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In Vivo Pericyte–Endothelial Cell Interaction during Angiogenesis in Adult Cardiac and Skeletal Muscle

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Pericytes (PC) exert an inhibitory effect on endothelial cell (EC) proliferation *in vitro* and withdrawal of PC occurs prior to EC proliferation during pathological capillary growth *in vivo.* Using stereological analyses of PC and capillary EC fine structure, we have studied their relationship during the early stages of physiological angiogenesis using two *in vivo* models. In rat skeletal muscle where capillary growth was induced by indirect electrical stimulation, there was a reduction in the relative area of contact between pericytes and the capillary abluminal surface (22% vs 30% for normal controls; $P < 0.05$) and pericyte surface:volume ratio (12.5 vs 14.9 μm^{-1} for normal controls; $P < 0.05$) after 3 days of stimulation, at a time prior to the appearance of new capillaries. Further withdrawal of pericyte processes was evident at the point where an actual increase in capillary numbers was observed (7 days stimulation; PC surface:volume ratio $= 10.4\ \mu m^{-1}$), although the degree of PC–EC interdigitation increased. Similar changes, but to a lesser extent, were observed in both left ventricular myocardium and papillary muscles of pigs following 4–5 weeks chronic heart rate reduction. Although PC coverage of capillaries in the heart was found to be less than that in skeletal muscle, the relative contact area between PC and capillaries also showed a reduction in paced vs sham-operated control heart (papillary: 15% vs 20%; ventricle: 12% vs 16%; $P < 0.05$). Interdigitation of PC and EC was absent in cardiac muscle. These data suggest that retraction of PC may play a permissive role in controlling angiogenesis *in vivo* in normal adult tissue.

INTRODUCTION

Pericytes are the perivascular cells found within the basement membrane of capillaries, having a close structural relationship with the underlying endothelial cells, and are synonymous with Rouget or mural cells mentioned in older literature. They are found in nearly all vertebrate tissue with continuous or fenestrated capillaries, although variations in structure, frequency, and distribution suggest that they represent a heterogeneous family of cells with possibly different functions in various tissue (see reviews by Sims, 1986; Tilton, 1991; Shepro and Morel, 1993; Nehls and Drenckhahn, 1993). Pericytes are thought to originate from interstitial fibroblasts and give rise to the vascular smooth muscle cells of arterioles and venules, with which they share many common structural features (Rhodin, 1967). Other roles postulated for these apparently pluripotential mesenchymal cells range from that of providing structural support for the capillary wall (preventing excess dilatation), acting as a scaffold along which endothelial cells migrate during sprouting (Nehls *et al.,* 1992), control of vascular

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permeability, and regulation of capillary perfusion by means of their contractility (Tilton *et al.*, 1979b).

Of particular interest for the present study is the role of pericytes in regulating endothelial cell proliferation and differentiation, processes that are fundamental to *in vivo* angiogenesis. Inhibition of capillary proliferation by pericytes has been implicated in the retina (Crocker *et al.*, 1970). During developmental or pathological neovascularization, new vessels grow from capillaries without pericytes or where pericyte degeneration has occurred, whereas there is a decline in endothelial cell proliferation and capillaries stop growing when pericytes appear or reappear (Kuwabara and Cogan, 1963; De Oliveira, 1966; Ausprunk and Folkman, 1977). This morphological evidence was supported by the work of Orlidge and D'Amore (1987) who developed a coculture system which showed that pericytes inhibit endothelial cell growth. When the cell types were physically separated during culture, but diffusible products were still present in the media, there was no effect on endothelial cell proliferation. This finding supports the concept that *in vivo* angiogenesis might be controlled by contact inhibition of endothelial cells by pericytes.

There are, however, data which caution against the universal applicability of this scheme. For example, Bar and Wolff (1972) point out that angiogenesis in the brain begins most commonly from capillaries rich in pericytes during embryonic development, and Schlingemann *et al.* (1990) state that within tumors or during would healing, pericytes are more numerous in vessels undergoing angiogenesis than in resting vessels. Such discrepancies emphasize the possibility of alternative roles for pericytes under different circumstances and in different tissues. Endothelial cells secrete elements of the extracellular matrix which may stimulate pericyte proliferation (Swinscoe and Carlson, 1992; Newcomb and Herman, 1993), possibly acting via endothelin-1 (Yamagishi *et al.*, 1993). Pericytes themselves synthesize many components of the extracellular matrix that change during maturation. The many similarities between the two cell types during angiogenesis suggest that both may participate in the formation of capillary sprouts (Schor *et al.*, 1992).

Most of the evidence indicating the possible role of pericytes in regulating angiogenesis has come from *in vitro* studies and structural investigations of developmental or pathological changes. There are no data on the role of pericytes in controlling angiogenesis in mature, physiologically normal tissue when capillary growth has been induced by either increased activity (skeletal muscle; Myrhage and Hudlicka, 1978) or decreased heart rate (cardiac muscle; Wright and Hudlicka, 1981; Brown *et al.*, 1994b). Pericytes surround most capillaries in both skeletal and cardiac muscle (Tilton *et al.*, 1979a). The postulated regulatory role of pericytes was therefore examined by seeking evidence of altered endothelial cell–pericyte relationships following angiogenic stimulation in the period preceding, and following, the appearance of new capillaries in skeletal muscle, and during a period of active angiogenesis in the heart, based on previous work detailing the time course of changes in capillary supply (Hudlicka and Brown, 1993).

