J. Physiol. (1983), **344**, *pp.* 209–222
With 2 plates and 10 text-figures
Printed in Great Britain

VASODILATATION BY ACETYLCHOLINE IS ENDOTHELIUM-DEPENDENT: A STUDY BY SONOMICROMETRY IN CANINE FEMORAL ARTERY *IN VIVO*

BY J. A. ANGUS, G. R. CAMPBELL*, T. M. COCKS AND J. A. MANDERSON*

From the Baker Medical Research Institute, Melbourne, Victoria, Australia

(*Received 15 March 1983*)

SUMMARY

1. External diameter of the femoral artery was measured by sonomicrometry in the anaesthetized dog.

2. Intra-arterial acetylcholine lowered arterial pressure and thereby passively lowered diameter. When blood flow and distal resistance were controlled by roller pump and Starling resistor respectively, acetylcholine (0·1–10 μM) and substance P (0·1–1 nM) both caused up to 10% increase in diameter.

3. Removal of endothelium by mechanically rubbing the artery lumen abolished the dilator response to acetylcholine and substance P but did not affect the response to nitroprusside.

4. Constrictor responses to noradrenaline were unaltered by endothelium removal.

5. Topical application of acetylcholine and substance P onto the adventitial surface of the artery also caused an increase in diameter but both agents were 50–100 times less potent by this route compared with intra-arterial infusion. These dilator responses were abolished by endothelium removal. In these circumstances acetylcholine caused constriction.

6. We conclude that acetylcholine and substance P require an intact endothelium to elicit vasodilatation *in vivo*, at least for the large femoral artery.

7. The results from the topical application experiments suggest that local neural release of vasoactive substances such as acetylcholine and substance P depend on an intact endothelium to cause vasodilatation.

INTRODUCTION

Acetylcholine dilates resistance blood vessels *in vivo* and either contracts or relaxes segments of large arteries studied *in vitro* in muscle baths. Furchgott & Zawadzki (1980) showed that rings or strips of isolated arteries only relaxed in response to acetylcholine, substance P, bradykinin and adenosine 5′-triphosphate (ATP) if the endothelium remained intact. They proposed that acetycholine and the other dilators activated specific receptors on endothelial cells to cause the release of a substance(s) that subsequently relaxed the underlying vascular smooth muscle cells.

* Department of Anatomy, University of Melbourne.

The aim of this study was to investigate whether intra-arterial acetylcholine and other dilators increase the diameter of a large artery (femoral artery) *in vivo* by an endothelium-dependent mechanism. A technique of controlled blood flow and distal resistance was used since the intra-arterial administration of vasodilators lowers intravascular pressure and thereby passively reduces the diameter of the large artery. In addition, arterial diameter was measured during the topical application of acetylcholine to the adventitial surface before and after removal of the endothelium. Continuous measurement of the external diameter of the femoral artery was performed by sonomicrometry. A preliminary abstract of some of this work has been published (Angus, Cocks, Le Duc & Campbell, 1982).

METHODS

The experiments were performed on femoral arteries *in situ* from greyhounds or dogs of mixed breed (20–30 kg of either sex) anaesthetized with sodium pentobarbitone (30–40 mg/kg I.V.) and ventilated with room air. Body temperature, fluid balance and blood gases were maintained within normal limits. Intravenous fluids, heparin (300 u./kg initially and 150 u./kg every hour) and pentobarbitone (6 mg/kg per hour) were given via a double luman brachial venous catheter. Both right and left femoral arteries were exposed.

Measurement of diameter

The phasic external femoral artery diameter was continuously measured by the transit-time ultrasound technique (Bertram, 1977). A small light-weight piezoelectric receiver crystal (9 MHz, 1·5 mm × 3 mm, 5·4 mg) was glued to a strip (2 mm × 3 cm) of surgical glove rubber and passed under the femoral artery with the crystal uppermost so as to contact the ventral surface of the artery. This crystal was held firmly in place by the surrounding skeletal muscle and by the overlying artery. A second crystal (emitter) was positioned directly above the bottom crystal and sutured to the adventitia of the artery wall by two loops of 7/0 polypropylene atraumatic suture (Ethicon). The two crystals were brought into focus by carefully moving the bottom crystal on the rubber strip. The crystals only registered a diameter when the alignment was within 5° of parallel. The accuracy, linearity and stability of the entire system were tested in a calibration tank with the crystals mounted on Perspex rods attached to micrometer gauges. Static measurements showed that incremental changes in diameter of 0·01 mm could be resolved repeatedly and linearly over the range 1–8 mm of transducer face separation. The resolution of phasic diameter measurement is frequency-limited by the 100 Hz low-pass pulse filter circuits (pulse repetition frequency 4·5 kHz). Crystals were insulated by multiple dippings in laminating epoxy resin (Fibre-Glast laminating epoxy, 88/87) to ensure a resistance of > 10 MΩ across the faces. The thickness of the insulation was accounted for in determination of the absolute external diameter of the artery. Multistranded lightweight flexible platinum–iridium Teflon-coated wire (Medwire, NY, U.S.A., 101R9/49T) was used as the cable for the upper (emitter) crystal.

Auto-perfused femoral artery and vascular bed (uncontrolled preparation)

In some dogs both the femoral arteries was exposed. A catheter was inserted in the right or left saphenous artery for measurement of arterial pressure. A square-wave electromagnetic cuff-type probe (2·5–4 mm i.d.) (Biotronex BL610) was placed on the femoral artery proximal to the saphenous artery to record femoral blood flow. A distal wire snare was used to obtain zero flow. A single pair of sonomicrometer crystals was placed on the artery proximal to the flow probe to obtain diameter. Femoral vascular resistance was continuously computed on-line by dividing femoral artery pressure by femoral blood flow (Baker Institute, resistance computer). Intra-arterial bolus injections of acetylcholine were made via a catheter passed retrograde into the root of the abdominal aorta from the contralateral femoral artery. Topical applications of drugs were made by slowly dropping the solutions onto the adventitial surface of the artery in the area of the sonomicrometer crystals.

