MEDIATION OF PINOCYTOSIS IN CULTURED ARTERIAL SMOOTH MUSCLE AND ENDOTHELIAL CELLS BY PLATELET-DERIVED GROWTH FACTOR

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ABSTRACT

Pinocytosis was measured in monkey aortic smooth muscle cells (SMC), bovine aortic endothelial cells, and Swiss 3T3 cells in culture as cellular uptake of [U-^{14}C]sucrose and horseradish peroxidase (HRP) from the tissue culture medium.

Monkey arterial SMC and Swiss 3T3 cells were maintained in a quiescent state of growth at low cell density in medium containing 5% monkey plasma-derived serum (PDS). Replacement of PDS with 5% monkey whole blood serum (WBS) from the same donor, or addition to PDS of partially purified platelet-derived growth factor(s) (PF), resulted in a marked stimulation of pinocytosis as well as of cellular proliferation. In SMC, enhancement of the rate of pinocytosis occurred 4–6 h after exposure to WBS or PF, and the rate was up to twofold higher than the rate in medium containing PDS. In contrast, [^{3}H]thymidine uptake by SMC did not increase until 12–16 h after exposure to PF.

In endothelial cells the presence of PF or WBS did not enhance either the rate of pinocytosis or the rate of proliferation over that in PDS. Thus, endothelial cells did not become quiescent at subconfluent densities in PDS but maintained rates of proliferation and pinocytosis that were equivalent to those in WBS.

By autoradiography, the fraction of labeled nuclei in SMC cultures 24 h after change of medium increased from 0.061 ± 0.004 in quiescent cultures to 0.313 ± 0.028 after exposure to WBS or PF. In contrast, labeling indices of endothelial cells were similar for cultures grown in PDS, WBS, or PF at any single time point after change of medium.

These findings suggest that the rate of pinocytosis may be coupled in some fashion to growth regulation, which may be mediated in part by specific growth factors, such as that derived from the thrombocyte.

KEY WORDS pinocytosis · platelet factor · endothelial cells · smooth muscle cells · growth

Cellular endocytosis describes the phenomenon of invagination of plasma membrane resulting in the formation of membrane-bounded cytoplasmic vesicles which may or may not contain insoluble particulate matter (1, 19). Endocytosis therefore includes phagocytosis and pinocytosis. In cells which are not specialized for phagocytosis, internalization of extracellular fluid with its accompa-

nying soluble substances is considered to occur by pinocytosis. Although common to a wide variety of eukaryotic cells, the factors that regulate pinocytosis and their mechanism of action remain poorly understood. This is partly because of difficulties encountered in the quantitation of pinocytosis, particularly in the choice of tracer molecules. Interaction of tracer with the plasma membrane may result in measurement of adsorptive pinocytosis as well as fluid pinocytosis (13). Tracers of pure fluid pinocytosis have been chosen in more recent studies (25, 19, 27), and these techniques have been applied to investigations of the interrelationships between pinocytosis and other cellular events (for review, see reference 19).

In our studies of basic rates of fluid pinocytosis in arterial smooth muscle cells (SMC) under various conditions, we noted considerable variation when cells were cultured in media containing different amounts of homologous whole blood serum (WBS) in which platelet-derived growth factor(s) (PF) is found, but much less variation when SMC were maintained in medium containing serum prepared from cell-free plasma (plasma-derived serum, PDS) derived from the same pool of blood. In this paper, we report rates of fluid pinocytosis by arterial SMC, endothelial cells (EC), and Swiss 3T3 cells in PDS, WBS, and in the presence of partially purified PF. The relationships between growth factor, cell proliferation, and fluid pinocytosis are considered.

MATERIALS AND METHODS

Chemicals

[U-^{14}C]sucrose, specific radioactivity 396 Ci/mol, was obtained from New England Nuclear, Boston, Mass. Horseradish peroxidase (HRP) Type II, σ-dianisidine, poly-D-lysine (mol wt 150,000), poly-L-lysines (mol wt 17,000, 32,000, 240,000), poly-L-asparagine (mol wt 8,000) were supplied by Sigma Chemical Co., St. Louis, Mo. Sodium dodecyl sulphate (SDS) was obtained from British Drug Houses Ltd., Poole, Dorset, U. K.

Cell Culture

SMC were grown from explants of the inner media of thoracic aorta of the pigtail monkey (*Macaca nemestrina*) according to the methods of Ross (14). The culture medium was Dulbecco-Vogt's modification of Eagle's basal medium (DME) supplemented with 5% homologous WBS. For the studies reported here, the cells were in second or third subculture. Swiss 3T3 cells were kindly supplied by Dr. Robert Pollack, Department of Microbiology, S.U.N.Y., Stony Brook, N. Y. Stocks were maintained in DME medium containing 10% calf serum. Adult bovine aortic EC were a gift from Dr. Stephen Schwartz. They were grown in DME supplemented with 10% fetal calf serum. There was a complete absence of contaminating smooth muscle cells as determined by the absence of staining with antibody to SMC actin, myosin, and tropomyosin (4).

