell culture flask which contained 5 ml of filtered (Miller-GV; 0.22 μm) collagenase enzyme mixture. This mixture consisted of 2 mg/ml collagenase (type I, specific activity = 152 U/mg), 15 mM HEPES buffer (pH 7.2 -7.3), 0.15 mg/ml elastase (porcine pancreas, type II, specific activity = 83 U/mg protein), 2 mg/ml bovine serum albumin, and 0.38 mg/ml soybean trypsin inhibitor (type I-S) in Ham's F-12 medium. After a thirty minute incubation period in a 37° C oscillating water bath, flask contents were triturated and shaken an additonal thirty minutes producing a single-cell suspension. The suspension was washed twice with phosphate buffered saline (PBS); collagenase containing supernatant was decanted following centrifugation at x 500 g for five minutes. Cells were resuspended in DME, supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 μg/ ml), 8 mM HEPES, and inoculated into a 25 $cm^2$ Nunclon flask. Cell cultures were incubated at 37° C under humid 5%: 95% $CO_2$ to air conditions. Unattached cells and cellular debris were removed with a medium change 24 hours after initial plating. Plating efficiency of about 60-75% was obtained; a confluent cell monolayer was formed within seven days at which time cells were subcultured.

### *Characterization of cultured vascular SMC*

Characterization of SMC was done using immunofluorescent techniques. Eight well glass microscope slides (Lab Tek) were coated with sterilized gelatin (1% by weight/volume) prior to cell plating. Plated vascular SMC were grown for 24 hours and then washed three times in 0.1 M PBS, fixed in 4% paraformaldehyde in PBS for 15 minutes, rinsed in PBS, and immersed in –20°C acetone for 3 minutes. Cells were incubated with primary antibody dilutions (1:25, 1:50, 1:100) of rabbit anti-smooth muscle actin or myosin for 2 hours at 20° C then throughly rinsed with PBS. An indirect method of immunohistological localization was carried out using fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit IgG (1:100 dilution) incubation for one hour. Some slides were labelled by an FITC-conjugated avidin-biotin complex (ABC) technique (Vecta stain). No staining was observed when the primary antibodies were omitted. All slides were coverslipped in a glycerol and PBS (7:3) solution and photographed through a Leitz Vario Orthomat 2 microscope with LEP fluorescent unit using Kodak ASA 400 colour film.

### *Methods of evaluating proliferation*

1. Thymidine incorporation/scintillation counting

Cellular incorporation of radiolabelled thymidine ($^3$H-TdR) into DNA following stimulation with bovine serum was determined by a previously published method (Pang, 1989) with slight modifications. Vascular SMC were plated in 24 well Nunclon plates at 4 x $10^4$ cells/ml, allowed 24 hours to attach and rinsed once in DME. Culture medium was replaced by synchronizing fluid consisting of Ham's F-12 medium supplemented with insulin-transferrin-selenium (ITS) at final concentration of 5 ng/ml, 5 ng/ml, and 5 μg/ml per ml of DME, respectively. Following 72 hours of

synchronization, stimulants and control conditions were applied for 24 additional hours. Cells were incubated in 1 ml volumes of 0.5 μCi $^3$H-TdR/ml of DME for two hours before rinsing in Ham's F-12 solution. Vascular SMC were fixed for 15 minutes in ethanol and glacial acetic acid (3:1), distilled water washed and incubated for twenty minutes at 4° C with perchloric acid. DNA was extracted by heating vascular SMC in 0.5 ml volumes of perchloric acid at 90° C for 20 minutes. Well contents were transferred to scintillation vials together with 4 ml of Aquasol (DuPont) scintillation cocktail, and radioactivity counted in a liquid scintillation counter (Beckman; model LS 1801).

2. Cell number determination

Vascular SMC were plated in 2ml volume petri dishes at 4 x $10^4$ cells/ml of culture medium, grown for 24 hours, then washed and synchronized for 72 hours in Ham's F-12 medium and ITS. They were trypsinized following 24 or 72 hours stimulation time intervals, centrifuged, and vortex resuspended using chilled calcium and magnesium deficient PBS. Cell number was determined using an electronic cell counter (Coulter Counter ZM).

