Influence of PGA_1 on cartilage growth

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Summary. Using the Fell technique of organ culture of cartilage in a chemically defined medium, it has been shown that prostaglandin A_1 at a concentration of 25 μg/ml caused chondrocyte death in chick embryonic limb rudiments. An equimolar concentration of PGE_2 was not toxic to the cells.

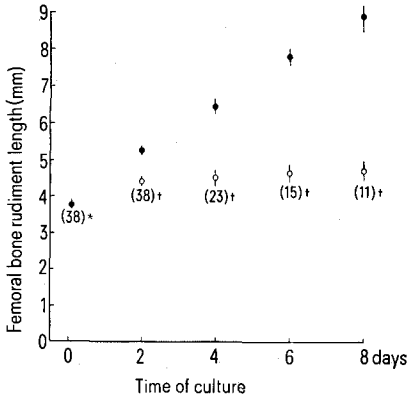
In mammalian tissues prostaglandins (PG) are ubiquitous[2], displaying many pharmacological actions that suggest the regulation of homeostasis[3,4]. Eisenbarth and his colleagues[5,6] reported that PGA_1, PGB_1, PGA_2 and PGB_2 inhibited chick embryonic cartilage matrix synthesis; they suggested that this effect might be independent of the usual mechanisms by which prostaglandins alter cellular activity. These mechanisms include the intracellular increase in cyclic AMP[7]. In a search for an explanation for the influence of PGA_1 on cartilage, we have extended Eisenbarth's short-term investigations of cartilage fragments to a study of cultured whole limb rudiments maintained for 8 days.

Methods. The technique of Fell[8–10] was adopted for the study of femoral and tibial bone rudiments from 8-day chick embryos. Tissue viability was ensured by perfusion with Hank's balanced salt solution[11]. The chemically defined growth medium was that of Fitton-Jackson[12]. Explants were cultured in an atmosphere of 5% CO_2 in air. The growth medium was renewed on alternate days. Femoral and tibial bone rudiments from one limb of each embryo were cultured in control medium; those from the other limb were cultured in medium containing 25 μg/ml PGA_1.

Control and test explants supported on 22 mm × 18 mm × 2.5–3.0 mm grids of stainless steel mesh were placed in separate 35 mm Petri-style culture dishes within a 90 mm Petri dish. Explant growth was monitored by the measurement of length on days 0, 2, 4, 6 and 8. At these times matched control and test sample explants were removed and processed histologically. Paraffin sections were stained with haematoxylin and eosin or with alcian blue in 0.4 M $MgCl_2$[13].

Results and discussion. The growth rate of femoral explants in medium with and without PGA_1 is shown (figure). Control explants appeared morphologically similar to those depicted by other workers[10] under similar conditions; explant length increased from day 0 to day 8. The length of PGA_1-treated explants did not increase after day 2; tibial explants behaved in the same way. The differences in growth between control and PGA_1-treated explants, after 2, 4, 6 and 8 days of organ culture, were highly significant ($p < 0.001$). PGA_1-treated bone rudiments examined at these times revealed many empty chondrocyte lacunae. The remaining cells had small darkly-staining nuclei and deeply eosinophilic cytoplasm, observations suggesting cell injury. PGA_1-treated explants contained much less alcian blue-positive material than untreated controls.

This morphological evidence helps to substantiate the view that chondrocytes are susceptible in vitro to the adverse influence of PGA_1. Thymocytes, L-929, Swiss 3T3, Balb 3T3, 3T3 SVC1X and chick embryo fibroblasts are known to be similarly affected. Eisenbarth et al.[5] considered the possibility that chondrocyte death might result from PGA_1 but dismissed this explanation for their observations because measured chondrocyte oxygen consumption was unaltered after 7 h incubation. The present evidence, however, is compatible with the view that PGA_1-induced cartilage cell disorder is a result of direct cell injury that may include an action upon respiratory pathways, although no information is yet available on the mechanism of PGA_1-mediated cell death of any of the cell types listed above. Further evidence for the nature of chondrocyte cytotoxicity is now being sought by ^3H-thymidine autoradiography and by electron microscopy.



Linear growth of control and of PGA_1-treated bone rudiments, expressed as mean length ± 2 SEM. Number of observations in brackets. ●, control; ○, PGA_1-treated (25 μg/ml). ✦ $p > 0.10$; † $p < 0.001$.