All cultures were maintained at 37°C in humidified 95% air/5% CO_2. For counting, cells were detached from the flask by incubation for 10 min with trypsin (0.125%)-EDTA (0.025%) in Tris buffer at 37°C. After agitation of the cells by multiple pipetting with siliconized glass pipettes, cells were counted in a Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.) with thresholds optimized for each cell type.

Preparation of WBS, PDS, and PF

PDS and WBS were prepared as recently described (24), essentially by the method reported by Rutherford and Ross (17) but with additional removal of platelet factor(s) from PDS by carboxymethyl-Sephadex (C-50, Pharmacia Fine Chemicals, Piscataway, N. J.) column chromatography.

A 20,000-fold purification of PF from outdated human platelet-rich plasma was carried out by CM-Sephadex chromatography, ammonium sulphate precipitation, and gel filtration on Bio-Gel P100 (Bio-Rad Laboratories, Richmond, Calif.) as fully described elsewhere (24).

Measurement of Rate of Pinocytosis

Before the measurement of the rate of pinocytosis, cells that had been grown in WBS were trypsinized, centrifuged at 200 *g* for 7 min, then resuspended in DME containing 5% monkey PDS. They were recentrifuged and finally resuspended in fresh quantities of the same medium. This procedure dilutes most of the platelet-derived growth factors that may be loosely associated with the cells. The cells were then plated (Cornwall syringe, Becton, Dickinson & Co., Rutherford, N. J.) in 35-mm or 60-mm diameter plastic petri dishes (Falcon Plastics, BioQuest, BBL & Falcon Products, Cockeysville, Md.) in 2 or 4 ml of DME supplemented with 5 or 20% monkey PDS (DME-PDS), usually at a density of 2×10^3 cells/cm^2. After 48 h the medium was replaced with fresh DME-PDS. On the third or fourth day after plating, the medium was replaced with either fresh DME-PDS or DME supplemented with 5 or 20% monkey WBS (DME-WBS). In some experiments, 100 μl of DME-PDS-PF solution was added to cultures maintained in DME-PDS to a final concentration of PF equivalent to 5, 20, or 50% WBS; controls in these experiments received 100 μl of DME-PDS. Measurement of the rate of pinocytosis was made during a 2-h period beginning at various times after exposure to PF as indicated in the figure legends.

Two tracers of fluid pinocytosis, [U-^{14}C]sucrose, and

HRP, were used in these studies, either individually or together in the same culture medium. Immediately before addition of tracer to the cells, the volume of culture medium was adjusted to 1 or 2 ml, and tracer was added in a small volume (usually 10 μl) of phosphate-buffered saline (PBS) or isotonic saline. The final activities of radiolabeled sucrose and HRP/ml of culture medium were 10 μCi and 1.33 mg, respectively. An aliquot of medium was removed from each dish for precise determination of ^{14}C radioactivity. Cultures were incubated at 37°C. At 4°C, pinocytosis effectively ceased (1), allowing incubations at this temperature to serve as controls for binding of tracer to the cell surface. Therefore, during each experiment some cultures were incubated in gassed boxes (95% air/5% CO_2) at 4°C.

For washing of tracers from cultures and for the enzymatic assay of intracellular HRP, the procedures of Steinman and Cohn (20) and Steinman et al. (21) were closely followed. After cells were exposed to either tracer, they were gently washed five times with calcium- and magnesium-free PBS. 2 ml of DME-PDS medium were then added to each dish, and the cells were reincubated at 37°C for 30 min, followed by three washes with PBS. Cells were then lysed by the addition of 1 ml of 0.1% (aqueous) SDS. In cultures exposed to both tracers, duplicate 0.1-ml aliquots of the lysate were assayed for HRP activity by the methods of Steinman and Cohn (20). To the remaining 0.8 ml were added 10 ml of Aquasol (New England Nuclear) and the samples were counted in a Packard liquid scintillation counter (Packard Instrument Co., Inc. Downers Grove, Ill.). Counting efficiencies (range: 70–80%) were determined from external standard ratios by reference to a quenched set of [^{14}C]toluene standards (New England Nuclear) prepared in Aquasol.

Cell numbers were determined in cultures subjected to the same procedures as those cultures used in the measurement of pinocytosis. From the activities of tracers taken up by the cells and those activities measured in the culture medium, and assuming HRP and sucrose to exist entirely in the fluid phase, the quantities of fluid pinocytosed by the cells were calculated and expressed per 10^6 cells/h of incubation. This calculation is similar to the "endocytic index" of Williams et al. (27).