Preliminary analyses have been presented in abstract form (Egginton *et al.*, 1993b; Brown *et al.*, 1994a).

MATERIALS AND METHODS

Skeletal Muscle

Home-bred male Sprague–Dawley rats (253 ± 4 g body weight) were divided into four equal experimental groups. Animals chosen at random were used as controls,

without any intervention, while all other animals underwent unilateral surgical implantation of electrodes in the vicinity of the lateral popliteal nerve (Myrhage and Hudlicka, 1978). All surgical procedures were performed under aseptic conditions and 1–2% halothane (Fluothane, ICI) anesthesia, with postoperative administration of antibiotic. One group of sham-operated animals was sampled 4 days later. Hindlimb muscles of the remaining animals were stimulated for either 3 days (3d) or 7 days (7d) starting on the day following surgery at 10 Hz for 8 hr day^{-1}, using 0.3 msec pulse width and sufficient voltage to induce maximal palpable contractions. Food and water were available *ad libitum*, and the animals' sleep pattern was not disturbed. Under these conditions, Myrhage and Hudlicka (1978) observed an increase in the anatomical capillary supply after 7 days of stimulation, but there was no change in muscles stimulated for 2 days and only a small increase after 4 days of stimulation (Brown *et al.*, 1993). Thus, we sought to quantify structural changes both during the initial stages of angiogenesis and once the growth process was well established.

Animals were anesthetised by sodium pentobarbitone (50 mg 100 g^{-1}) about 16 hr after the last stimulation. Extensor hallucis proprius (EHP) muscles from both legs were dissected free, pinned at resting length to strengthened cork strips, and fixed by immersion in 2.5% glutaraldehyde, 1.5% sucrose, 0.1 *M* phosphate buffer (350 mOsm), pH 7.4 at 20°C, for 1 hr. This muscle has a fiber type composition which is representative of the hindlimb fast muscles (Myrhage and Hudlicka, 1978), but is sufficiently narrow (2–5 mm, 20 mg wet weight) to permit adequate infiltration of fixative. Muscles were subsequently sliced with care into small blocks ca. 1.5 mm^3 and placed in fresh fixative for 24–48 hr at 4°C. Postfixation was performed with 1% O_sO_4 in phosphate buffer for 1 hr at room temperature. Samples were dehydrated in a graded series of alcohols, cleared, and vacuum embedded in an Araldite/Epon mixture which was cured at 70°C for 36 hr. Blocks were chosen at random to include one from each of four animals per group. Semithin (0.5-μm) sections were used to orientate the blocks to produce true cross sections of the whole muscle. Thin (60- to 80-nm) sections were double stained with 3% methanoic uranyl acetate and 2% aqueous lead citrate and viewed at 60 kV on a Jeol 100 CXII transmission electron microscope at an initial magnification of $\times$900 or $\times$11,700. At this scale, basal laminae and cytoplasmic interdigitations were visible with minimal goniometer tilt correction.

Cardiac Muscle

Farm-bred female pigs (23–29 kg initial body weight) were instrumented under isofluorane/O_2/N_2 inhalation anesthesia with Medtronic dual chamber telemetric pacemaker generators. Details of the protocol used to induce bradycardia are reported elsewhere (Brown *et al.*, 1994b). Briefly, sensing endogenous atrial P-waves and giving a delayed pacing stimulus produced a single mechanical contraction which prolonged the subsequent diastole and reduced heat rate from 110 to 60–80 beats/min, depending on the programmed pacemaker rate. Seven animals were instrumented but not paced and acted as sham-operated controls. Heart rate was monitored postoperatively from ECG signals transmitted by small telemetric recording devices implanted under the skin of the chest at operation. Heart rate was measured in both groups of conscious, unrestrained animals two to three times per day and averaged for each individual. One time point was studied, during a presumed active period of angiogenesis, based on the increase in capillary density (CD) found in paced rabbit hearts

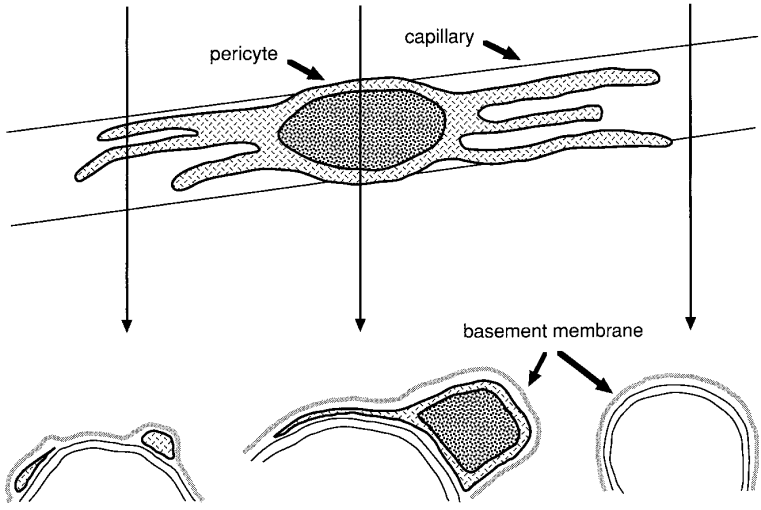

FIG. 1. Diagrammatic representation of pericyte topology, showing the resultant profiles on transverse sections of a capillary taken at random. The basement membrane between pericyte and capillary abluminal surface has been omitted for clarity.