Controlled flow and distal resistance

A 10 cm length of femoral artery was exposed by retracting the overlying muscle. The proximal end of the artery was cannulated with short Silastic catheters in both directions to allow the distal segment of the artery to be perfused at a fixed flow rate (50 ml/min, Watson-Marlow pump) (see Fig. 1). The artery was cannulated again some 2–5 cm distal from the saphenous artery branch to lead the femoral blood to a pneumatic Starling resistance before being returned to the dog via the femoral vein. The Starling resistance was adjusted to bring the femoral artery side-branch pressure (measured from a catheter placed near the origin of the saphenous artery) to 90 mmHg

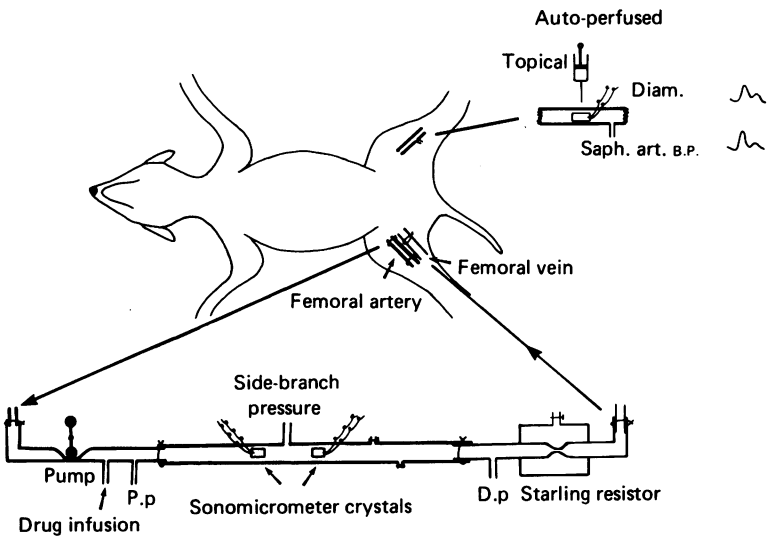

Fig. 1. Schematic diagram illustrating the different preparations of left and right femoral arteries *in situ*. Right femoral artery: expanded view of controlled preparation where blood flow and distal resistance are controlled by a roller pump and Starling resistor (P.p. proximal pressure; D.p. distal pressure). Left femoral artery: uncontrolled (auto perfused) preparation. In some preparations femoral flow was measured by electromagnetic cuff flow probes.

with the flow rate fixed at 50 ml/min. Two pairs of sonomicrometer crystals were placed on the femoral artery approximately 1·5 cm apart, one above and one below the branch of the saphenous artery. Through side holes in the catheter tubing, the proximal and distal pressures of the artery segment were measured with Statham P23Db transducers. Drugs were infused into the femoral artery at 1 ml/min from another proximal side port (Fig. 1). If the intra-arterial vasodilators lowered the side branch pressure, a careful search for side branches was undertaken between the proximal and distal catheters. Any such vessels were ligated.

Removal of endothelium

Endothelial cells lying in the area below either the proximal or distal pair of crystals were removed by briefly rotating (10 s) an inflated Fogarty embolectomy catheter (3F). The catheter had been previously inserted into the artery by disconnecting the tubing from the roller pump or Starling resistance as required. The embolectomy catheter balloon was made more abrasive by loosely tying a piece of 3/0 silk thread longitudinally over the balloon so that on inflation, the thread was firmly held against the balloon surface.

Drugs

Acetylcholine chloride (Sigma) was dissolved in ascorbate (0·1 mM) for each experiment and kept on ice. This solution was diluted with 0·9% saline immediately before use. (−)-Noradrenaline bitartrate (Levophed, Winthrop) was diluted in 0·1 mM-ascorbate and kept on ice. Other drugs used

were: substance P triacetate and bradykinin triacetate (Sigma); nitroprusside (Nipride-Roche); indomethacin (Sigma) dissolved in 4·2% w/v $NaHCO_3$ and diluted in 0·9% saline.

Histology

At the conclusion of some experiments in which the femoral artery had been perfused with blood at controlled flow and distal resistance, the artery was prepared for detailed histological examination. By controlling the distal resistance, the saphenous pressure was maintained at 90 mmHg while the artery was perfused with 0·9% saline to remove blood. When the perfusate was free of red cells, the artery was perfused with 5% glucose followed by 30 s (25 ml) with 0·25% $AgNO_3$. A further perfusion of glucose was followed by glutaraldehyde in 0·1 M-phosphate buffer (pH 7·4). These vessels were used for *en face* examination of endothelial cells (Geissinger, 1974).

In other experiments the vessels were perfused *in situ* at 90 mmHg pressure with 0·9% NaCl until the perfusate was clear then fixed with 2·5% glutaraldehyde in 0·1 M-phosphate buffer. The vessel was cut from the tissue and further fixed for 1–2 h in 2·5% glutaraldehyde in 0·1 M-phosphate buffer. Each vessel was sliced longitudinally into small pieces (2 × 5 mm) which were divided for scanning and transmission electron microscopy. The tissue was then rinsed with 0·1 M-phosphate buffer before post-fixation in osmium tetroxide. After thorough rinsing with distilled water, vessel segments for scanning electron microscopy were dehydrated in graded concentrations of ethandiol (2-ethoxyethanol in H_2O) and then Cellusolve (ethylene glycol in ethandiol). After critical-point drying, tissue segments were mounted on metal stubs, coated with gold and then viewed on an ETEC Autoscan scanning electron microscope. The remaining vessel segments were stained *en bloc* with a 2–4% solution of uranyl acetate, and rinsed thoroughly with distilled water before dehydration through graded acetone solutions and embedding in Epon 812. Thick sections (2 μm) were cut for light microscopy. Thin sections were cut with a diamond knife, mounted on copper grids, stained with uranyl acetate and lead citrate and examined at 60 kV on a Philips 400 electron microscope.