Incorporation of [^3H]Thymidine into DNA

[^3H]thymidine (0.1 μCi/ml, specific activity 6–7 Ci/mmol) was added to the culture medium for 3-h periods up to 48 h after exposure of cells to WBS or PF. Dishes of cells were removed, lysed with 1 N NaOH, and 1 ml of 20% trichloroacetic acid (TCA) was added to each. The precipitate was washed four times, then filtered (Millipore, 0.22 μ pore size, Millipore Corp., Bedford, Mass.) under reduced pressure. Each filter was transferred to a scintillation vial, and 10 ml of Liquifluor (New England Nuclear) were added. [^3H]thymidine incorporation into DNA was measured in a Packard scintillation counter. Counting efficiencies (range: 30–35%) were determined from external standard ratios by reference to a quenched set of [^3H]toluene standards prepared in Liquifluor.

For autoradiography, cells were pulse-labeled for 3 h with [^3H]thymidine (0.3 μCi/ml medium) in the appropriate medium, washed with PBS, and immersed in Holley's fixative (11) for several minutes. After further rinsing, the cells were air-dried. The floor of each culture dish was then cut out with a small circular saw, mounted in halves on a glass slide with polyvinyl-pyrrolidone, sealed, and the slides were dipped in Kodak NTB2 emulsion and exposed for 14 days at 4°C. After development of silver grains, the cells were stained with hematoxylin-eosin. Determination of the fraction of labeled nuclei was made on 1,000 cells in five radial sectors of each dish. Thus, a total of at least 5,000 cells per dish was counted.

RESULTS

Proliferative Response to PF

Growth curves showing the proliferation of arterial SMC and 3T3 cells cultured in the presence of WBS as compared with PDS are shown in Figs. 1*a* and 1*b*. Both cell types showed little net change in cell number when cultured in DME-5% PDS, and achieved a steady state defined as quiescence (17). Replacement of the medium by DME-5% WBS, or addition of platelet extract, initiated DNA synthesis and cell division as demonstrated previously (15, 16, 24).

Cultured adult bovine aortic endothelial cells (EC) behaved differently from SMC and 3T3 cells with respect to growth response in the presence of WBS or PF (Fig. 1*c*). EC did not attain quiescence in PDS, and there were no significant differences in the growth curves when EC were exposed to 20% PDS, 20% WBS, or PDS + PF (equivalent to 20% WBS or 50% WBS). These observations are similar to those on human endothelial cells reported by Wall et al. (26), although different from those of Gospodarowicz et al. (10) who observed that platelet lysate was mitogenic for bovine aortic endothelial cells in 2.5% plasma serum, a lower level of plasma serum than that in the present study (20%).

Autoradiography

The fraction of SMC nuclei which were labeled by [^3H]thymidine was 0.061 ± 0.004 in 5% PBS (quiescence), rising to 0.313 ± 0.028 at 24 h after

addition of PF. In endothelial cells, there were no significant differences in labeling of nuclei of cells exposed to PDS, WBS, or PF, but there was a decline in the fraction of labeled cells as confluence was approached (Table I).

[^{14}C]Sucrose and HRP as Pinocytosed Tracers

In experiments where both sucrose and HRP were added to the same culture medium, it was necessary to determine whether the presence of either tracer affected pinocytosis of the other. There was no significant difference in the uptake of [^{14}C]sucrose in the presence or absence of HRP; similarly, HRP uptake was unaffected by the presence of [^{14}C]sucrose.

Both [^{14}C]sucrose and HRP meet the major criteria required for a tracer of fluid uptake by