(Wright and Hudlicka, 1981), which was first observed after 14–20 days of pacing, showing a progressive rise up to 60 days.

After 4 to 5 weeks of chronic bradycardia hearts were removed and weighed, and small tissue blocks of mid-left ventricular free wall and papillary muscles were immersion-fixed in a manner similar to that for EHP. Adjacent tissue blocks were lightly covered with an inert mounting medium (OCT compound; Tissue Tek) and quickly frozen in isopentane cooled in liquid nitrogen. Frozen sections (10 μm) were stained for vascular endothelium by a biotinylated lectin (*Griffonia simplicifolia*-1) for visualization. Capillary density (mm^{-2}) was estimated in a minimum of 10 fields per section, covering an area of 0.98 mm^2 at a magnification of $\times$400, using an unbiased counting protocol. Blocks for both EM and LM analyses were chosen at random to include one from each of five animals per group.

Stereological Analyses

Sampling of sections by electron micrographs was performed in a systematic random manner using a predefined square lattice, with adequacy of replicate fields determined according to data variance (Egginton, 1988), using 30–40 capillaries per animal to minimize intragroup variability. Thirty-five-millimeter negatives were projected at a final magnification of $\times$19,500 (skeletal muscle) or $\times$22,000 (cardiac muscle) onto a stereological counting grid via a microfilm reader, using a lattice spacing (d) of 1.0 cm (equivalent to 0.51 or 0.45 μm, respectively) (Brown and Egginton, 1988; Egginton *et al.*, 1993a). Our definition of pericytes (PC) is those highly branched perivascular cells closely associated with capillaries and bounded by a common basal lamina (Fig. 1). Quantification of capillary and pericyte morphology (Fig. 2) used standard point-count and line-intercept techniques for estimates of volume and surface densities, respectively. Surface/volume ratio (S/V) and profile cross-sectional area could then be calculated, with mean cell thickness derived from cell area associated with circumfer-

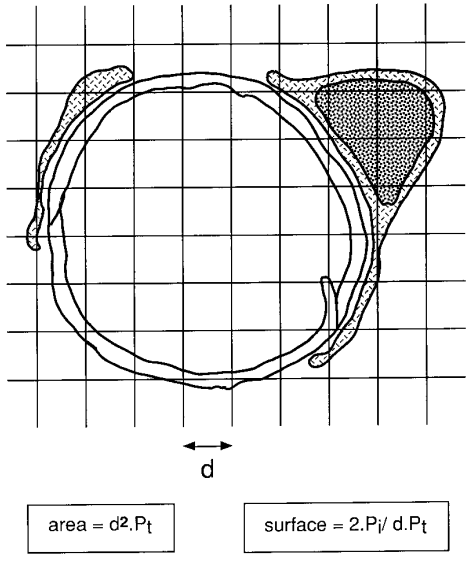

FIG. 2. Stereological analysis of capillary and pericyte morphology, and their interaction, by point-count and line-intercept methods.

ence of the medial cell plane. Data for pericyte processes and cell bodies were combined for each capillary.

The relative contact area between cell types was estimated by the ratio of boundary length of pericyte membrane in apposition to the abluminal capillary surface to total capillary boundary length (abluminal circumference). The frequency of pericyte processes found in close association with endothelial cell (EC) clefts was calculated as (number of clefts with PC processes)/(total number of clefts). Other indices of endothelial cell–pericyte interaction scored on a nominal scale (Egginton *et al.,* 1993a) were presence of pericyte processes and appearance of EC or PC cytoplasmic interdigitations.

Single factor analysis of variance (ANOVA) was used for comparison of values, with Scheffe's multiple range *F* test to estimate significance between groups. Capillary sprouting is a nonuniform event (fewer than 30% of capillaries may be involved, at best) and the accompanying endothelial cell activation very focal (representing a minute fraction of capillary length). On muscle cross sections we are therefore dealing with a rare event. Any biological significance of accompanying structural change is diluted in the statistical treatment of averages by including activated and nonactivated vessels. Distribution analysis is in this case often more sensitive to changes in a subpopulation of cells and was applied where appropriate.

RESULTS

Skeletal Muscle

Capillary morphology was similar to that previously described (Cliff, 1963; Egginton *et al.,* 1993a), with thin endothelial cells having numerous cytoplasmic vesicles