3. Cell cycle by autoradiography

SMC were plated at 4 x $10^4$ cells/ml or 1 x $10^5$ cells/ml on Lab-Tek 8 chamber tissue culture slides using normal culture medium. Some glass slides were plated with sterilized gelatin to promote cell attachment. Tritiated thymidine was used to label DNA synthesizing cells. Autoradiography ws carried out as follows: cells were exposed to 0.5 μCi of $^3$H-TdR per ml of DME for 30 minutes. SMC were washed with two Ham's F-12 medium rinses. Culture medium was replaced and cells incubated at 37° C in 5%: 95% $CO_2$ to air humid atmosphere. At predetermined time points of 0.5, 1, 2, 3, 4, 8, 12, 16, 20, 22, 24, 28, 30, 32, 34, 36, 44, 48, 52 and 56 hours, cells were washed twice in 0.01M PBS and fixed for 15 minutes in a 3:1 solution of 100% ethanol to glacial acetic acid or 4% paraformaldehyde in PBS. Slides were washed with distilled water ($dH_2O$) and allowed to air dry for 12 hours before liquid emulsion dipping. All photographic procedures were carried out in a darkroom. Photographic emulsion gel (Ilford: K-5) was mixed with an equal volume of $dH_2O$ warmed to 40° C. Autoradiography slides were manually dipped and air dried. Slides were placed in light impenetrable storage boxes containing Drierite and stored at 4° C for 14 to 16 days.

Autoradiograms were developer using Kodak D-19 developer for 4 minutes, rinsed in $dH_20$ containing 1-2 drops of D-19 followed by a 5 minute fixation period in 1.21 M sodium thiosulphate. Three $dH_2O$ washings and subsequent 5 minute immersion in 45° C $dH_2O$ completed the processing protocol. All procedures were carried out in a regulated 18° C water bath. After development, slides were placed in $dH_2O$ for 15 minutes prior to staining. Staining procedures included: 8 minutes placement in haematoxylin, and one minute in eosin, separated by 7 minutes of lukewarm running tap water rinse. Autoradiography was completed by standard graded ethanol dehydration and immersion in Hemo De (Fisher). Cover slips were mounted on microscope slides using Pro-Texx (Lerner) mounting medium.

Autoradiographic staining patterns were analayzed to determine labelled mitotic index (the percentage of mitotic figures labelled in each sample) and thymidine labelling index according to the method of Quastler and Sherman (1959). Mitotic figure labelling percentages were determined using a Leitz Laborlux K light microscope.

Statistics

Representative graphs are represented in the Results section of this study. Each graphically depicted point is an average of duplicate determinations; each experiment was repeated 2 or 3 times. Data were analyzed by Newman-Keul's multiple range-test at p level of 0.05 (Steel and Torrie, 1960). Graphs were plotted with a SIGMAPLOT Scientific Graph System (Jadel Scientific, U.S.A.).

## Results

### *Characterization of cultured vascular SMC*

Outward SMC migration occurred from 60% of the plated primary aortic explants within 7-10 (Fig. 1). SMC reached confluence within an additional 7 days. One to two days following initial plating, mesenteric SMC exhibited a crescent-shaped morphology that became less evident following the first passage (Fig. 2). Enzyme dispersed cell preparations contained some endothelial cells that were visible as small, polygonal shaped collections that were also noticeably absent by passage two or three, and thereafter. Mesenteric SMC, once initially plated, needed longer periods of time to reach confluence than did aortic SMC explants plated at the same time. Successive subpassages of mesenteric SMC achieved confluence at notably slower rates than aortic SMC. For both mesenteric and aortic SMC, plating efficiency upon passaging was consistently maintained at 93 to 98%. Vascular SMC isolated by either method exhibited a typical hill-and-valley growth formation at confluence (Fig. 3), at which time they were passaged.

Characterization of SMC by immunocytochemical localisation with 1:25, 1:50, and 1:100 dilutions of anti-muscle actin IgG in PBS positively persisted in SMC passages 4-33, as previously described (Pang, 1989). Maximum staining results were obtained using 1:25 dilutions of either primary antibody when coupled to 1:100 dilutions of goat anti-rabbit FITC secondary