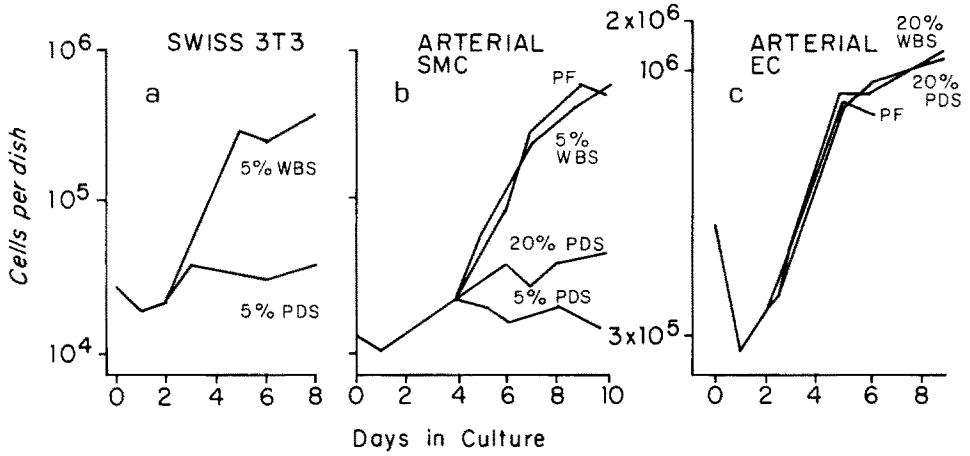

FIGURE 1 Growth-curves of cultured (*a*) Swiss 3T3 cells, (*b*) monkey arterial smooth muscle cells (SMC), and (*c*) bovine arterial endothelial cells. Cells were plated (35-mm diameter dishes) on day 0 in DME + 5% PDS (SMC and 3T3) or DME + 20% PDS (EC). On day 2 or 3, PDS was replaced by either fresh PDS, WBS, or PDS + PF. PDS: Plasma-derived serum. WBS: Whole blood serum. PF: Platelet-derived growth factor(s). DME: Dulbecco-modified Eagle's medium.

TABLE I

Fraction of Smooth Muscle and Endothelial Cell Nuclei Labeled by 3-h Pulse of [^{3}H]Thymidine in the Presence of PDS, WBS, or PF

	Hours after medium changed from 5% PDS	
Medium	0	24
SMC		
5% PDS	0.061 ± 0.002	0.064 ± 0.004
20% PDS	0.058 ± 0.002	0.072 ± 0.005
5% PDS + PF (≡5% WBS)	–	0.313 ± 0.028

	Hours after medium changed from 20% PDS				
Medium	0	10	24	48	72
Endothelial cells					
20% PDS	0.271 ± 0.020	0.276 ± 0.023	0.248 ± 0.011	0.088 ± 0.023	0.052 ± 0.009
20% WBS	–	0.306 ± 0.020	0.248 ± 0.026	0.118 ± 0.036	0.038 ± 0.010
20% PDS + PF (≡20% WBS)	–	0.234 ± 0.018	0.285 ± 0.053	0.169 ± 0.054	0.050 ± 0.010
20% PDS + PF (≡50% WBS)	–	0.293 ± 0.034	0.279 ± 0.038	0.120 ± 0.026	0.051 ± 0.008

Mean ± 1 SD.

pinocytosis (21, 25). Both are pure solutes impermeable to the plasma membrane and both interact minimally with the plasma membrane and other components in the medium. Neither stimulates its own rate of uptake. It therefore follows that the quantity of fluid pinocytosed when measured by sucrose uptake should be the same as that measured by HRP uptake, provided there is no exclusion from pinosomes of one or the other tracer on the basis of particle size and provided the rates of degradation within the cell, or loss from the cell by other mechanisms (e.g., exocytosis, cell death), are similar. Fig. 2 demonstrates that in 3T3 cells the rates of fluid pinocytosis are consistently lower when calculated from intracellular HRP activity than from [^{14}C]sucrose uptake by the same cells. As reported by others (21), HRP uptake increased linearly for the first 2–3 h, after which it reached a plateau, whereas [^{14}C]sucrose uptake continued to increase in a linear fashion for at least 6 h (Fig. 2). Pinocytosis as measured by either tracer was almost completely inhibited at 4°C.

Differences in the rate of pinocytosis as measured by sucrose and HRP may in part be attributable to the fate of each tracer in the cell. In L cells, HRP is degraded in secondary lysosomes with an activity half-life of ~7 h (21). In contrast, there does not appear to be an intracellular sucrase activity (7), with the result that sucrose remains intact within the cell, its only routes of exit being exocytosis and cell lysis. The results of pulse-chase studies (unpublished) plotting the disappearance of [^{14}C]sucrose from arterial SMC have indicated a half-life for intracellular [^{14}C]sucrose of 66 h at 37°C. At 4°C there was no decrease in [^{14}C]sucrose activity associated with the cell layer. These results suggest that part of the difference in rate of pinocytosis measured with HRP as compared with [^{14}C]sucrose may be attributable to the subsequent fate of each tracer in the cell.