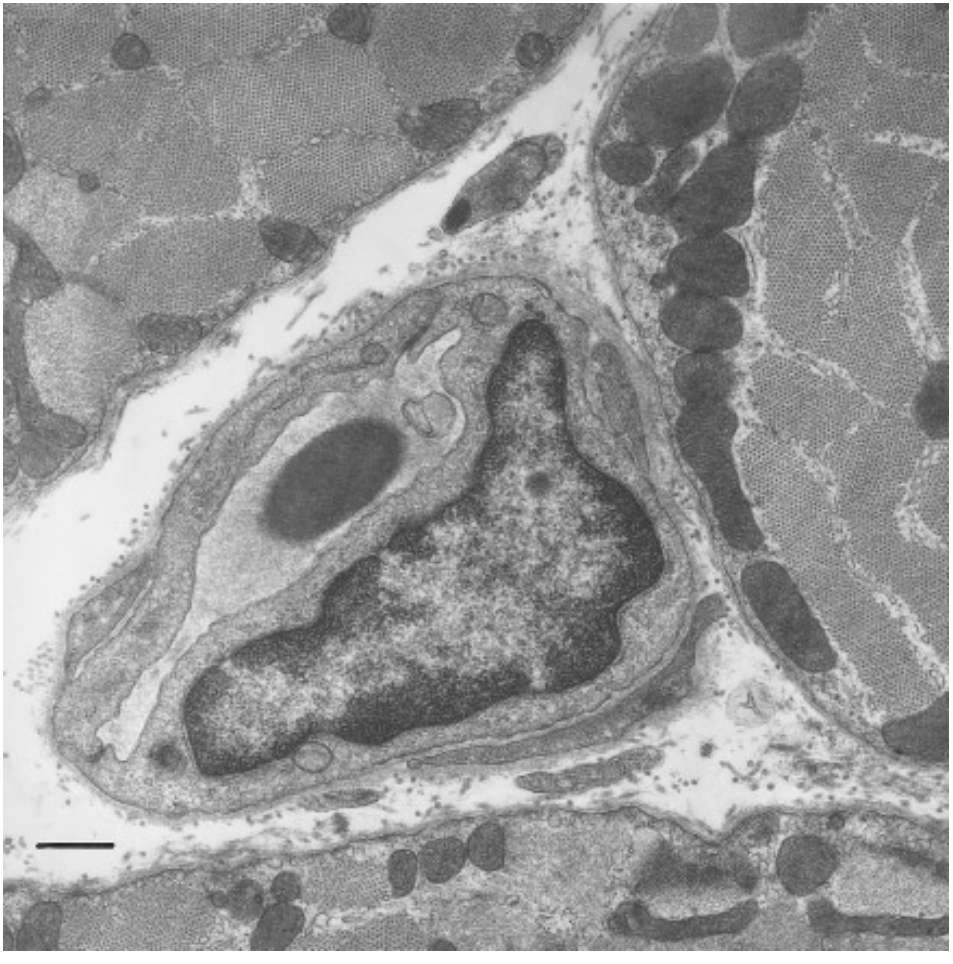

PLATE 1. Capillary morphology in control skeletal muscle, showing thin endothelial cells (EC) with prominent intercellular clefts and numerous cytoplasmic vesicles. Pericyte processes (P) are clearly visible within the surrounding basement membrane (BM). The BM becomes interrupted in the gap between pericytes and abluminal surface of EC. Scale bar, 1 μm.

and distinct intercellular junctions (clefts), surrounded by a clearly visible and intact basement membrane (BM) (Plate 1). The swelling of EC noted in other muscles subjected to electrical stimulation (Egginton and Hudlicka, 1991) was noted at both 3d and 7d, but was minimal in control and sham-operated muscles. Mean capillary size was statistically significantly reduced at 7d, compared to the normal control group (Table 1). At this time a few profiles of small capillaries showed morphological features of early angiogenesis (Plate 2), characterized by a thickened endothelium (high volume density of cytoplasm) with a reduced (slit-like) lumen. Vessels showing these features were observed in ca. 4% of capillaries in control muscle but their number increased to 16% in stimulated muscles.

Pericytes appeared to be included within the BM of capillaries, i.e., their apical (interstitium-facing) BM fused with that of the EC to form a contiguous layer. Al-

TABLE 1
Fine Structure of Capillaries and Pericytes during the Early Stages of Angiogenesis in Rat Skeletal Muscle (Mean ± SEM)

	Control ($N = 4$; $n = 155$)	Sham operated ($N = 4$; $n = 160$)	3d stimulated ($N = 4$; $n = 159$)	7d stimulated ($N = 4$; $n = 148$)
a (cap) (μm^2)	11.41 ± 0.68	11.48 ± 1.49	11.39 ± 0.92	9.20 ± 0.38*
b (cap) (μm)	15.78 ± 0.44	15.84 ± 0.98	16.08 ± 0.66	14.63 ± 0.31
Relative contact area	0.300 ± 0.024	0.266 ± 0.020	0.217 ± 0.024*	0.214 ± 0.014*
a (pc) (μm^2)	1.55 ± 0.19	1.07 ± 0.14	1.18 ± 0.07	1.27 ± 0.13
V_v (nuc, pc)	0.088 ± 0.011	0.031 ± 0.009*	0.031 ± 0.005*	0.035 ± 0.005*
S/V (pc) (μm^{-1})	14.86 ± 0.23	13.89 ± 0.49	12.46 ± 0.23*	10.42 ± 0.40
t (pc) (μm)	0.268 ± 0.020	0.319 ± 0.039	0.390 ± 0.057	0.295 ± 0.027

Note. Abbreviations: a (cap), mean capillary profile area; b (cap), mean capillary perimeter; a (pc), mean pericyte profile area; V_v (nuc, pc), pericyte nuclear volume density; S/V (pc), pericyte surface-volume ratio; t (pc), mean pericyte thickness.

* $P < 0.05$ vs control (ANOVA).