RESULTS

Pressure–diameter relationship

In the autoperfused preparation, the phasic signals from the sonomicrometer crystals and the side-branch pressure catheter indicate that the diameter and pressure wave forms were similar. The pressure signal was applied to the x input and the phasic diameter to the y input of a storage oscilloscope in the x–y mode (Tektronix T912). A hysteresis loop was clearly evident. The diameter followed the initial pressure rise in a linear fashion but returned well above the initial relationship as the pressure fell towards the end-diastolic level (Fig. 2). In the same artery, the relationship between pressure and diameter was studied more closely in the controlled preparation. The artery was pump-perfused at 50 ml/min with the Starling resistor in series with the femoral vein without altering the position of the pressure catheter or sonomicrometer crystals (see Methods). The pressure and diameter wave forms were again similar and followed the characteristics of the roller pump. Under these conditions a hysteresis loop was also evident within a single cardiac cycle (Fig. 2). Starting at a pressure of 60 mmHg, the pressure was raised in 20 mmHg steps by altering the air pressure in the Starling resistor. At each step, the pressure was held steady for 1–2 min. At 140 mmHg the pressure was reduced in steps of 20 mmHg back towards 60 mmHg. The pressure–diameter relationship was hyperbolic where smaller changes in diameter occurred at higher pressures than at lower pressures (Fig. 2). In addition there was a clear hysteresis loop in the pressure–diameter relationship for mean values (Fig. 3).

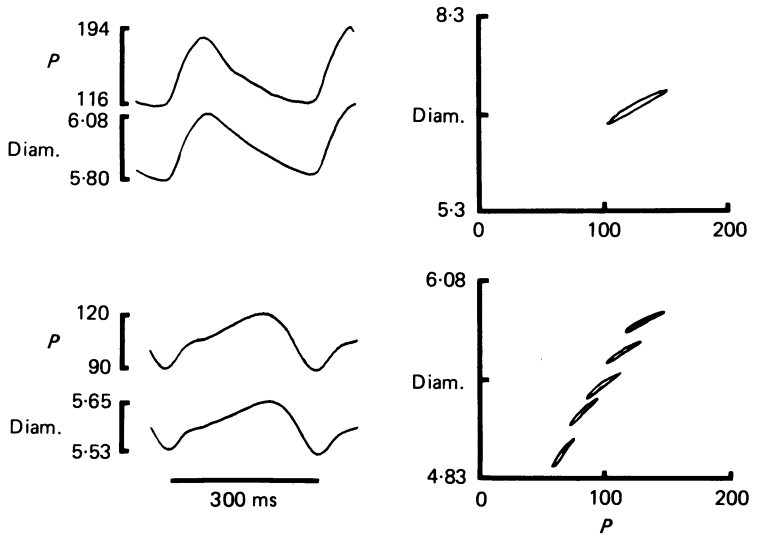

Fig. 2. Oscilloscope records of the wave forms from side-branch pressure (mmHg) and external femoral artery diameter (mm) (left panels). Pressure–diameter hysteresis loops from *x–y* mode are shown in right panels. Upper records are from an auto-perfused (uncontrolled) preparation and the lower records are from the same artery after inserting the roller pump and distal Starling resistance (controlled preparation). The lower hysteresis loops were obtained by altering the Starling resistance.

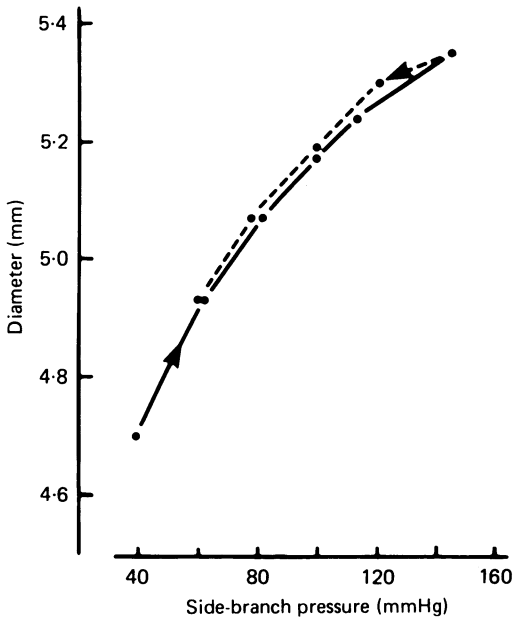

Fig. 3. Graph illustrates the hyperbolic relationship between arterial pressure and external diameter obtained in one blood-perfused femoral artery under conditions of controlled flow and resistance. Pressure was raised by 20 mmHg steps from 40 mmHg. Some hysteresis was observed on the down limb.

Acetylcholine: uncontrolled preparation

Acetylcholine 10 μg/kg intravenously caused marked hypotension, reflex tachycardia and a transient fall in mean femoral blood flow (Fig. 4). The external diameter of the femoral artery fell passively with the decrease in arterial perfusion pressure but returned quickly to the pre-acetylcholine level when the pressure was restored. By comparison, a lower dose of acetylcholine (2 μg/kg) administered low down in the abdominal aorta, via a catheter passed retrograde from the contralateral femoral

