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The vascular endothelium forms the lining of the inner surface of blood vessels. Endothelial cells from human umbilical vein were isolated by a simplified technique, which is a modification of the methods of Gimbrone (1976) and Jaffe et al. (1973a). By this method, confluent primary cultures were obtained within 4-5 days. These could be used to study the properties that constitute the specialized anti-thrombotic function of the endothelium.

Cells in culture were characterized as endothelial by the following criteria: (a) the presence of angiotensin-converting enzyme activity (Johnson & Erdos, 1977); (b) the production of an inhibitor of platelet aggregation (prostacyclin) (Weksl er et al., 1977); (c) morphology (Gimbrone, 1976); (d) the presence of Factor VIII-related antigen (Jaffe et al., 1973b).

Endothelial cells were isolated from cords obtained at normal vaginal deliveries. The cord was cut from the placenta soon after birth and the vein was rinsed out with sterile phosphate-buffered saline, pH 7.4, containing penicillin (100 units/ml) and streptomycin (100 µg/ml). Both the ends of the vein were cannulated with trumpet-tipped glass cannulae and secured in position by tying the cord over it with sutures. A small piece of polythene was slipped over the open ends of the cannula. This acted as an adaptor for the syringe and also allowed the infusate to be retained when clamped shut with a haemostat. The vein was rinsed with phosphate-buffered saline to remove all traces of blood, was filled with 0.25% trypsin solution in phosphate-buffered saline (specific activity 0.5 Anson unit/g from bovine pancreas purchased from BDH, Poole, Dorset, U.K.) and incubated in a 37°C water bath for 15 min. After incubation, the trypsin digest was flushed from the vein by infusing with 20 ml of phosphate-buffered saline. The effluent was collected directly into a sterile 20 ml plastic syringe. The cord was massaged gently in the process of eluting its contents. The cells were sedimented from the effluent at 1000 g for 10 min and the cell pellet was resuspended in 5 ml of growth medium (Medium 199 modified with Earles salts, and containing 20 mM-Hepes (4-[2-hydroxyethyl]-1-piperazine-ethanesulphonic acid) buffer, 8 mM-NaHCO₃, 2 mM-L-glutamine, penicillin (100 units/ml) and streptomycin (100 µg/ml)) supplemented with heat-inactivated newborn-calf serum to a final concentration of 20%. The whole suspension was seeded into 25 cm² plastic tissue culture flasks and incubated at 37°C. The medium was changed after 48 h. Confluent culture was obtained in about 4 days. In all the experiments described here, cells were used after one passage and were not subcultured.

Angiotensin-converting enzyme activity was measured by a spectrofluorimetric method based on the formation of the fluorescent adduct of o-phthalaldehyde and the histidyl moiety of the L-histidyl-L-leucine product formed from the hippuryl-L-histidyl-L-leucine substrate (Friedland & Silberstein, 1976). When the substrate concentration was 1.6 mM endothelial cells converted 17% of the substrate in 24 h into free L-histidyl-L-leucine. In contrast, human skin fibroblasts (Flow 1000) hydrolysed only 1% of the substrate in the same time.

Weksl er et al. (1977) have shown that cultured endothelial cells produce prostacyclin, an inhibitor of platelet aggregation. This activity was found to be specific to endothelial cells, since control cell types were found to possess little, or no, prostacyclin activity (Fig. 1).

Endothelial cells were described by Gimbrone (1976) as closely opposed polygonal cells and within the monolayer were uniform simple squamous epithelial-like cells. The umbilical-vein cells fitted this description when examined live under the phase-contrast microscope or after fixing and staining under the microscope.

Factor VIII protein has been reported to be synthesized and secreted by endothelial cells (Jaffe et al., 1973b). In a preliminary study the cells isolated from the vein exhibited a strong fluorescence when tested for factor VIII antigen by an indirect immunofluorescence technique. Cells were first incubated with an antibody to human factor VIII that had been raised in rabbits and the cells were then treated with antibody to rabbit immunoglobulin G that had been raised in a goat and then conjugated with fluorescein. This caused a bright fluorescence in >95% of the cells of an endothelial culture, whereas control cultures, such as fibroblasts, did not take up the dye.

Thus each of the above four criteria were found to be useful for the characterization of endothelium. A cultured cell preparation from calf brain, which has been described as being of capillary endothelial origin (Phillips et al., 1976), was found to be have no detectable angiotensin-converting enzyme activity and negligible prostacyclin production and thus these cells were shown to be much different to the large vein endothelial cells produced in this laboratory.

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Prostacyclin production by cultured endothelium

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A major function of endothelial cells is to provide a surface that does not encourage the aggregation of platelets. In addition, these cells produce an unstable molecule prostacyclin, which has potent aggregation inhibitory activity (Gryglewski et al., 1976).