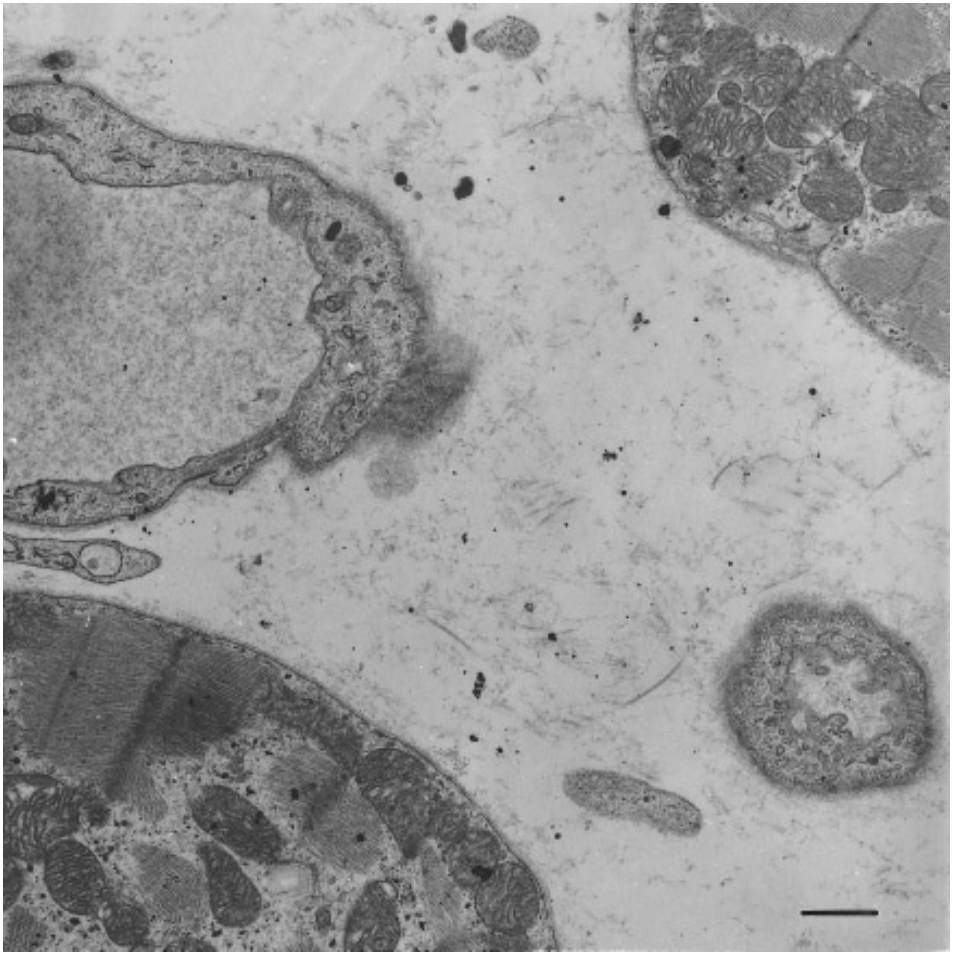

PLATE 2. Presumptive capillary sprout in paced cardiac muscle. Note the small cross-sectional area and narrow lumen. Scale bar, 1 μm.

though pericyte basal (capillary-facing) and EC abluminal BMs formed a conjoint structure within the intercellular gap, this was discontinuous and absent where the two cell types came into close contact. This was particularly evident at the margins of the pericyte processes where, infrequently, PC–EC interdigitations similar to those observed between ECs could be found (Fig. 3). The overall pericyte coverage of capillaries was around 30% in normal and 27% in sham-operated controls, but declined to about 22% when muscles were stimulated for 3d and 7d (Table 1; Fig. 4). In control muscle, $<1\%$ of capillary profiles were devoid of any associated pericyte cell body or processes, i.e., essentially the whole length of the capillary bed is surrounded by pericytes. After 7d stimulation this proportion increased to around 4%, presumably as a result of pericyte retraction.

Gross changes in PC morphology were not evident during the early stages of angiogenesis, with their processes appearing similar in all groups. Although the total PC volume (represented by profile area around capillaries sectioned transversely),