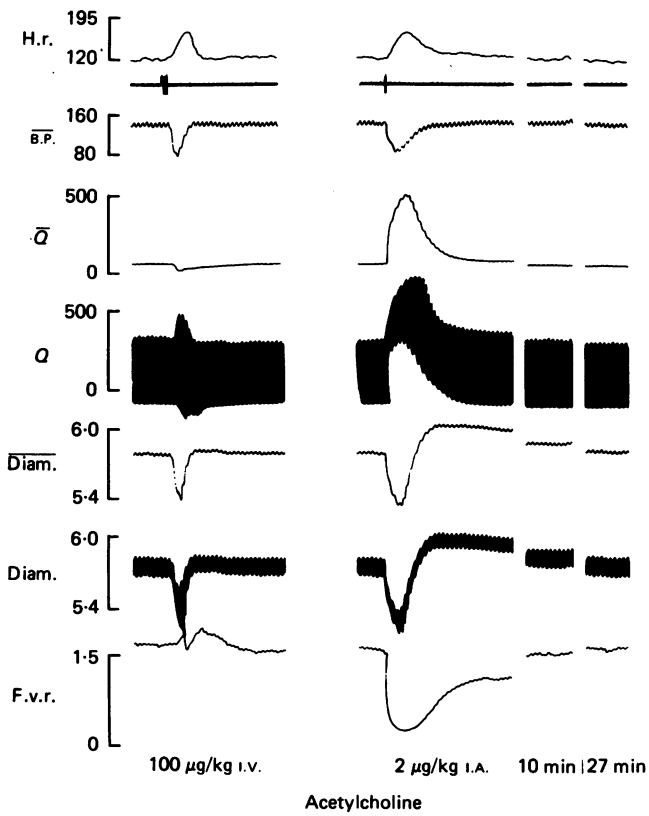

Fig. 4. Chart records from one experiment with the uncontrolled preparation. Acetylcholine was given as a bolus injection of 10 μg/kg I. V. (left), and 10 min later 2 μg/kg I.A. (right) via the abdominal aorta. H.r. = heart rate (beats/min); B.P. = mean systemic blood pressure (mmHg), $\bar{Q}$ = mean and Q = phasic femoral blood flow (ml/min); $\overline{\text{Diam.}}$ = mean and Diam. = phasic external femoral artery diameter (mm); F.v.r. = mean femoral vascular resistance (mmHg/ml per minute). Time marks (second trace from top) are 5 s, and heavy line is injection marker. Records at far right were taken 10 and 27 min after acetylcholine injection.

artery, caused prolonged hypotension and reflex tachycardia. There was a marked rise in femoral flow and fall in femoral vascular resistance, which indicated substantial arteriolar dilatation. There was also an initial fall in femoral diameter that passively followed the pressure change. As the arterial pressure was restored, the diameter rose well above base line and was still elevated after 10 min. Since the pressure did not

rise above control levels, this increase in diameter must be due to the active dilatation of the large femoral artery. These experiments indicate the difficulty of interpreting any direct drug-induced change of large-artery diameter when changes in intravascular perfusion pressure occur simultaneously. At the present time there is no satisfactory method of removing endothelial cells from the resistance arterioles to test the role of these cells in the vasodilator response to acetylcholine. We therefore developed the controlled preparation of the blood-perfused femoral artery in which endothelial cells can be removed mechanically from a selected site.

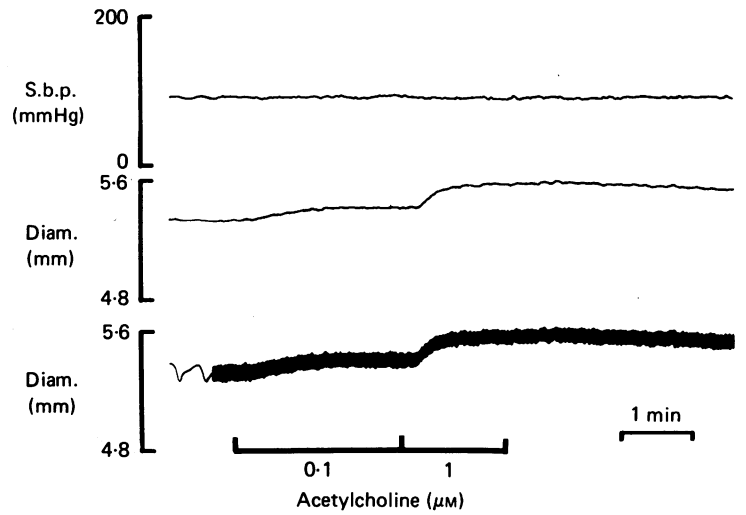

Fig. 5. Chart records from a pump-perfused femoral artery (controlled preparation) showing the dilator response to acetylcholine (0·1–1 μM) infused intra-arterially. No change in mean side-branch pressure (S.b.p.) was noted.

Acetylcholine: controlled preparation

Acetylcholine infused into the artery segment via a side port (see Fig. 1) caused concentration-dependent small rises in diameter. The threshold concentration was 10–30 nM with a short (30 s) latency but a long (10–15 min) offset time (see Fig. 5). The maximum increase in external diameter was about 0·4 mm (i.e. ~ 10%) at a concentration of 10 μM (Fig. 6). These changes in diameter were due to a direct action of acetylcholine since no change in the side-branch pressure was noted in this controlled preparation.

To examine the role of the endothelium in this response to acetylcholine, two pairs of sonomicrometer crystals were applied to the artery, separated by 1–1·5 cm. An intra-arterial infusion of acetylcholine (10 μM) or (−)-noradrenaline (1 μM) was given and the responses recorded from one pair of crystals. The same procedure was repeated while the diameter changes were recorded from the second set of crystals because only one sonomicrometer was available. Endothelial cells were removed from the area under one pair of crystals (site 2) by inserting a Fogarty balloon catheter (see Methods). The acetylcholine and noradrenaline infusions were then repeated for each pair of crystals.

The dilator response of the large artery to intra-arterial acetylcholine (10 μM) was

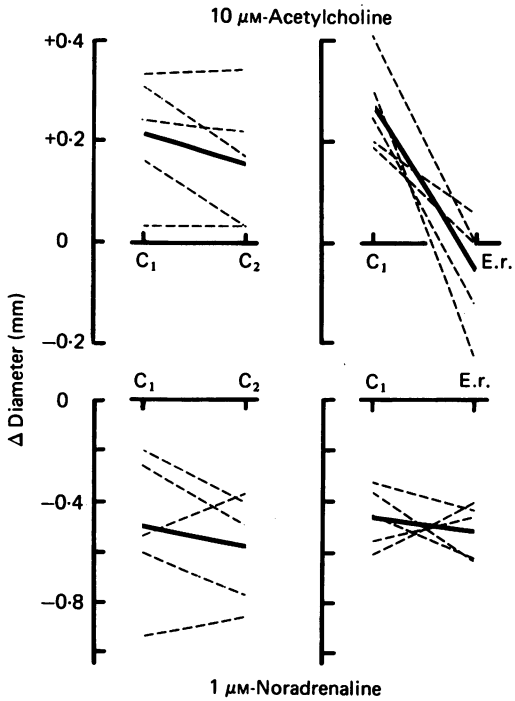

Fig. 6. Changes in femoral artery diameter (mm) measured at two sites 1·5 cm apart in five arteries, during the intra-arterial infusion of 10 μM-acetylcholine (top) and 1 μM-noradrenaline (bottom). Each site was measured twice with each drug. Site 1: C_1 and C_2 refer to the first and second infusion respectively with endothelium present. Site 2: C_1 refers to the first infusion and E.r. refers to the second infusion after endothelium removal by balloon catheter. Dashed lines join values within artery; heavy lines join mean changes.