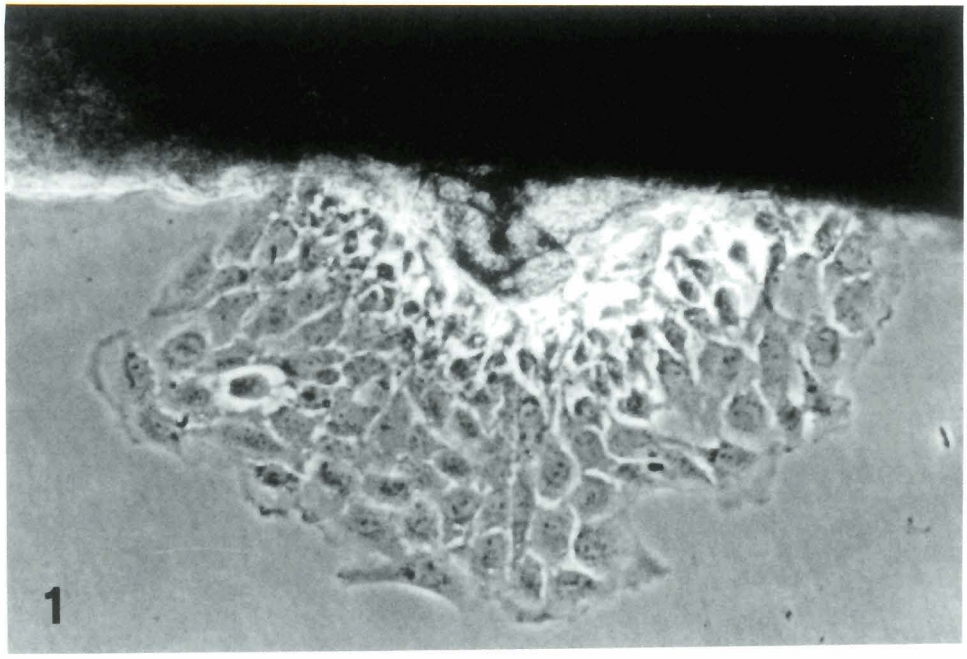


**Fig. 1.** Smooth muscle migration from the tunica media of a piece of thoracic aortic explant. Phase contrast microscopy. × 800

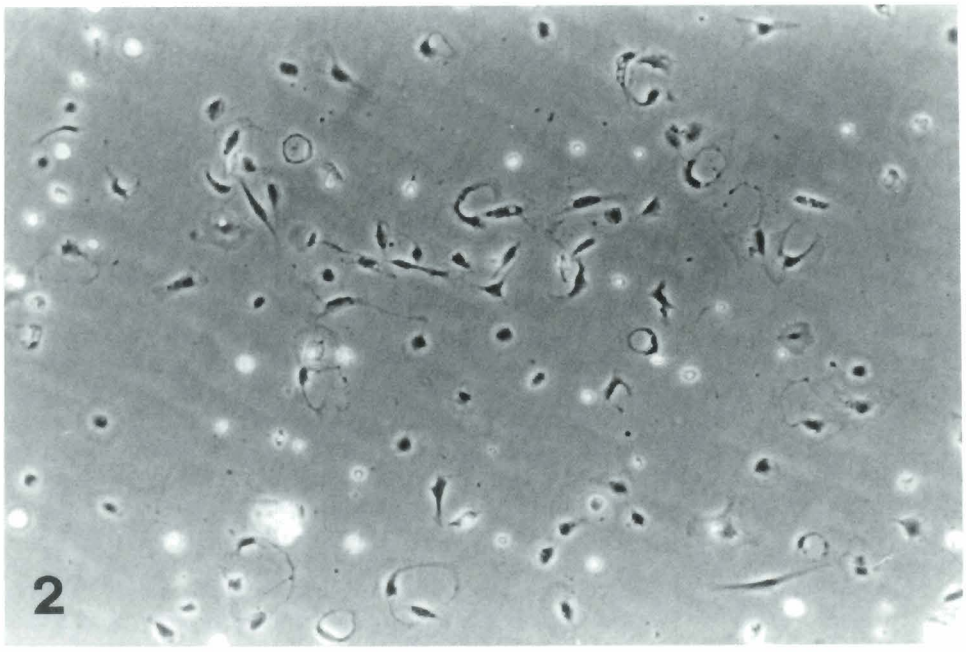


**Fig. 2.** Mesenteric smooth muscle cells. One day after initial plating, viable cells had already adhered to the culture flask. Single cell suspension was obtained from mesenteric arterial arcade subjected to collagenase digestion. Phase contrast microscopy. × 600

antibody for one hour. Preliminary blocking with normal goat serum (1:10) was effective in eliminating non-specific binding, particularly nuclear staining. Microscope sample slides coverslipped in glycerol and PBS solution (7:3) were suitable for photographic purposes for eight weeks when stored at -20° C. SMC adherence efficiency to the Lab-Tech 8 well glass microscope slides was observed to be greatly increased by precoating slides with gelatin.

*Proliferation of cultured vascular SMC*

After the 24 hour plating interval, variability in cell number existed despite being plated at equivalent concentrations (4 x $10^4$ cells/ml culture medium) and similar viabilities, usually 93-98%. During synchronization, all SMC tested showed minimal, but gradual, increase in absolute cell numbers despite the absence of mitogenic factors in the basal medium. Effectiveness of a variety of synchronizing agents