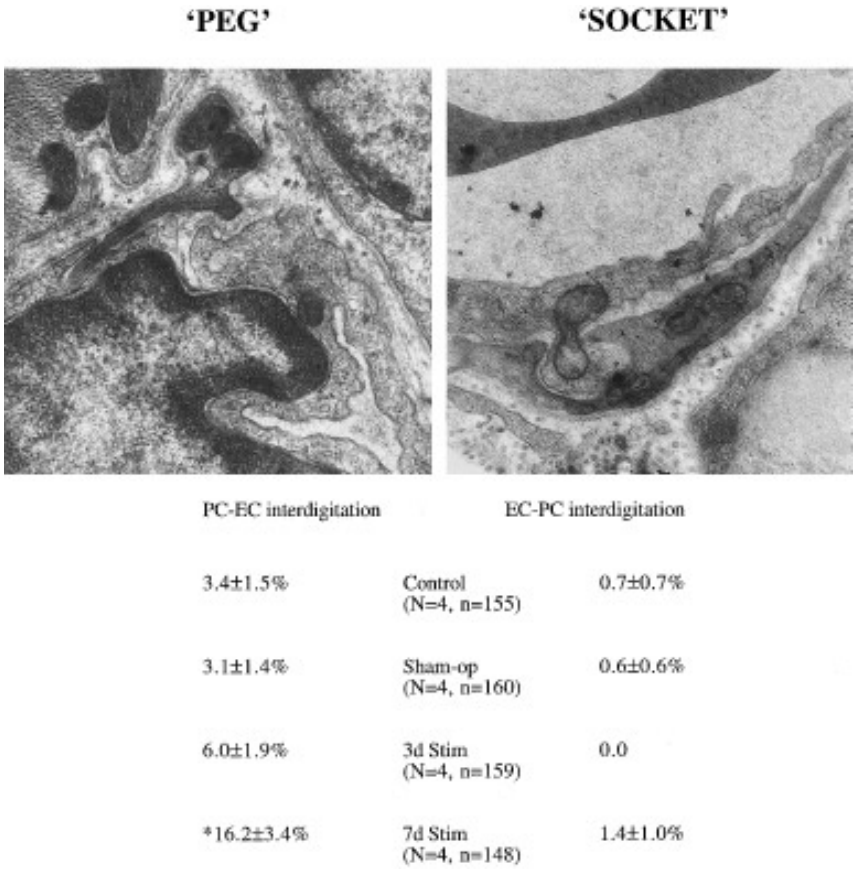

FIG. 3. Example of the two forms of intimate endothelial cell–pericyte interaction, together with a quantitative estimate of their abundance in skeletal muscle. The relative abundance of PC–EC interdigitations in 7d-stimulated muscle was significantly ($P < 0.001$) higher than that in 3d, sham, or control muscle.

remained constant, a greater proportion of capillaries from stimulated (28 ± 3%) than control muscles (17 ± 3%; $P < 0.05$) had an adjacent pericyte cell body as opposed to coverage exclusively by processes. The relative proportion (volume density) of PC nucleus was lower in all groups than that in controls. Differences were also apparent in surface/volume ratio (S/V) where a decrease in stimulated muscle again indicates a withdrawal of PC processes and rounding up of existing pericytes (Table 1). Although no change was observed in the nature of the normal close association between EC and PC, there was a greater proportion of PC processes being associated with EC clefts in 7d-stimulated muscles (25 ± 2%) than in controls (20 ± 1%; $P < 0.05$). In addition, while interdigitations between ECs and PCs occur infrequently in normal muscle they increased with duration of stimulation, particularly with PC interdigitations into EC (Fig. 3).

Cardiac Muscle

Capillary morphology resembled that of the rat skeletal muscle (above) and rabbit myocardium (Brown and Egginton, 1988). The larger size of capillaries scales with

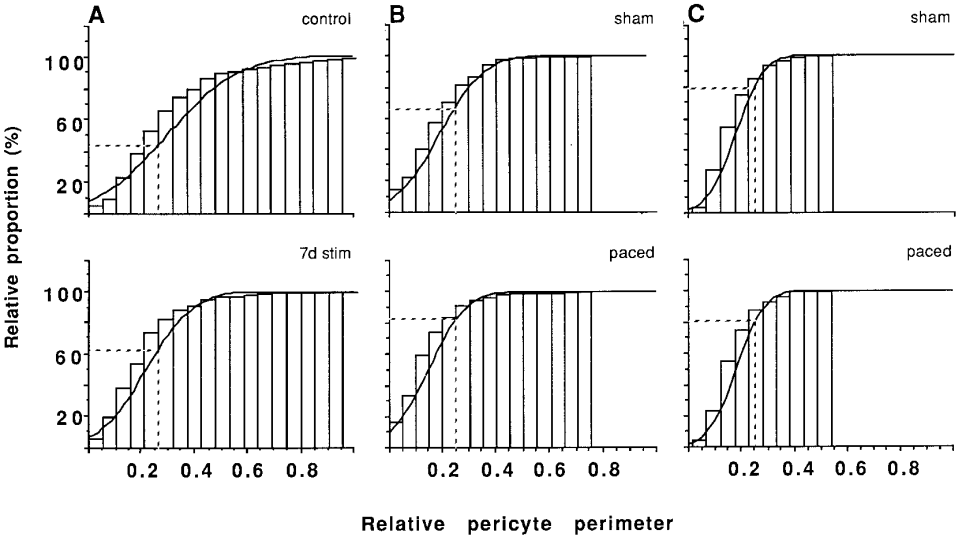

FIG. 4. Cumulative probability plots of pericyte coverage following *in vivo* angiogenesis. (A) Control (top) and stimulated (bottom) rat EHP; (B) sham-operated (top) and paced (bottom) pig papillary muscle; (C) sham-operated (top) and paced (bottom) pig ventricular myocardium. Lines are drawn to show values of the 25th percentile.

the greater heart mass in pigs, but cross-sectional area showed little change following induced angiogenesis (Table 2). There were, however, a number of structural changes which indicate a parallel response to that seen in skeletal muscle. In addition to the appearance of some small capillary profiles (Plate 2), the mean lumen volume decreased by 10–15% in proportion to endothelial cell swelling (papillary, 0.54 vs 0.46; ventricle, 0.48 vs 0.42; sham vs paced, respectively). The number of capillaries having a slit-like lumen also increased from 5.5% in sham to 13% in paced papillary muscles and from 9 to 11% in ventricular myocardium, respectively.

Pericytes also showed a morphology similar to that described above. Although coverage of capillaries was only around three-quarters of that found in skeletal muscle, there was a similar (25%) reduction in coverage following angiogenesis in both the papillary ($P < 0.06$) and ventricular ($P < 0.05$) myocardium (Table 2; Fig. 4). This was accompanied by a modest increase in capillary profiles lacking any pericyte association (sham, 9.7 and 6.9%, and paced, 11.2 and 18.9%, for papillary and ventricular muscle, respectively). Substantially more capillaries fall into this category than in skeletal muscle (1–2%).

Pericyte morphology was not markedly different between experimental groups, having both similar mean profile areas and nuclear content. Pericyte *S*/*V* was also similar, while mean cell thickness in papillary muscle increased by <10%, suggesting only a modest retraction of processes (Table 2). The proportion of clefts associated with PC processes rose in both papillary (15 ± 1 vs 12 ± 3%) and ventricle (17 ± 2 vs 10 ± 2%; $P < 0.05$). In the myocardium, however, no evidence of EC–PC interdigitation was seen.