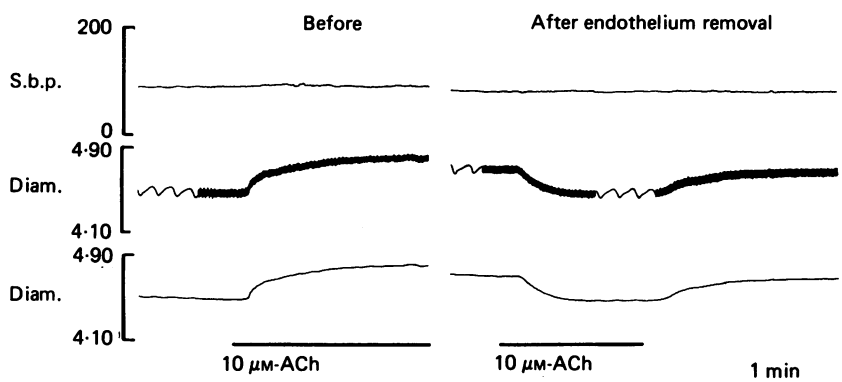

Fig. 7. Chart records from a controlled preparation (left) showing an increase in femoral artery diameter (mm) during acetylcholine infusion (10 μM I.A.) with little change in side branch pressure (S.b.p., mmHg). After removal of endothelium by balloon catheterization the same concentration of acetylcholine caused femoral artery constriction.

not significantly different when repeated some 30 min later (C_1 *versus* C_2, paired *t* test, Fig. 6, site 1). In the same artery, but 1·5 cm away (site 2) removal of the endothelium prevented the dilator response to acetylcholine and in two out of five vessels there

was marked constriction (Fig. 6, site 2 and Fig. 7). The site 2 (endothelium removed) was alternated between the proximal and distal crystals. When the endothelium was removed from the distal crystal area, acetylcholine still dilated the segment of artery upstream (proximal site) but either constricted or caused no change to the segment downstream. This indicates the local nature of the endothelium-dependent dilator signal since the dilator signal released upstream was ineffective at the distal site (only 1–1·5 cm away). The constrictor responses to noradrenaline infusions were similar at the two sites and were little affected by the removal of endothelium (Fig. 6). The resting diameter at site 1 was $4{\cdot}12 \pm 0{\cdot}73$ mm (mean ± 1 S.E. of mean for C_1 and $4{\cdot}18 \pm 0{\cdot}60$ mm for C_2. At site 2 the resting diameter fell from $4{\cdot}32 \pm 0{\cdot}48$ mm before to $4{\cdot}11 \pm 0{\cdot}22$ mm after endothelium removal. The change in resting diameter was not significant (paired t test) at either site.

ATP, bradykinin and substance P

Other dilator drugs were tested in this preparation. ATP (1–10 μM) caused small increases in the diameter of $0{\cdot}067 \pm 0{\cdot}02$ mm ($n = 7$) but reduced the vessel diameter after endothelium removal ($-0{\cdot}05 \pm 0{\cdot}05$ mm, $n = 7$). Bradykinin (0·01–1 μl) dilated the artery to a smaller extent than acetylcholine (0·01–0·11 mm, $n = 4$), and after endothelium removal the same concentrations of bradykinin still dilated three arteries but caused a small contraction ($-0{\cdot}06$ mm) in another artery. Substance P was the most potent dilator of the femoral artery. Responses were obtained in five arteries with intra-arterial infusions of 0·1–1 nM (Fig. 8). In every case, removal of endothelium prevented a subsequent dilator response to these substances.

In two arteries, dilator responses to acetylcholine (100–1000 nM) and substance P (0·1–1 nM) were obtained before and 15 min after the cyclo-oxygenase inhibitor indomethacin (5 mg/kg I.V.) (Fig. 8). A role for newly synthesized prostacyclin PGI_2 in the dilator response is therefore unlikely. In two experiments nitroprusside 0·01 (mg/min) was infused into the femoral artery. The 5% increase in diameter was unaltered by the endothelium removal. In contrast to intra-arterial acetylcholine, nitroprusside infusion caused substantial falls in the systemic pressure from 120 to 40 mmHg. This severe hypotension limited the concentration that could be used in the femoral perfusion.

Topical application of drugs

Topical application of acetylcholine chloride (2, 20, and 200 μg in 2 ml volumes, i.e. 5·5 μM–0·55 mM) caused increases in the diameter of the autoperfused femoral artery without substantially changing the arterial pressure (Fig. 9). At the highest dose, some acetylcholine reached the femoral resistance bed since a small rise in femoral flow and fall in resistance was observed. In six controlled preparations acetylcholine (5 μM–1 mM topically) increased the diameter by $0{\cdot}15 \pm 0{\cdot}03$ mm. The concentrations applied topically that are necessary to cause large femoral artery dilatation are 50–100 times greater than the concentrations of acetylcholine that relax the artery from the lumen. In contrast, noradrenaline (0·2 μg in 2 ml, i.e. 0·59 μM) applied topically in three experiments caused a fall in femoral diameter (0·2–0·4 mm). This concentration was in the range (0·1–1 μM) that constricted the artery during intra-arterial infusion.

Endothelium removal abolished the dilator response to topical acetylcholine

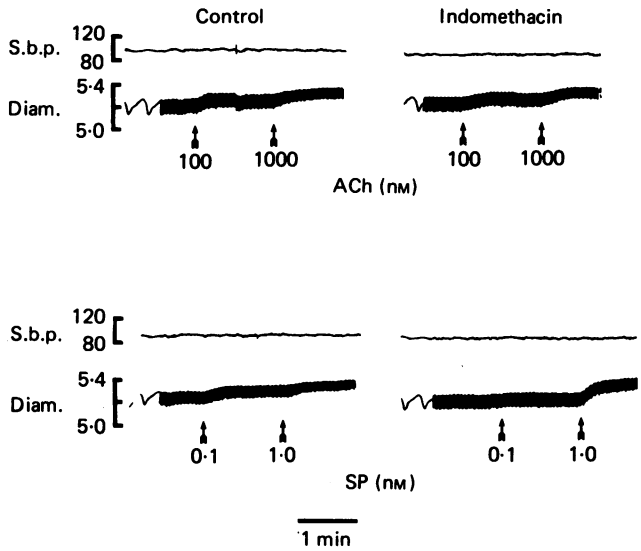

Fig. 8. Chart records from a controlled preparation showing mean side-branch pressure (S.b.p., mmHg) and femoral artery diameter (Diam., mm) during infusion (I.A.) of acetylcholine (100–1000 nM) (top) and substance P (SP, 0·1–1 nM) (bottom) before (left) and 15 min after indomethacin (5 mg/kg I.V.) (right).