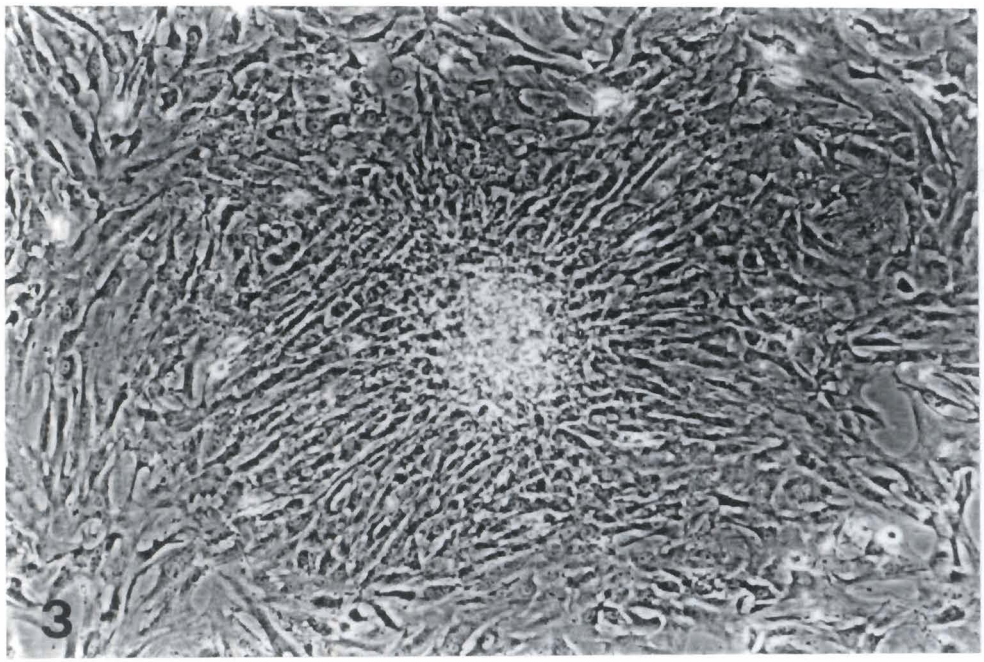


**Fig. 3.** Growth pattern of mesenteric smooth muscle cells in culture. As in cultured aortic cells, cultured mesenteric smooth muscle cells exhibit a characteristic «hill-and-valley» growth configuration after reaching confluence. Phase contrast microscopy. × 600

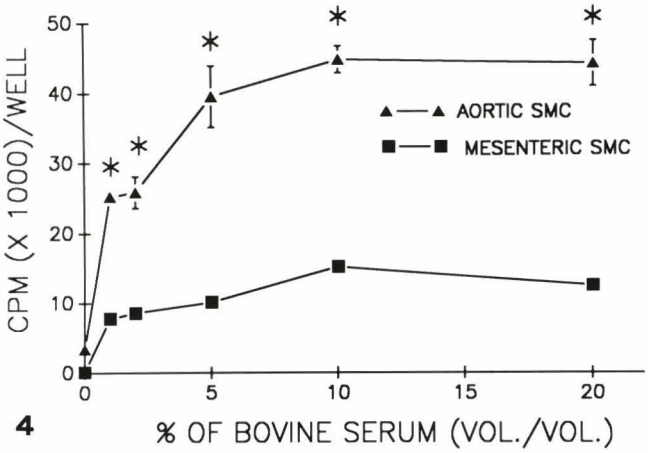


**Fig. 4.** Effect of bovine serum stimulation of thymidine incoroporation in cultured vascular smooth muscle cells. * -denotes mean value of aortic SMC is significantly higher than mesenteric SMC.

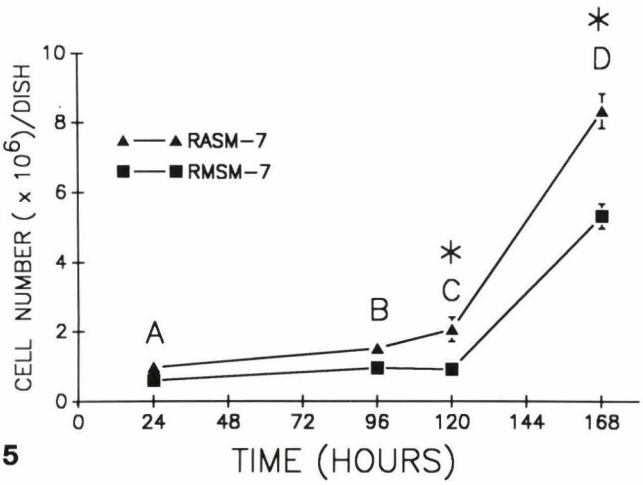


**Fig. 5.** Effect of bovine serum stimulation on cell number of cultured aortic (RASM-7) and mesenteric (RMSM-7) smooth muscle cells. Smooth muscle cells were placed and allowed to attach to the substratum for 24 hours (A), synchronized for 72 hours (B7, and stimulated with 10% bovine serum for 24 (C) or 72 (D) hours at which time cell number were determined electronically. * - denotes mean value of aortic SMC is significantly higher than mesenteric SMC.

to depress cell proliferative activity prior to stimulation in either scintillation or cell counting experiments was tested. It was found that Ham's F-12 + ITS and DME + ITS gave the most consistent result after 72 hours of synchronization in both aortic and mesenteric SMC as compared with that synchronized with DME + 0.1% bovine serum.