Thus in both skeletal and cardiac muscle there is reciprocal change in relative pericyte coverage and CD accompanying or preceding *in vivo* angiogenesis.

TABLE 2
Fine Structure of Capillaries and Pericytes Following Bradycardial Pacing in Pig Myocardium (Mean ± SEM)

	LV papillary muscle		LV free wall	
	Sham operated ($N = 5$; $n = 176$)	Paced ($N = 5$; $n = 169$)	Sham operated ($N = 5$; $n = 225$)	Paced ($N = 5$; $n = 229$)
a (cap) (μm^2)	27.49 ± 3.82	24.19 ± 2.02	18.52 ± 0.94	18.29 ± 1.44
b (cap) (μm)	31.01 ± 2.54	27.16 ± 1.15	27.65 ± 0.66	26.31 ± 1.38
Relative contact area	0.198 ± 0.020	0.149 ± 0.010	0.156 ± 0.014	0.123 ± 0.006*
a (pc) (μm^2)	1.54 ± 0.31	1.51 ± 0.41	1.18 ± 0.04	1.04 ± 0.12
S/V (pc) (μm^{-1})	12.61 ± 1.58	10.41 ± 0.58	13.94 ± 0.83	13.91 ± 1.16
t (pc) (μm)	0.211 ± 0.025	0.228 ± 0.050	0.202 ± 0.011	0.200 ± 0.019

Note. Abbreviations as in Table 1.
* $P < 0.05$ vs sham (ANOVA).

DISCUSSION

Electrical stimulation of skeletal muscle is known to be a very effective way of inducing capillary proliferation (Hudlicka, 1991). In previous experiments, capillary supply to hindlimb skeletal muscles of the rat was slightly increased at 4d, but not after 2d of stimulation (Brown *et al.,* 1993); angiogenesis continues such that capillary to fiber ratio is further increased by 7d and beyond (Hudlicka, 1991). The choice of 3d and 7d intervals of stimulation therefore provided a comparison of EC–PC interaction prior to and following the appearance of new capillary vessels. Likewise, bradycardial pacing has been shown to progressively increase capillary supply in rabbits, (Wright and Hudlicka, 1981) as well as in pigs (Brown *et al.,* 1994b). Data from the same animals show that capillary density was significantly increased by up to 20% following chronic bradycardial pacing, reaching around 1750 mm^{-2} for left ventricular papillary muscle compared to 1470 in sham-operated controls (Brown *et al.,* 1994a). As this occurred in the absence of cardiac hypertrophy (heart:body weight ratio of around 0.45% in both groups) this is a clear indication that angiogenesis had been induced. However, little attention has been paid to the role of perivascular pericytes during physiological angiogenesis *in vivo.* The use of morphometric techniques was chosen to avoid the ambiguities inherent in more invasive approaches, or the limitations of cytochemical identification, and to unambiguously identify PC on the basis of definitive structural criteria. This is particularly important when tissue integrity is disturbed, and fibroblasts may otherwise be easily mistaken for PCs. For example, Phillips *et al.* (1992) consider all angiogenesis to be the result of an inflammatory response, which is important when interpreting data from sham-operated controls. Analysis of structural changes may then indicate which results from *in vitro* or pathological studies are applicable to *in vivo* specimens undergoing physiological angiogenesis.

Capillary Size

In comparing the present *in vivo* models of angiogenesis, some interorgan and indeed interspecific variations are to be expected. Capillaries from pig myocardium are larger in cross-sectional area than are those from rat skeletal muscle, but this may also reflect other differences such as allometric scaling with body mass, response to surgical interventions, and the method of fixation (muscles were fixed by immersion, with skeletal muscle pinned to resting length but myocardium unavoidably being free to contract). For our present purposes, the important point is that this does not affect the degree of pericyte coverage in normal tissue, being similar to other reports for rat skeletal muscle (Tilton *et al.,* 1979a) and both mouse and rat heart (Forbes *et al.,* 1977; Tilton *et al.,* 1979a). Capillary morphology shows little effect of stimulation or pacing, apart from EC swelling (Egginton and Hudlicka, 1991), although there was a significant reduction in mean size of capillaries as a result of the former but not the latter treatment in our electron microscopy study. In contrast, Myrhage and Hudlicka (1978), using intravital microscopy, found an increase in capillary diameter for existing vessels following stimulation. Although newly formed capillaries (sprouts) tend to be smaller with slit-like lumen and an increased proportion (volume density) of endothelial cells (e.g., Cliff, 1963), such vessels are clearly visible in both tissues following angiogenesis. Their influence on mean capillary size is evident in skeletal muscle but not in myocardium, indicating nonsprouting angiogenesis may have occurred in the latter. A similar finding also holds for EC volume density (data not shown).