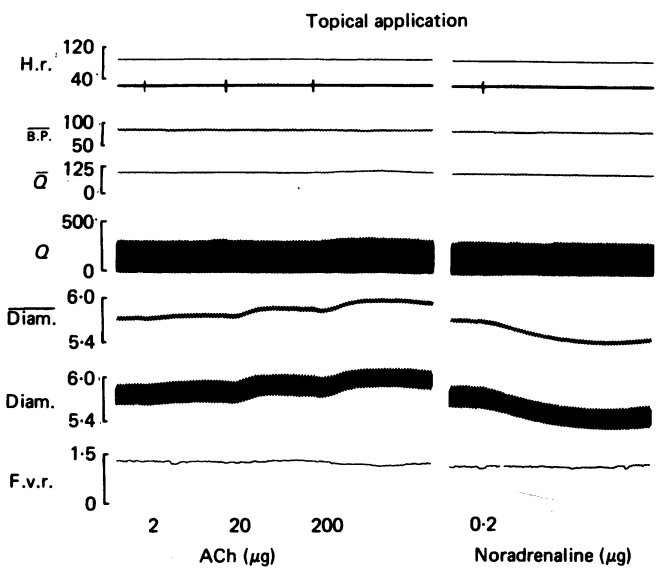

Fig. 9. Chart records from an uncontrolled (auto-perfused) preparation showing the effects of acetylcholine (2–200 μg) and noradrenaline (0·2 μg) in 2 ml, applied topically (adventitia) to the femoral artery. Parameters from top are H.r. = heart rate (beats/min); B.P. = mean arterial pressure (mmHg); $\bar{Q}$ and Q, are mean and phasic femoral blood flow (ml/min); Diam. and Diam. = mean and phasic femoral diameter; F.v.r. = femoral vascular resistance (mmHg/ml per minute).

(0·1–0·5 mM) in the controlled preparation. In two preparations, raising the acetylcholine concentration to 10 mM caused a substantial constriction in the absence of endothelium (Fig. 10).

Topical application of substance P (50–500 nM) in three arteries also increased the femoral diameter to a similar degree as did acetylcholine in the autoperfused and controlled preparations; in the latter case the effect was abolished following removal of the endothelium. As with acetylcholine, concentrations of substance P were 50–100 times higher than those required to dilate the artery during lumen infusion.

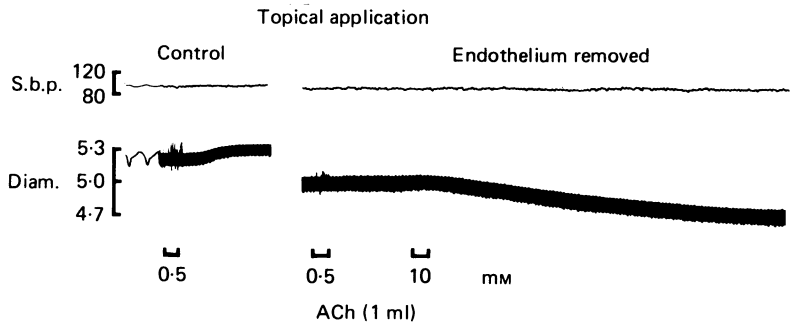

Fig. 10. Chart records from a controlled preparation showing side-branch pressure (S.b.p., mmHg) and femoral diameter (Diam., mm) during topical application of 1 ml acetylcholine (0·5–10 mM) before (left) and after endothelium removal (right).

Histology

In each femoral artery only the area below one pair of sonomicrometer crystals was completely denuded of endothelium by balloon catheterization. The intima below the second pair of crystals therefore served as control (Pl. 1). Endothelial cells were observed by the *en-face* silver-staining technique. The balloon catheterization completely removed the endothelial cells (Pl. 2) and the site was covered by platelet microthrombi. Higher power electron microscopy showed the extent of the platelet carpet adherent to the endothelium denuded surface. In addition, many of the smooth muscle cells between the internal elastic lamina and the endothelium (intima) were damaged by the balloon catheter (see Pl. 2). Little evidence of damage to the medial smooth muscle layers was found.

DISCUSSION

Measurements of arterial diameter

The preparation of the pump-perfused artery with distal Starling resistance allowed (a) the measurement of diameter in response to topical application or drug infusions under steady-state conditions, (b) the accurate assessment of the passive pressure–diameter relationship and (c) ready access for the Fogarty embolectomy catheter for endothelium removal.

Numerous reports of hysteresis loops in the pressure-diameter relationships have been reported for a single cardiac cycle in sheep aorta (Pagani, Mirsky, Baig, Manders, Kerkhof & Vatner, 1979) and for the greyhound femoral artery *in vivo* (Bertram, 1977;

see also the review by Dobrin, 1978). We did not use a high-fidelity pressure transducer but relied on a short, stiff catheter in the saphenous artery. This may have given some phase lag in the pressure record compared with the diameter for a single cardiac cycle. However, the steady-state mean values of pressure and diameter recorded during the alteration in mean pressure from 60 to 140 mmHg showed a similar hysteresis loop and could not be accounted for by phase lag.