Aortic SMC proliferated at a faster rate than mesenteric SMC during the 72 hour time interval. Cell proliferation response to bovine serum was determined by scintillation counting and Coulter counting techniques.

Following 72 hour synchronization, in the subsequent 24 h period, SMC in all experiments showed rapid increases in thymidine incorporation to bovine serum concentrations as low as 1% by volume in basal media (Fig. 4). All SMC displayed dose response curves to increasing serum concentrations. All passages tested displayed similar and reproducible patterns of response to serum stimulation. Maximum rates of DNA synthesis seemed to occur at, or near, 10% serum in the SMC lines tested. However, with as little as 2% serum stimulation the mesenteric SMC

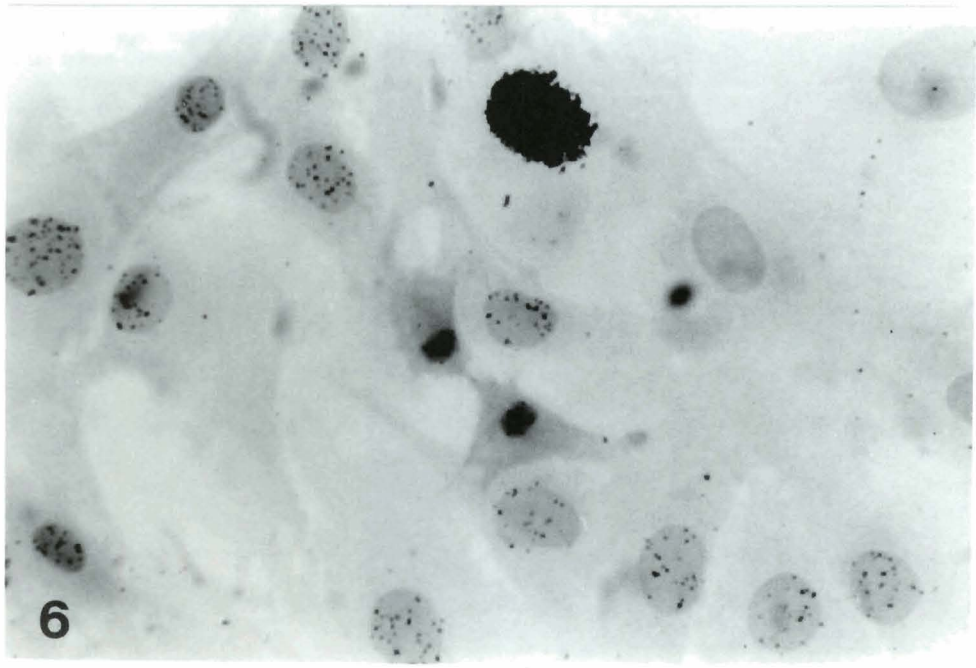


**Fig. 6.** Autoradiography of cultured aortic smooth muscle cells following addition of $^{3}$H-thymidine. Silver grains present over the nuclei represent positively labelled cells. A dividing cell at telophase is also positively labelled. LM. Haematoxylin and Eosin stain. × 1,600

