Morphological effects of prostaglandins E_1, E_2 and F2α on fibroblast-like cultures of human synovial cells[1]

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Summary. In medium supplemented with serum, PGE_1 and PGE_2 were equally potent in inducing cells with a fenestrated appearance, whereas $PGF_{2\alpha}$ was comparatively ineffective. In BSA without serum the effects were more persistent and characterized by a high proportion of astrocyte-like cells. The effects were reversed upon removal of the prostaglandins.

In the primary phase immediately after isolation, 2 morphological classes of cell can be recognized in cultures of human synovium[2]. Many are macrophage-like and probably represent the type A synoviocytes of the parent tissue[3]. Others have the appearence of fibroblasts and may be examples of the type B fibroblasts or the intermediate type C synoviocytes. During passaging with trypsin the macrophage-like cells are lost due to their strong adhesion to the culture surface and the synovial cells which survive through repeated passages are uniformly fibroblast-like in appearance. These cells synthesize hyaluronic acid but also retain some capacity for phagocytosis and therefore most closely resemble type C synoviocytes.

A number of morphological variations can be induced in fibroblast-like synovial lines by treatment with various agents[4]. Increased numbers of lysosomes and elevated lysosomal enzyme activity can be promoted by sucrose and other indigestible saccharides[5], as well as by adenosine[4]. This effect (designated type I), is associated with decreased secretion of hyaluronic acid. A 2nd (or type 2), response is provoked by several agents which are known to cause increased endogenous cyclic adenosine monophosphate (cAMP). Cholera enterotoxin and dibutytyl cAMP were particularly potent examples. These agents produce cells with a fenestrated appearance due to ridges and depressions in the cytoplasm (type 2a) and also contracted cells with multiple dendrite-like processes (type 2b). The type 2 changes are usually associated with increased synthesis of hyaluronic acid. Prostaglandins are also known to generate intracellular cAMP[6]. The object of the present study was therefore to examine the morphological effects of the more stable examples for comparison with responses to CT and other cAMP-generating agents used in previous studies.

Synovial cells isolated from intact joints of cadavers as described previously[7], were kindly provided by Dr J.R.E. Fraser. The methods of culture and of establishing fibroblast-like cell-lines were also as in previous reports[8,9]. Multiple monolayer cultures were prepared in 50 ml polystyrene vessels and the prostaglandins added in culture media after 24 h. Morphology was monitored by phase contrast illumination, using an Olympus PMB6 microscope. Prostaglandins were obtained from Upjohn Diagnostics,

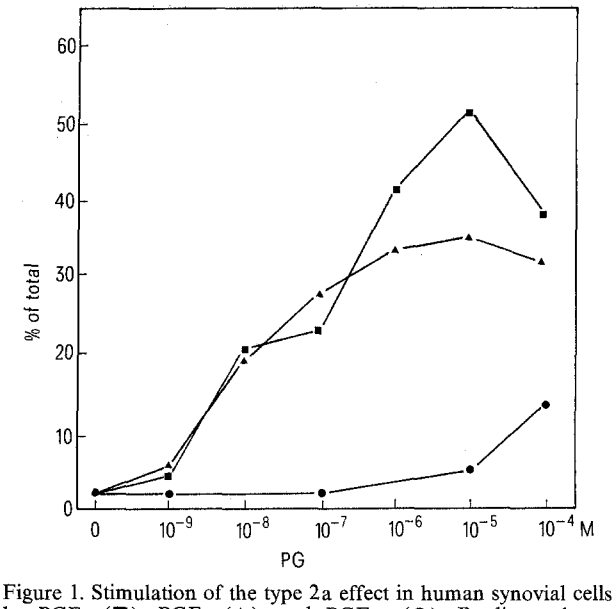

Figure 1. Stimulation of the type 2a effect in human synovial cells by PGE_1 (■), PGE_2 (▲), and $PGF_{2\alpha}$ (●). Replica cultures were treated for 24 h in Eagle's basal medium with 10% HS, then the total cells in 10 randomly selected phase contrast microscope fields was counted. Each point is the mean of counts from 2 cultures.