In this study the medium of human umbilical-vein endothelial cells has been examined for its effects on aggregation of human platelets. Endothelial cells were grown in plastic vessels to confluence in Medium 199 supplemented with 20% newborn calf serum (Patel & McEvoy, 1980). Platelets were suspended in plasma at 37°C in an aggregometer and the effect of the cultured cells was measured by addition of solutions that had bathed the cultured cells for certain times. These solutions were either (a) platelet-poor plasma or (b) Gay's medium modified with Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer (Patel & McEvoy, 1980). The assay system consisted of 0.3 ml of platelet-rich plasma, mixed with 0.3 ml of either plasma or Gay's medium. The fact that the inhibitory activity was due to a prostaglandin was shown by demonstrating that production of the inhibitor was blocked by compounds such as aspirin. The inhibitory activity was adjudged to be entirely due to prostacyclin because of the fact that it was completely destroyed by incubation for 30 min at 37°C. In this study, ADP was used as aggregating agent. A concentration of 2.5 µM-ADP was used throughout. Under these conditions the prostacyclin completely inhibited aggregation. At higher ADP concentrations, however, the prostacyclin preparation was only partly inhibitory. A particular difficulty of these investigations was the aging of the platelets, even when stored in plasma at 37°C. The extent of aggregation under the conditions of this assay was found to vary with the age of the platelets. However, prostacyclin appeared to be equally effective as an inhibitor of aggregation of aged platelets as it was with newly isolated platelets.

Prostacyclin has been reported to have a half-life of approx. 5 min (Gryglewski et al., 1976). However, our experiments have shown that the half-life may be considerably longer in the presence of plasma proteins, perhaps as long as 20 min. This may have considerable significance when evaluating the physiological role of prostacyclin. At present it is not clear whether prostacyclin is a localized anti-thrombotic mechanism produced in response to local injury, or whether it is a true circulating hormone constantly being produced as a mechanism of controlling platelet function (Moncada et al., 1978).

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Ionomycin stimulates T-lymphocytes to grow

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Various results suggest that stimulation of T-lymphocyte growth by mitogens is initiated by an increase in the intracellular Ca²⁺ concentration (Crumpton et al., 1977). The most cogent evidence is provided by the demonstration that the Ca²⁺ ionophore A23187 stimulates pig mesenteric-lymph-node and human peripheral-blood lymphocytes to transform in an apparently identical manner to that induced by such polyclonal mitogens as Phagellus vulgaris phytohaemagglutinin ('leuko-agglutinin') and concanavalin A (Lackasen et al., 1974; Maino et al., 1974; Hovi et al., 1976; Jensen et al., 1977). Alternatively, Kaplan (1977) has argued that the growth-promoting capacity of ionophore A23187 may be unrelated to its action as a Ca²⁺ ionophore. Thus ionophore A23187 may act directly, in a similar manner to the non-Ca²⁺ ionophore 12-O-tetradecanoylphorbol 13-acetate, by interacting with a cell-surface 'receptor' or perturbing the plasma-membrane structure. The present results show that ionomycin, a Ca²⁺ ionophore that is unrelated structurally to ionophore A23187 (Liu & Herman, 1978), induces T-lymphocyte growth as judged by the incorporation of thymidine into DNA. These results argue strongly against Kaplan's (1977) explanation.

Ionomycin, an antibiotic produced by Streptomyces conglobatus, has a higher specificity for Ca²⁺ relative to Mg²⁺ than has ionophore A23187 (Liu & Herman, 1978), extracts Ca²⁺ from an aqueous to an organic phase and stimulates mast cells to release histamine (Bennett et al., 1979). Fig. 1(a) shows that ionomycin was almost as effective as P. vulgaris phytohaemagglutinin and much more effective than ionophore A23187 in stimulating pig mesenteric-lymph-node cells to synthesize DNA; similar concentrations of ionomycin and ionophore A23187 induced optimal synthesis (about 0.8 µg/ml). Ionomycin was less effective at stimulating the growth of human peripheral-blood lymphocytes (about 25% of the phytohaemagglutinin response), whereas with mouse (Balb/c) spleen lymphocytes, little stimulation of thymidine incorporation (about 2-fold background) was detected (Fig. 1(b)). This spectrum of species variation was similar to that obtained previously with ionophore A23187 and most probably reflects the increasing toxicity of Ca²⁺ ionophores for the different species (Maino et al., 1974; Hovi et al., 1976). Thus ionomycin concentrations greater than 0.4 µg/ml were toxic for mouse lymphocytes, whereas 1 µg/ml was non-toxic for pig lymphocytes. The time course for DNA synthesis, as well as the dependence of synthesis on extracellular Ca²⁺ concentration


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