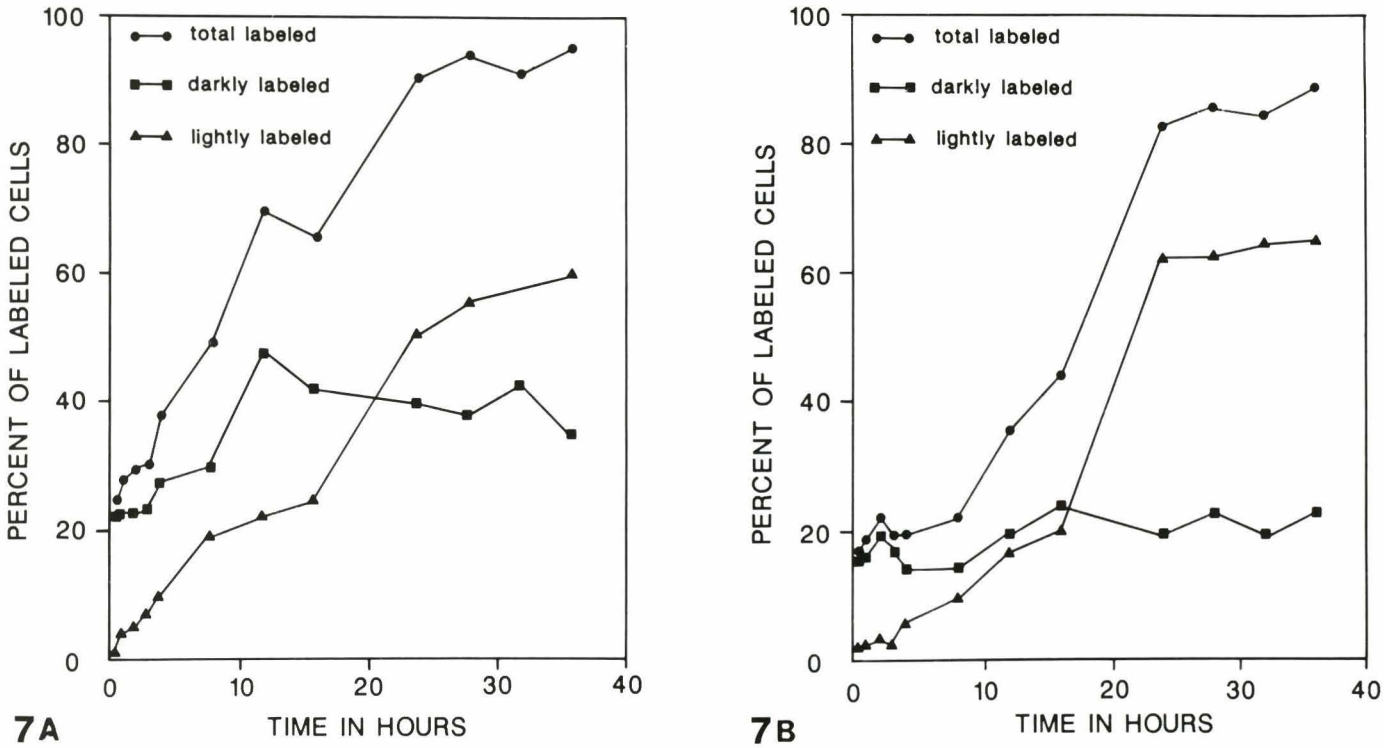


**Fig. 7.** Thymidine labelling index of cultured aortic (A) and mesenteric (B) smooth muscle cells. Nuclei of SMC exposed to $^{3}$H-thymidine were classified by autoradiography. Percent of darkly labelled and lightly labelled SMC nuclei decreased and increased, respectively as SMC transversed the cell cycle and eventually divided into two daughter cells. Aortic SMC had a higher percent of total labelled cells than did mesenteric SMC.

encroached their peak DNA synthesizing capability. After a 24 hour stimulation period, DNA synthesis in aortic SMC was consistently elevated above those levels for mesenteric SMC at all serum concentrations tested.

Vascular SMC responded to 1, 2, 5, 10 and 20% serum stimulation by increasing their numbers in a dose dependent manner, similar to patterns observed for scintillation counting. Patterns of cell growth at low serum concentrations were difficult to discern. At

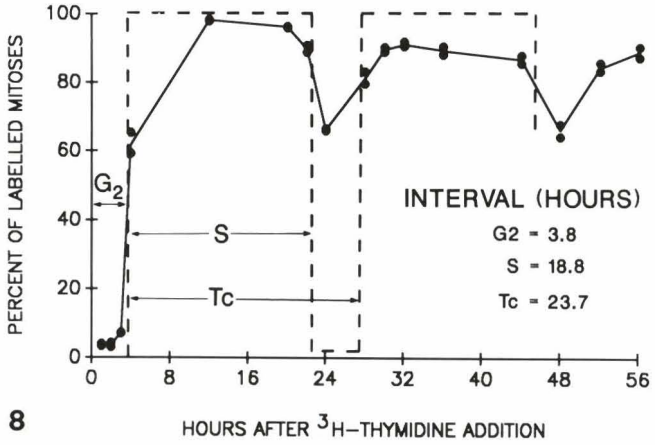


**Fig. 8.** Labelled mitotic index of cultured aortic smooth muscle cells. The percent of labelled mitotic SMC was determined over a 56 hour interval. Maximal and minimal labelling values were used to determine cell cycle parameter duration. Regularity of successive cell cycle parameter duration, namely synthetic phase (S) and premitotic phase ($G_2$), and total cell cycle duration (Tc) were obvious. The discontinuous line represents theoretically ideal labelled mitotic SMC levels.