Acetylcholine and endothelium

Acetylcholine delivered by intra-arterial infusion in the controlled preparation was similar in potency in causing an increase in diameter to that reported for relaxation of canine femoral artery rings studied *in vitro* (De Mey & Vanhoutte, 1981). In contrast, acetycholine applied topically was 50–100 times less potent. This difference may be due to the inadequate exposure of the artery wall (especially on the ventral surface) to the acetylcholine solution. Alternatively cholinesterases located in the perivascular adventitial plexus in the vessel wall would lower the concentration of acetylcholine that reached the endothelium. There is evidence that the enzyme is located in the large and small coronary arteries in humans, dogs and monkeys (Amenta, Ferrante & Cavallotti, 1981; Pillay & Reid, 1982). If the innervation of femoral arteries is similar, there would be a larger concentration of the enzyme towards the adventitia than in the media or intima. Assuming that the endothelial cell is the necessary source of the vasodilator signal to acetylcholine then it follows that for the topical application of acetylcholine the concentration gradient from the adventitia to the intima could account for the observed difference in potency.

Similarly, substance P-like immunoreactive nerve fibres and varicosities have been found extensively in the adventitia and the adventitia–media border of most blood vessels (including the femoral artery) of the guinea-pig (Furness, Papka, Della, Costa & Eskay, 1982). In the cerebral circulation of the cat the distribution of fibres reactive to substance P is well defined (Edvinsson, McCulloch & Uddman, 1981, 1982). It is likely, therefore, that enzymes specific for the destruction of substance P would be associated with the nerve terminals in the femoral artery wall. This would simply explain the lower potency of substance P delivered topically compared with the potency during intra-arterial infusion.

Our experiments support the general hypothesis of Furchgott & Zawadzki (1980) and of De Mey & Vanhoutte (1981) in femoral artery that endothelial cells are necessary for acetylcholine-mediated vasodilatation. Under some circumstances acetylcholine can cause vasoconstriction *in vitro* (Furchgott & Zawadzki, 1980) and when delivered into the lumen of the artery denuded of endothelium (present experiments). This suggests that direct vascular smooth muscle vasoconstriction to acetylcholine does occur, analogous to the contractile responses in gut smooth muscle. Therefore we were surprised to observe that acetylcholine applied topically could cause an endothelium-dependent relaxation, especially in a large muscular artery where the ratio of smooth muscle cells to endothelial cells is large. The topical experiments confirmed that the removal of endothelial cells could alter the acetylcholine vasodilator response to one of vasoconstriction. Perhaps the diffusion of acetylcholine from the adventitia to the endothelial cells is rapid. Thus the onset of the smooth muscle relaxation in response to the released substance from the endothelial cells precedes any slower direct vasoconstrictor response.

Other experiments confirmed that the endothelium removal had not substantially altered the dilator and constrictor responses of the arterial segment. Nitroprusside could still relax the artery while the constrictor responses to intra-arterial or topical noradrenaline were unaltered by endothelium removal.

In summary, our experiments confirm that, at least in the large femoral artery *in vivo* under blood perfusion, endothelium plays an essential role in the response to acetylcholine and other substances such as substance P. The topical experiments suggest that local release of vasoactive substances such as substance P or acetylcholine from neural varicosities in the adventitia or media may also depend on intact endothelial cells to elicit vasodilatation. At this time we can only extrapolate our findings from the large artery to resistance arterioles and suggest that the integrity of the endothelium is essential for the potent and powerful vasodilator responses to acetylcholine and substance P observed *in vivo*.

We acknowledge the excellent assistance of Matthew Le Duc and Susan Weir. This work was supported by grants from the National Health and Medical Research Council of Australia.