Pinocytotic Response to PF and WBS

ARTERIAL SMC AND SWISS 3T3 CELLS: Quiescent subconfluent arterial SMC and Swiss 3T3 cells cultured in PDS pinocytosed fluid at an increased rate when measured 24 h after replacement of PDS by WBS, or after addition to PDS of a 5% serum-equivalent dose of PF (Fig. 3). The time-course of the pinocytotic response to WBS is shown in Fig. 4*a*, and to PF in Fig. 4*b*. The rate

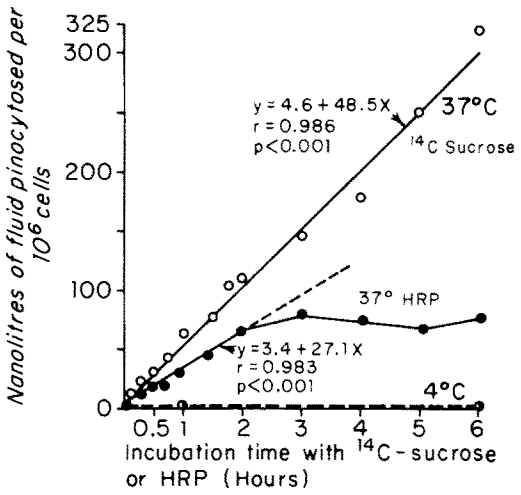

FIGURE 2 Uptake of [^{14}C]sucrose and HRP by Swiss 3T3 cells in logarithmic phase of growth. Both tracers were added to the same culture medium at time 0.

of pinocytosis in the presence of WBS or PF was not significantly different from control rates during the first 4 h. Thereafter, however, the rate increased and reached a peak around 24 h, after which it declined. In contrast, incorporation of [^{3}H]thymidine into DNA of arterial SMC was not increased above control levels until 12–16 h after the cells had been exposed to PF (Fig. 5). The decrease in the rates of pinocytosis in growth-stimulated cells ~36 h after stimulation (Figs. 4*a*, 4*b*) occurred because of the existence of an inverse relationship between pinocytosis and cell density (unpublished observations). Cells stimulated by WBS or PF divide and thus cell density increases whereas that of the nondividing control (PDS) cells remains constant.

When arterial SMC were exposed to various concentrations of PF or WBS and the rate of pinocytosis was measured after 24 h, there was an increased rate in the presence of 5% WBS compared to 1% WBS, and in 10% WBS there was a further increase (Fig. 6*a*). The dose response to PF was similar; only in the presence of a concentration of PF≥5% WBS did the rate of pinocytosis increase (Fig. 6*b*). These results suggest that a threshold concentration of platelet factor(s) is required to initiate enhanced pinocytosis. As shown by Vogel et al. (24), the proliferative response to PF shows a similar dose dependency in the presence of sufficient PDS.

ARTERIAL ENDOTHELIAL CELLS: In con-

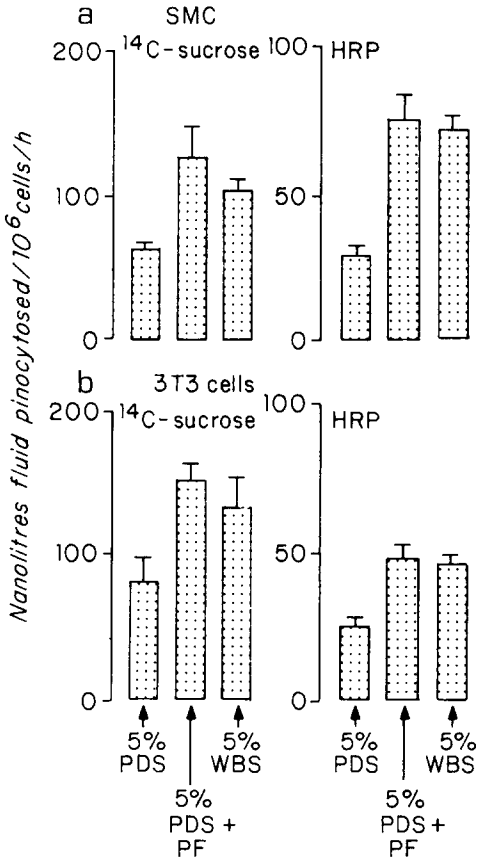

FIGURE 3 Stimulation of pinocytosis by PF and WBS in (*a*) arterial SMC and (*b*) Swiss 3T3 cells as measured by HRP and [^{14}C]sucrose. Cells were plated in DME + 5% PDS. 72 h later, the medium was changed to DME plus either fresh 5% PDS, 5% PDS + PF, or 5% WBS. 23 h later, HRP and [^{14}C]sucrose were added and the rate of pinocytosis was measured over 2 h. Each histogram is the mean of four determinations ±1 SD.