10% serum levels, aortic SMC reached maximum cell proliferation levels; 20% serum levels elicited negligible further increase from mesenteric SMC. Mesenteric SMC reached near maximum proliferation rates at considerably lower serum levels, usually below 5%. Vascular SMC grew exponentially during the next 48 hours of stimulation, approaching cell saturation density points by 72 hours post-stimulation. Repeated trypsinization was often necessary for disassociation of SMC from culture vessel surfaces. Extracellular matrix deposition was visible in petri dishes of all SMC lines. Cell population were vastly divergent for aortic and mesenteric SMC at 72 hours, the difference being 2-3 fold higher in aortic SMC. Figure 5 depicts that as early as 24 hour following addition of 10% bovine serum, cell number of aortic SMC was already higher than mesenteric cells.

*Autoradiography of cultured vascular SMC*

Vascular SMC plated onto 8 well Lab-Tek microscope slides at 4 x $10^4$ cells per ml of culture media was sufficient to evenly cover the slide surface by the 56 hour time point: SMC plated at $10^5$ cells/ml become overcrowded, making cell distinction difficult. Glass microscope slide coating with poly-L-lysine did not seem to promote SMC-surface adhesion as well as 0.1% gelatin. An example of the autoradiographs used for analysis is shown in Figure 6.

Following the half hour $^3$H-TdR incubation, autoradiographs showed that the percent of total labelling of SMC exhibited a lag phase in aortic and mesenteric SMC (Figs. 7A, 7B). The percent of lightly labelled cells during initial post-incubation hours was significantly lower than the percent of darkly labelled SMC. The ratio of lightly to darkly labelled SMC persisted or increased slightly for 3-4 hours, at which time the percent of total labelled cells was approximately 20%.

Between 8-10 hours following the $^3$H-TdR incubation, the percent of total labelled SMC in both aortic SMC increased exponentially, as indicated by the steep slope of their respective labelling curves. Mesenteric SMC exhibited similar increases in labelling patterns; the onset of exponential growth was delayed, occurring 10-12 hours following $^3$H-TdR addition. In both cases, the rapid rise in percent of total labelled SMC was paralleled by an increase in percent of lightly labelled cells. Simultaneously, the percent of darkly labelled SMC decreased gradually with time. The intensity of the $^3$H-TdR nuclear label diminished over the 36 hour time period, resulting in some darkly and lightly labelled SMC to be indistinguishable.

Between 22 to 26 hours, the percent of total cells labelled in the two SMC lines reached plateus levels. Aortic SMC had a greater total of labelled nuclei than did mesenteric SMC. The labelling percent of aortic SMC at 24 hours and 32 hours post $^3$H-TdR incubation were 93.1% and 91.4% respectively; mesenteric SMC labelling levels were 82.7% and 85.0%.

Using light microscopy, SMC in either the interphase or mitotic phase of the cell cycle were distinguishable. Following counter staining with haematoxylin, $^3$H-TdR uptake and incorporation, visualized by autoradiography, allowed metaphase, anaphase, and telophase stages of mitosis to be discernible. All mitotic figures, labelled or unlabelled, were characterized and counted; the average number of mitotic cells counted at each time point, for aortic and mesenteric SMC were 221 and 120, respectively.

Following the 1/2 hour incubation, nuclei showed a low staining intensity, a lag period, in which 2.0-3.8% of SMC were labelled. After a subtle increase, all SMC lines at the 3.5-4.0 hour interval exponentially increased the percentage of labelled mitotic cells that reached maximum levels by 12 hours in the aortic SMC line (Fig. 8). The mesenteric SMC maximum labelled mitotic figure percentage reached a plateau at 20 hours. This peak labelling plateau was used to evaluate the 50% labelling percentages of both the ascending and descending arms of the mitotic labelling curves. The interval between the 50% labelled mitotic figures point on the ascending arm and baseline allowed determinations of $G_2$ duration, measuring 3.7 hours in aortic SMC and 3.8 hours in mesenteric SMC. The S intervals in two SMC lines were similar as were $G_1$ and M valell processes tues. Assuming duration of M phase to be 1/2 hour, $G_1$ values ranged from 2.8 to 5.4 hours.

Minimal percentage values of labelled mitoses occurred at the 24, then 48 hour post pulse-chase time points in all cell lines. A second peak of labelled mitosis plateau occurred at 28-30 hours time, but of less intensity than the first maximum of each respective cell line. There were no significant

differences between two SMC lines in terms of total cell cycle duration (Tc); the mean Tc of two SMC lines was 22.85 ± 1.17 hours. The second «dip» in $^3$H-TdR labelling was used to generate the second set of S values in the SMC lines. As estimated from the graphs, the first (S1) and second (S2) S values for each cell line were 18.8 and 18.3 in aortic cells, and 19.2 and 19.2 hours in mesenteric cells, respectively. Analysis of the mitotic labelling indices for each SMC line showed a consistent rhythmicity between successive Tc values.