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Eisenbarth and co-workers[16] have demonstrated that PGA_1 inhibits the cellular incorporation of radioactive precursors into the proteoglycan, protein, RNA and DNA of a well-differentiated rat chondrosarcoma and of a poorly-differentiated murine chondrocarcoma. They suggest a potential therapeutic role for PGA in cases of chondrosarcoma[5,6,16]. The present work shows the cytotoxicity of PGA_1 for non-malignant chondrocytes in organ culture and the inhibition of growth of chick embryonic limb bone rudiments in vitro and, in one sense, supports the proposal that PGA may possess selective effects that act against cancer cell metabolic pathways.

The present observations may also be relevant to the pathogenesis of human connective tissue disease. In rheumatoid arthritis (RA) there is progressive destruction of peripheral articular cartilage[17]. Significant concentrations of prostaglandins (PGE) have been recognised in RA synovial fluid[18] and interpreted as 'reasonable evidence for the postulate that PGE and/or PGA are important contributory factors to the pathogenesis of inflammatory reactions in patients with rheumatic diseases'. It has been shown that the predominant prostaglandin in rheumatoid synovial fluid is PGE_2[19]. In an analogous experiment designed to test the effects of PGE_2 on cartilage (25 µg/ml) PGE_2 was found to have no inhibitory effect on the growth of chick embryonic limb rudiments in vitro, assessed by length and weight measurements, during a 4-day-period. No evidence of PGE_2-induced chondrocyte death was found by light microscopy. Comment on the significance of these observations for the pathogenesis of rheumatoid arthritis must now await experimental data on the effects of prostaglandins on human articular cartilage in organ culture. Consequently, although we report the ability of PGA_1 to cause chick embryonic chondrocyte death and impaired cartilage matrix synthesis, it is not yet known whether damage to, or dissolution of, marginal articular cartilage in rheumatoid arthritis is influenced by the local release of prostaglandins.

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A light microscope study of fibre diameter and sarcomere length relationships in rigor skeletal muscles

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Summary. Correlation coefficients between fibre diameter and sarcomere length were significant in relaxed, unfixed muscles in rigor, indicating that fibres with large diameters had short sarcomeres and fibres with small diameters had long sarcomeres. No significant correlations were obtained in muscles from limbs that entered rigor in a folded or stretched position.

Fibre diameter and sarcomere length are important parameters in determining growth potential[1,2] and physiological properties[1] of skeletal muscles. Information is not available on the relationship between the diameter of a fibre and the length of the sarcomeres in individual skeletal muscle fibres in the pre-rigor state. Accurate determination of these dimensional relationships is impossible because of the following properties of pre-rigor muscle: excitability, thaw-rigor changes if the muscle was frozen in the pre-rigor state, and penetration properties of the fixative. Therefore, this study on fibre diameter and sarcomere length was performed on mouse and turkey muscles that had entered rigor mortis. The limbs were in a folded, relaxed or stretched position, thereby providing muscles at varying degrees of contraction and relaxation. Conventional histological methods would involve separate transverse and longitudinal sections in order to obtain fibre diameter and sarcomere length values. Isolation of individual muscle fibres permits the dimensional measurements to be performed on the same fibre. Passively contracted ('kinked') fibres could be observed when the fibres were isolated. Some muscles contain a large number of passively contracted fibres[3-5]. A high proportion of passively contracted fibres would make the fibre diameter measurements meaningless[6]. The proportion of passively contracted fibres in these muscles was small irrespective of the state of relaxation or contraction of the muscles.

Methods. Adult female mice (30–35 g body weight) were killed by ether anesthesia. Adult male turkeys (7–8 kg body weight) were exsanguinated by cutting the carotid artery. The turkeys were placed in an inverted metal cone to prevent excessive limb and wing movement during exsanguination. Passively shortened and stretched biceps brachii muscles from mice were obtained by pinning one fore-limb inwards maximally, and stretching the other fore-limb outwards maximally. The fully extended limb was pinned at 180° from the trunk. Stretched semitendinosus muscles in the turkey were obtained by securing the limb in a cephalad position. Shortened semitendinosus muscles were obtained by flexing the limbs inwards maximally and securing them in that position. All folding and stretching treatments were performed immediately postmortem. Soleus, sternomastoideus and gastrocnemius muscles in mice, and the pectoralis muscle in turkeys were obtained by placing the animals in a resting position

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