PC Abundance

Pericyte morphology was similar to that reported previously, i.e., extensive branching with processes forming an incomplete layer around the capillary abluminal surface. Tilton *et al.* (1979a) reported a similar abundance (relative frequency) of pericytes in rat skeletal and cardiac muscle. Based on the objective criterion of the proportion of capillary profiles having an adjacent pericyte cell body (i.e., visible nucleus), which on random sections is proportional to relative volume, we find a lower abundance in normal skeletal muscle than in heart (18 vs 38%, respectively). The difference between cardiac and skeletal muscle in the proportion of capillaries with pericyte profiles having close cytoplasmic contact with endothelial cells (15–30%) is similar to the range observed in lung (20%) and heart (10%) by others (e.g., Eppling, 1966). At least some of these are likely to represent tight junctions (Schulze and Firth, 1993). The proportion of endothelial clefts associated with pericyte processes is greater in skeletal than cardiac muscle, but in both cases is greater than expected from that calculated by Schulze and Firth (1993) for rat heart, and may simply reflect the use of different species. In the present study, the increased likelihood of finding a pericyte process adjacent to or covering an endothelial cleft may reflect slippage of the pericyte around the capillary. Whatever the cause, it clearly has the potential to alter capillary permeability by increasing cleft width following PC contraction by either exposing EC junctions or pulling them apart.

PC Coverage

Pericyte coverage of the capillary abluminal surface is greater in skeletal than cardiac muscle, confirming the difference reported for rats by Tilton *et al.* (1979a) of 21 and 11%, respectively. This may point toward one of the earliest postulated roles for pericytes, that of a physical restraint on capillary proliferation. It may also reflect intertissue differences in microvessel growth, where angiogenesis in skeletal muscle is by sprout formation but in the heart may result more from elongation of existing capillaries or formation of more cross-connections. Such a degree of tissue specificity in coverage may be widespread with lung having even less and brain more coverage (Weibel, 1974; Allsopp and Gamble, 1979), possibly reflecting other potential roles for pericytes including that of controlling microvascular permeability. The reduction in coverage coincident with angiogenesis can be most readily explained by withdrawal of pericyte processes, which is consistent with a reduction in S/V ratio in skeletal though not cardiac muscle, if the usual assumption (that biological surface area is less variable than volume) is applied. This of course relies on pericytes displaying some degree of contractility (Murphy and Wagner, 1994) for which there is no direct evidence *in vivo* (Nehls and Drenckhahn, 1993), although there are morphometric data that are at least consistent with such a phenomenon (Tilton *et al.*, 1979b), again being found in skeletal but not cardiac muscle.

PC Withdrawal

Wallow *et al.* (1993) reported an increase in actin content of pericytes following hypertension leading to capillary constriction, which may have contributed to the smaller capillary size seen in stimulated muscles. How pericyte contraction may be initiated is far from clear, although there are at least three plausible routes. First, direct innervation has not been shown, but close proximity of nerve terminals have

been reported for a number of tissues (Tilton, 1991). Second, the morphometric data of Tilton *et al.* 1979b) suggest that pericytes may contract in response to histamine (see also Murphy and Wagner, 1994), which may possibly be released from other interstitial cells. Third, endothelial cells release endothelin-1 (ET-1) which in addition to its potent vasoconstrictor effect on vascular smooth muscle appears, at least in tissue culture, to exert a similar effect on the precursor pericytes (Ramachandran *et al.,* 1993). Interestingly, ET-1 also appears to stimulate pericyte proliferation, again *in vitro* (Yamagishi *et al.,* 1993).

Close endothelial cell association with pericytes is supported by morphological observations showing pericytes spanning two capillary sprouts (Nehls *et al.,* 1992) or mature vessels (Weibel, 1974; Gaudio *et al.,* 1990), while direct communication is suggested by the appearance of tight junctions (above). There are also close cytoplasmic interdigitations in many tissues and species (Tilton *et al.,* 1979a; Fig. 3) which may act as physical unions by which pericyte contractility exerts its influence on capillary diameter (Matsusaka, 1975; Gaudio *et al.,* 1990), and we observed a significant increase in the occurrence of such structures when skeletal muscle was stimulated. Furusato *et al.* (1990) noted an even distribution of PC–EC interdigitations over parent capillaries, while EC–PC interdigitations were sparsely but exclusively distributed over immature capillaries (angiogenic sprouts) in human granulation tissue. While these complex structural relationships between ECs and PCs suggest a close functional interaction, modulation of microvascular function by PCs may differ significantly between skeletal and cardiac muscle. In the latter, PC withdrawal appears not to involve contraction and EC–PC interdigitations were lacking in normal cardiac tissue, as reported by Schulze and Firth (1993), nor could we demonstrate any following angiogenesis.

Schor *et al.* (1992) suggest that, as both sprouting endothelial cells and pericytes show many similarities, both may participate in sprout formation during angiogenesis, an explanation that perhaps best fits the present morphometric data. As samples in the present study were taken at random, the morphometric analyses provide average values from those pericytes located close to, and remote from, the growing capillary sprout. Hence pericytes may perform a dual role of preventing EC proliferation at the proximal end, and providing a scaffold for EC migration at the distal end of capillary sprouts (Rhodin and Fujita, 1989). Whether this reflects pericytes inducing endothelial tube formation (Shepro and Morel, 1993) and/or acting as guiding structures (Cliff, 1963; Nehls *et al.,* 1992) awaits definitive *in vivo* studies.

Skeletal muscle from sham-operated animals shows a minor response to surgery, with further PC activation/withdrawal at 3d stimulation (when angiogenesis has been initiated), becoming significant at 7d. These data are consistent with PC contraction and/or withdrawal; the smaller changes in the heart are likely due to withdrawal alone. These results support the concept that an inverse correlation might exist between angiogenesis and the extent of pericyte coverage, and that a coordinated regulation might exist between capillary endothelial cells and pericytes during physiological angiogenesis in striated and cardiac muscle.

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