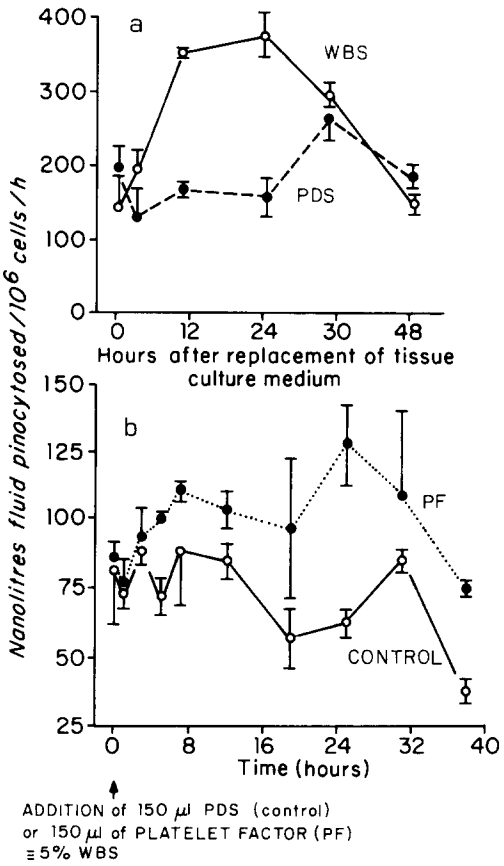

FIGURE 4 Time course of stimulation of pinocytosis by (*a*) WBS, (*b*) PF in arterial SMC as measured by [^{14}C]sucrose. Cells were treated as for Fig. 3 but were assayed for ^{14}C activity after 2 h of incubation with [^{14}C]sucrose at the times indicated after medium change. Each point is the mean of four (*a*) or two (*b*) determinations ±1 SD.

trast to arterial SMC and 3T3 cells, there were no significant differences between rates of pinocytosis in endothelial cells exposed to PDS, WBS, or semipurified platelet factor at all time points up to 72 h of exposure to the different media (Fig. 7). In these actively dividing endothelial cells, there was a progressive decline in the rate of pinocytosis per 10^6 cells as cell numbers increased with time. This relationship is currently under investigation.

Effects of Basic Poly-Amino Acids on Rate of Pinocytosis

The question arose as to whether the response of SMC to PF was a result of direct interaction, because of the cationic nature of the PF molecules (pI range 9.4–10.3), with the plasma membrane or was an indirect growth response. To investigate the first possibility, the cationic synthetic polyamino acid, poly-D-lysine, was added to quiescent cultures of SMC. There was no effect on the rate of pinocytosis in the presence of a range of concentrations of poly-D-lysine (Fig. 8).

DISCUSSION

Diploid mammalian cells in culture require WBS to survive and multiply. In our laboratory, a factor(s) present in WBS and derived from platelets (PF) has been shown to enhance the proliferation of arterial SMC and dermal fibroblasts in culture (15, 17, 16). Platelet releasate also promotes the growth of 3T3 cells (12, 2, 24). In the

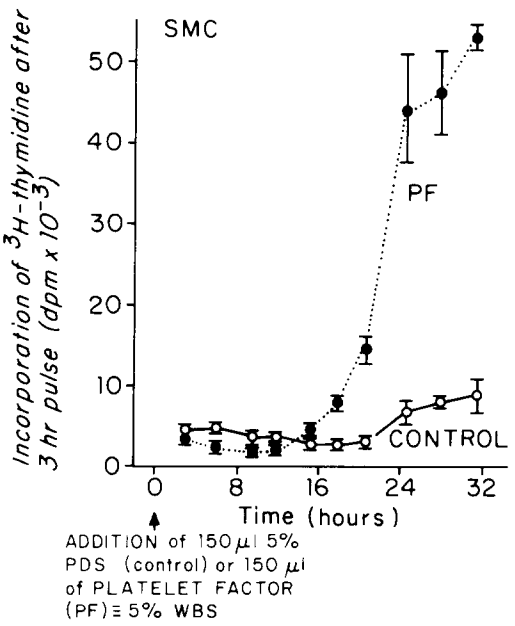

FIGURE 5 Incorporation of [^{3}H]thymidine into arterial SMC at increasing intervals after exposure to PF. Cells were treated as for Fig. 4*b* but [^{3}H]thymidine (0.1 μCi/ml) was added in 3-h pulses at the points indicated. Each point is the mean of three determinations ±1 SD.