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EXPLANATION OF PLATES

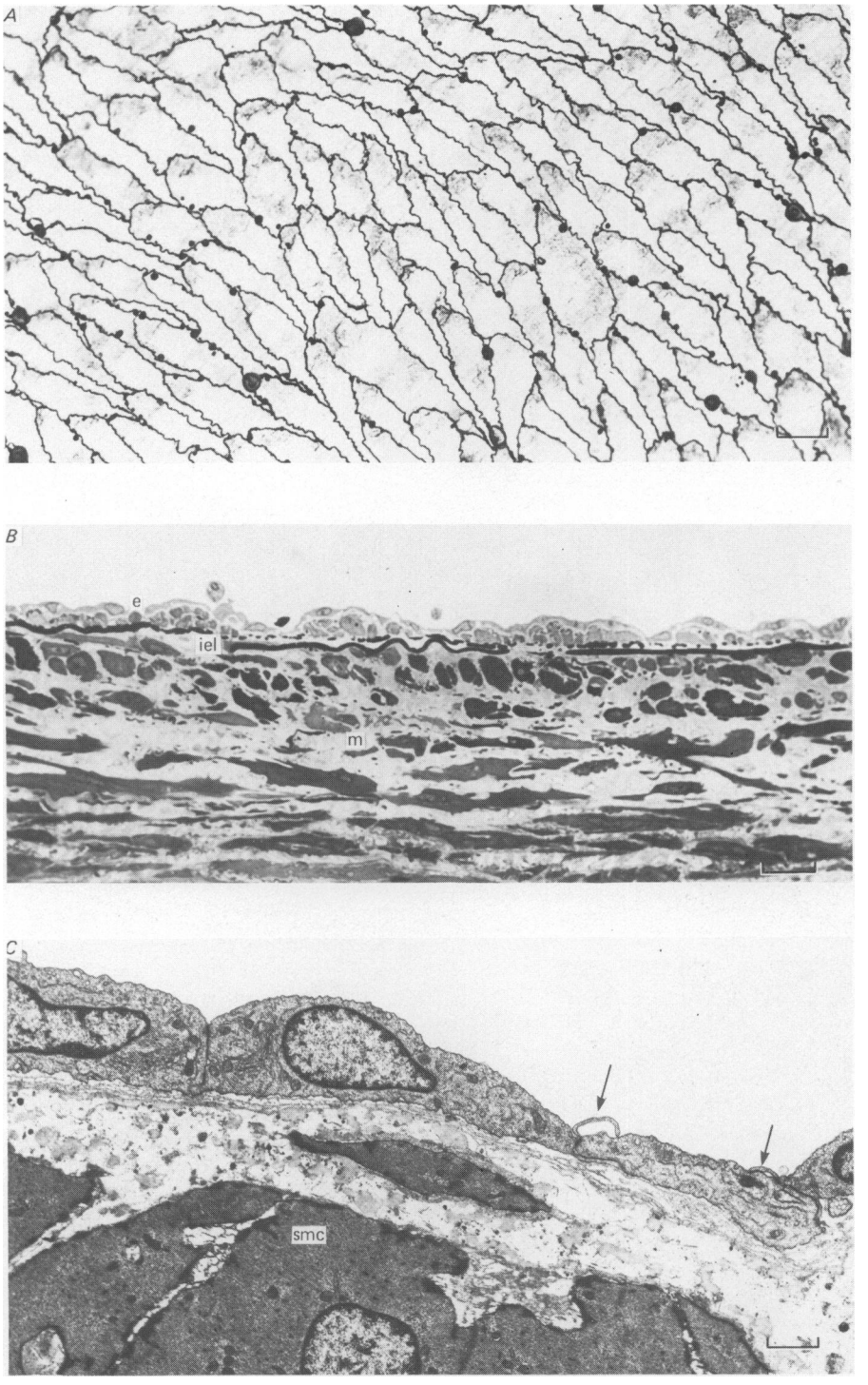
PLATE 1

Dog femoral artery. *A*, a silver-stained *en-face* preparation where endothelial cell borders are clearly demarcated by deposited silver. The cells comprise an intact monolayer. Bar 22 μm. *B*, cross-section of femoral artery reveals a continuous endothelial layer (e). The internal elastic lamina (iel) demarcates the intima from the media (m). Note the presence of smooth muscle cells in the intima. Bar 30 μm. *C*, transmission electron micrograph of vessel intima demonstrating typical features

of endothelial cells. Note intercellular flaps (arrows) and underlying intimal smooth muscle cells (smc). Bar 1·2 μm.

PLATE 2

Dog femoral artery after balloon catheterization. *A*, a silver-stained *en-face* preparation showing a peripheral area of the lesion where intact endothelial cells border upon a completely denuded region. Bar 27 μm. *B*, cross-section of an area where the endothelium has been completely removed and the exposed subendothelial connective tissue is covered by platelet microthrombi (mt). Polymorphs and erythrocytes are present. Note the absence of intact cells between the internal elastic lamina (iel) and the platelet carpet. Bar 28 μm. *C*, electron micrograph of the platelet carpet which exhibits numerous layers of platelets (p) adherent to the denuded surface. Several underlying smooth muscle cells exhibit ultrastructural evidence of degeneration (arrows). Bar 1·6 μm.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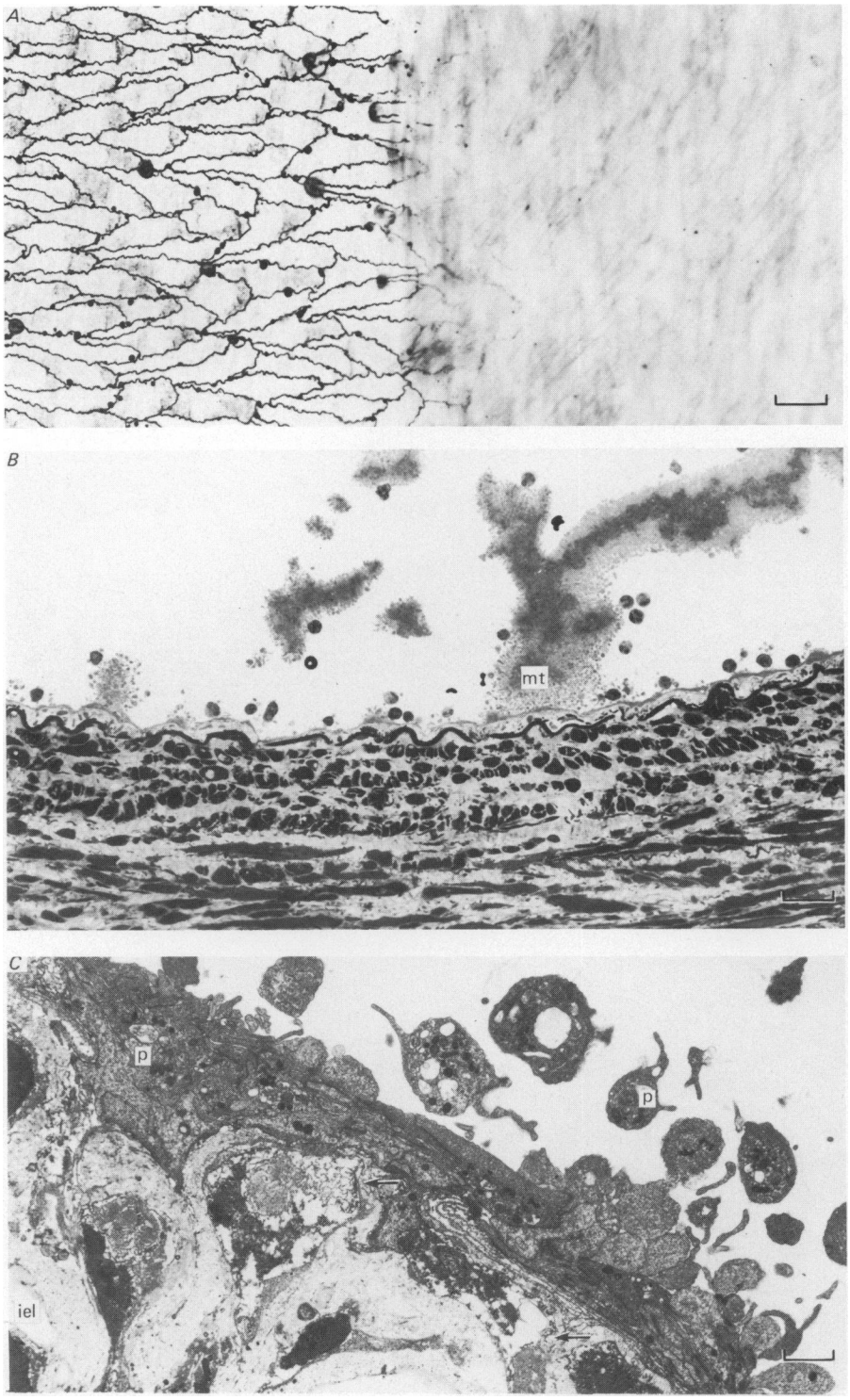
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