Examination of autoradiographic slides revealed the existence of a population of vastly enlarged SMC. The SMC occurred randomly at different time points. Random, too, was their pattern of staining with $^3$H-TdR. With duplicates of each of the 12 time points examined, the total number of enlarged SMC observed was 32 and 9 for aortic and mesenteric SMC, respectively. The frequency of occurrence of hypertrophied cells represented 0.009% for aortic SMC and 0.004% for mesenteric SMC in the total cell population. The hypertrophied cells were voluminous, having an enlarged nucleus and lightly stained cytoplasm.

## Discussion

Explant and enzyme digestion techniques were effectively utilized to isolate aortic and mesenteric SMC, respectively, for use in SMC growth comparisons. Vascular SMC, regardless of cell line or passage number, displayed «hill» and «valley» growth formation after reaching confluency. This is characteristic of SMC growth in culture (Chamley-Campbell et al., 1981), distinguishing SMC from contact-inhibited monolayer formation of endothelial and other epithelial cells *in vitro*. Further characterization using immunohistochemical techniques demonstrated that at least 90% of the cultured cells were smooth muscle. Despite characterized SMC sharing common denominators, such as the presence of actin and myosin proteins, and characteristic growth configurations, cell proliferation patterns suggest that aortic and mesenteric SMC are intrinsically different.

It has been shown by electron microscopy that SMC is the only cell type present in the media of mammalian arteries (Pease and Poole, 1960). The SMC must exhibit a functional duality, being capable of contraction and relaxation while remaining able to perform reparative functions. In order to satisfy both functional requirements, SMC maintain, to varying degrees basic mesenchymal properties of synthesizing extracellular matrix and cell division (Wissler, 1968). Evolution from a multipotential state to one of specialized functions involves differentiation. Therefore, SMC are capable of expressing great variations in morphology and phenotype.

Embryologically, both aortic and mesenteric SMC are derived from a common mesenchymal origin. However, a diverse functional heterogeneity exists in the vasculature. The aorta, an elastic artery, acts as a non-specific functional conduit for blood distribution. The function of the small muscular arteries such as mesenteric arteries in blood pressure regulation is well documented (Folkow, 1977; Guyton, 1986; Junqueira et al., 1986). Mesenteric SMC have evolved to a contractile phenotype in order to perform specific blood pressure regulating functions. Most SMC *in vivo* exhibit a contractile phenotype, the opposite phenotype being the predominant form in cell culture (Chamley-Campbell et al., 1979).

In this study, vascular SMC plated immediately following trypsinization exhibited a lag phase of growth that increases rapidly over the next few days until confluence was reached, at which time growth rate was attenuated. Densely plated SMC ($10^6$/ml of medium) in primary culture will remain indefinitely in the contractile state (Chamley et al., 1977). If more than five cell population doublings occur prior to reaching confluency, SMC remain in the synthetic phenotype (Campbell and Campbell, 1985). However, SMC plated in the contractile phenotype in intermediate numbers will modulate during growth to the synthetic state and, upon reaching confluency, will revert spontaneously to the contractile phenotype (Campbell and Campbell, 1985).

Thymidine labelling index studies involved the characterization of some 17,000 SMC per cell line per trial: trials were repeated three times in this study. Growth fraction studies are useful indicators of cell proliferation potential. In this regard, mesenteric SMC had a considerably lower percent of cells incorporating $^3$H-TdR and traversing the cell cycle than did aortic SMC. Thin observation provides significant evidence for the existence of differential growth potential, implications consistent with the phenotypic modulation model proposed by Chamley-Campbell et al. (1979). Since autoradiography experiment duration (36 hours) is considerably longer lasting than the determined cell cycle times for the two SMC lines, the variability of individual cell cycles within cell populations cannot account for growth fraction percentages less than 100%.

Results obtained by the experimental regimen designed for this study, allow several conclusions to be made. Thymidine labelling indices, detected by autoradiography, were different in the two SMC lines, despite cell cycles parameters and total duration intervals being equivalent. The heightened *in vitro* proliferation rate of aortic relative to mesenteric SMC in asynchronously growing cell populations can be explained in terms of the higher number of cells in cycle following stimulation. Using rate of incorporation of $^3$H-TdR into DNA and cell number counting as indices of proliferation, stimulation with bovine serum caused 2-3 fold increases in aortic SMC relative to mesenteric SMC. SMC isolated from different regions of normotensive rat vasculature, despite sharing a common genetic component and

mesenchymal origin, exhibited the potential to proliferate at various rates in culture. The underlying mechanisms of such growth patterns require further experimentation.

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