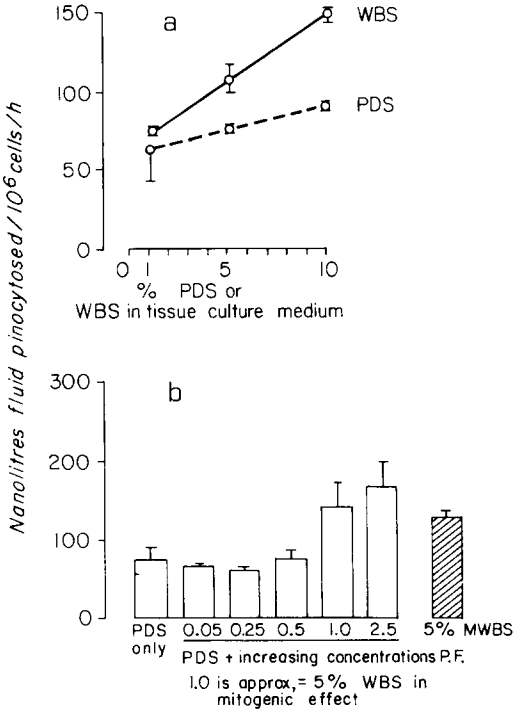

FIGURE 6 Rates of pinocytosis by arterial SMC in the presence of increasing concentrations of (*a*) PDS and WBS, (*b*) PF. Cells were maintained quiescent in DME + 5% PDS. 23 h after changing the medium to DME containing different concentrations of fresh PDS, WBS, or added PF, pinocytosis was measured over 2 h by incubation with [^{14}C]sucrose. Each point and histogram is the mean of four determinations ±1 SD.

present study, we have measured fluid endocytosis in arterial cells and in an established nondiploid cell line, Swiss 3T3, by applying methodology developed by others (20, 25). By exposing cells to medium containing both HRP and [^{14}C]sucrose and by demonstrating the absence of interaction between these two tracers, the volume of fluid pinocytosed has been calculated on the basis of the activities of both tracers within the same cells. As measured by both HRP and [^{14}C]sucrose, the rate of pinocytosis in cultured arterial SMC and Swiss 3T3 cells increased when the cells were stimulated from quiescence (in serum prepared from cell-free plasma, PDS) to an actively proliferating state in WBS or in the presence of PF. In quiescence, a low, steady-state turnover of cells was maintained as reflected by (*a*) no increase in cell numbers over an extended period, (*b*) a low and steady rate of incorporation of [^{3}H]thymidine, and (*c*) a fractional nuclear labeling index of 0.061 ± 0.004. These conditions were greatly altered by replacement of the PDS by WBS, or by addition of PF to PDS. The fractional labeling index rose to 0.313 ± 0.028, and the rate of incorporation of [^{3}H]thymidine was considerably increased ~16 h after exposure to platelet-derived growth factors. A marked elevation in the rate of pinocytosis, however, preceded the increased DNA synthesis by some 8–12 h. In endothelial cells, on the other hand, which did not appear to require PF for growth in culture, the presence of PF, either semipurified or in WBS, did not significantly alter the rate of pinocytosis over that in PDS. These results indicated that PDS did not lack essential factors for pinocytosis or did not contain inhibitors of pinocytosis, at least for endothelial cells.

A positive correlation between rate of macropinocytosis and serum concentration has been demonstrated in macrophages by direct light microscopy of the cells (5, 6) and by measurement of uptake of radioactive colloidal gold (8). In cultured human glial cells, Schellens and co-workers (18) using time-lapse cinematography have demonstrated a drastically reduced rate of macropinosome formation upon lowering the serum

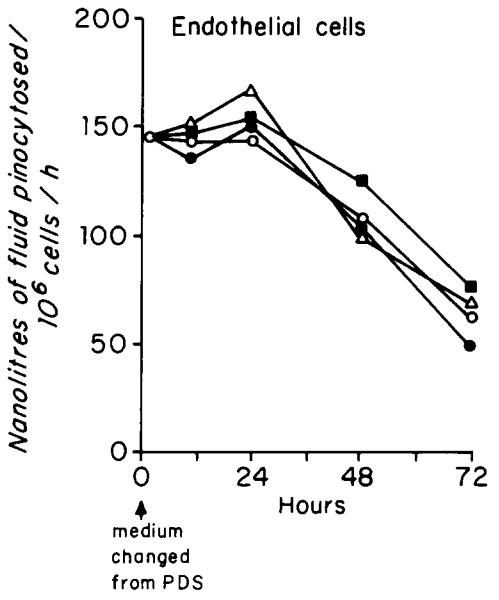

FIGURE 7 Time course of rates of pinocytosis by bovine arterial endothelial cells in the presence of PDS, WBS, and two concentrations of PF. Cells were plated in DME + 20% PDS. They did not attain a quiescent state, but continued exponential growth. After 48 h, the medium was changed to DME containing either 20% PDS, 20% WBS, or 20% PDS + PF at two concentrations. Pinocytosis was measured by [^{14}C]sucrose uptake over 2 h at the times indicated. Each point is the mean of duplicate determinations. O—O 20% PDS; △—△ 20% WBS; ■—■ 20% PDS + PF (≡ 20% WBS); ●—● 20% PDS + PF (≡ 50% WBS).