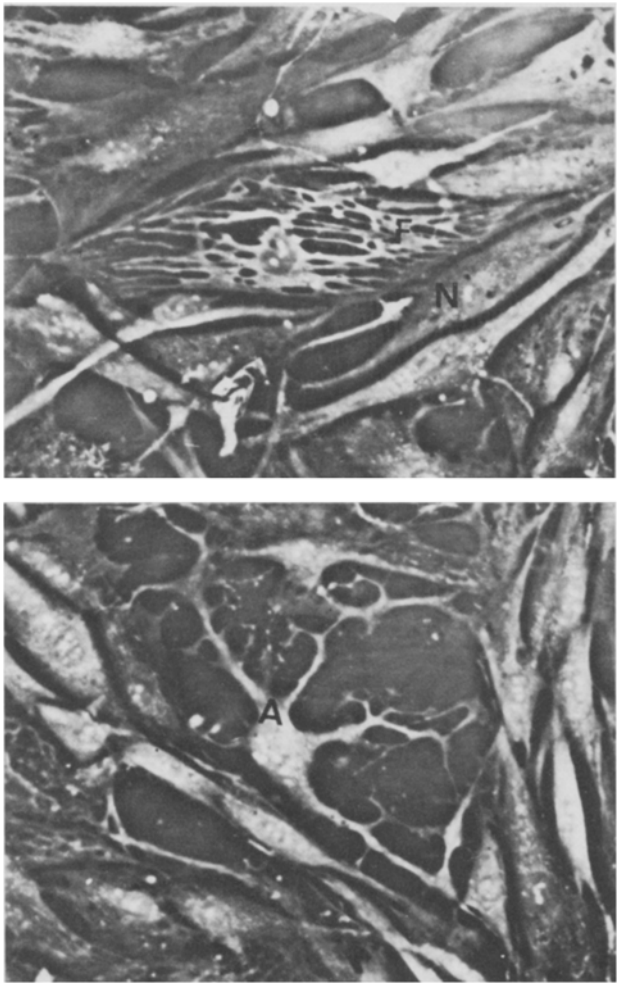

Figure 2. Human synovial cells treated for 24 h with 10^{-6} M PGE_1 in Eagle's basal medium with 10% HS. Figure 2a shows normal cells (N) and the fenestrated appearance of a type 2a cell (F). Figure 2b shows an example of the type 2b effect in which the cells adopt an astrocyte-like form (A), with dendrite-like processes. Negative phase contrast. Magnification × 480.

Michigan, USA. Foetal bovine (FS) and human serum (HS) were heat-inactivated at 56 °C for 30 min. Lipid-free bovine serum albumin (BSA) was obtained from the Sigma Chemical Co., USA.

In 10% HS or FS, PGE_1 and PGE_2 produced a concentration-dependent type 2a response and were of similar potency (fig. 1). In some cases, significant differences from controls could be detected at 10^{-9} M. $PGF_{2\alpha}$ was comparatively ineffective and produced a type 2a response only at concentrations of 10^{-5} M and above. When the prostaglandins were removed the morphology reverted to normal within 60 min. In the presence of serum, type 2b responses were limited to a small proportion of the total. Examples of PG-induced type 2a and 2b cells are illustrated in figure 2. In many experiments of the type shown by figure 1 the type 2a response was comparatively small (< 10%) and persisted for only 4–5 h, suggesting inhibition by some sera. In BSA (4 mg/ml) without serum a pronounced type 2b response occured in the untreated controls, which was characterized by tightly contracted astrocyte-like forms. This effect was markedly increased by PGE_1 and PGE_2. The type 2a response was comparatively slight but could be enhanced, with simultaneous reduction of the type 2b proportion, by allowing 48 h or more between passaging and addition of the prostaglandins. The effects in BSA were persistent for at least 72 h but reversed upon removal of the agents.

In unpublished experiments in this laboratory PGE_1 and PGE_2 were found to be potent activators of adenylate cyclase in synovial cells, whereas $PGF_{2\alpha}$ was less effective. This is in agreement with the effect of these agents on cAMP in explant-derived synovial cells[10]. Since cAMP is known to control the organisation of microfilaments and microtubules of the cytoskeleton[11], changes in cAMP levels can account for the morphological effects of prostaglandins, The type 2b effect in synovial cells is probably an intensification of the 2a phenomenon, since apparently intermediate forms can usually be observed. A fenestrated appearance produced in iris epithelial cells by agents which stimulate intracellular cAMP[12], is nonetheless distinctive from the type 2a response in synovial cells and studies so far suggest that this phenomenon might be specific for synovial cells.

1 This work was supported by the National Health and Medical Research Council of Australia.
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Role of kinetin in the dormancy of *Cercis siliquastrum* seeds[1]

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Summary. Studies conducted on *Cercis siliquastrum* seeds treated with kinetin confirm that this hormone does not interrupt dormancy in either whole seeds or those decoated at the radical pole. Seeds totally decoated or decoated at the cotyledon pole only demonstrated atypical germinations linked to cotyledon growth, permitting the embryo to escape the inhibitory action present in the endosperm; this does not occur when the cotyledon surface is experimentally reduced.

Within the realm of the problem regarding factors inhibiting germination of *Cercis siliquastrum* seeds[2], kinetin treatment has proven to have made an important contribution[3]. Kinetin does not interrupt dormancy in the seed but, at high concentrations, it causes the development of characteristic cuts in a circular pattern; this normally indicates germination, but here is not followed by radicle protrusion. Results are described of further experiments with the same substance, associated with mechanical action on both the integument and endosperm as a means of deepening knowledge of the effect of kinetin on factors responsible for dormancy.

Materials and methods. During the entire course of the experiment, which lasted for a period of 2 years, seeds collected from the preceding year were always used.

The technique was the same as that used in the above-cited study[3]. Aqueous extracts of endosperm homogenates were obtained by applying a technique reported in a preceding study[2]. Experiments were repeated on average 6 times each. Kinetin was supplied by the Sigma Chemical Co., USA.

Results. Kinetin treatment of totally decoated seeds, free of integument, determined different percentages of atypical germinations (with cotyledon protrusion), varying according to concentrations; optimum effect at 10^{-5} M (fig.). This was however not always followed by the normal seedling development that is invariably obtained with low temperature pre-treatment (30–40 days at 6 °C) or by the action of gibberellic acid (GA_3) in whole seeds. On the 20th day when some samples treated with kinetin show elongated seedlings free of endosperm, other seeds in which the cotyledons have partially protruded maintain a certain integrity, and the cotyledons even though belonging to healthy seedling are unable to go out of the seed completely.

When a partial removal (about $^1/_3$) of the integument is made, liberating the radical portion of the endosperm, kinetin does not induce germination even when two small incisions are made in proximity of the radical pole of the endosperm, although the endosperm is extremely thin in the area of the radical apex.

If instead the cotyledon portion is completely freed from the integument (approximately $^1/_3$), kinetin (with an optimal effect at 10^{-4} M) induces atypical germination analogous to