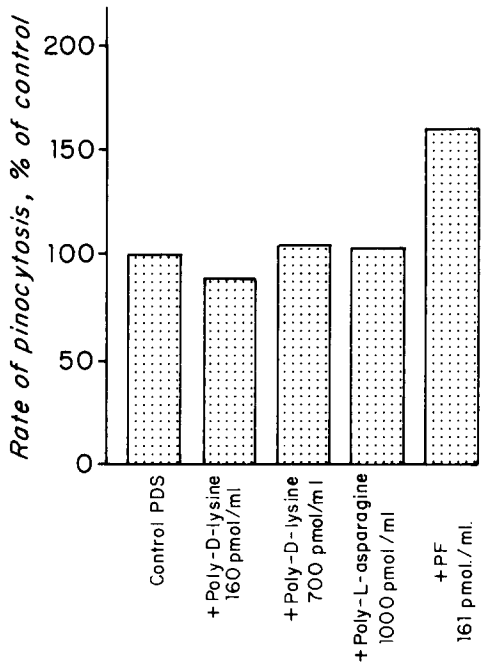

FIGURE 8 Absence of effect of synthetic basic poly-D-lysine on pinocytosis by arterial SMC. Polyamino acid or PF was added 24 h before determination of the rate of pinocytosis by [^{14}C]sucrose. Similar experiments in which poly-D-lysine was added at the same time as [^{14}C]sucrose also revealed no change in the rate of pinocytosis. Each histogram is the mean of four determinations.

concentration of the medium from 10 to 0.5%. They related this observation to the decreased rate of proliferation of the cells and suggested that one of the major functions of serum might be to stimulate turnover of plasma membrane associated with pinocytosis of high molecular weight nutrients essential for cell division. As an extension of these studies, Brunk et al. (3) demonstrated stimulation of macro-pinosome formation in human glial cells maintained in serum-free medium specifically upon addition of purified epidermal growth factor or serum. Although these interesting studies parallel our own, measurement of ruffling and macro-pinosome formation by light microscopy does not reflect rates of micro-pinocytosis (1). Indeed, it has been estimated that the formation of micro-pinocytic vesicles which cannot be resolved by the light microscope accounts for most of the fluid endocytosed by eukaryotic cells (1). In this respect, Steinman et al. (21) were unable to detect a difference in HRP uptake by macrophages exposed to a wide range of serum concentrations, even though the number of pinocytotic vesicles observed by light microscopy has been reported to rise with increasing serum concentrations (5).

In a recent publication, Vlodavsky et al. (23) reported that whereas sparse cultures of bovine endothelial cells internalized (adsorptively endocytosed) low density lipoprotein (LDL), in confluent—presumably contact-inhibited—endothelial cultures, internalization almost ceased. Although endocytosis of lipoproteins may be somewhat different from the fluid pinocytosis reported in the present study, our observations shown in Fig. 7 are similar to those of Vlodvasky et al. (23). As the cells reached confluence, there was a marked reduction in the rate of pinocytosis. It is possible that Vlodavsky et al. (23) observed a reduced LDL uptake as a simple manifestation of reduced pinocytosis as a result of the state of confluence of the population of the endothelial cells rather than specific inhibition of LDL recep-

tor mobility at the cell surface. It remains to be determined whether this decline is related to altered cell surface area, growth factor availability, or other facets of contact inhibition of DNA synthesis.

Gel filtration and isoelectric focussing of partially purified platelet-derived growth factor(s) have revealed growth-promoting activity by proteins with a range of pI 9.4–10.3 (16). It was therefore possible that the effect of PF on pinocytosis might be the result of direct interaction of a cationic protein with the negatively charged plasma membrane rather than a relationship between pinocytosis and cell division. Three observations do not support this idea. First, the altered rate of pinocytosis in SMC and 3T3 cells might have been expected to occur rapidly after addition of PF, when in fact there was a delay of several hours. Second, the addition of poly-D-lysine, a highly cationic molecule which binds nonspecifically to the plasma membrane, did not affect the rate of fluid pinocytosis. Third, endothelial cell pinocytosis was unaffected by the addition of PF, whereas if stimulation was dependent upon nonspecific binding of PF protein, an effect might have been expected in these cells.

The ability of PF to commit SMC and 3T3 cells to DNA synthesis, concomitant with an elevation in the rate of pinocytosis, suggests that growth factor stimulation of mitosis may be closely associated with changes in fluid pinocytosis. Since pinocytosis is a fundamental process in maintenance of cell homeostasis, particularly in relation to plasma membrane recycling (22, 9) and internalization of fluid phase components (1), even small alterations in the rate of pinocytosis may reflect quite profound changes of intracellular biochemistry such as occur during cell division.

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