# Sites of Action of Growth Hormone in Cartilage. (29700)

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Since the isolation of growth hormone many of its actions on the total organism have been widely investigated and well recognized. The present report describes an attempt to locate the cells, within the cartilage of the upper tibial epiphysis, which respond to the stimulus of growth hormone. With the aid of tritiated thymidine (both *in vitro* and *in vivo*), colchicine and the electron microscope, the sites of cell regeneration within the upper tibial epiphysis were mapped out (Rigal, 4). An attempt was then made to stimulate cell generation in these cartilages by administering extra growth hormone to living animals or to cultures of normal tissues *in vitro*. The effect on the cells was traced by culturing the tissues *in vitro* in the presence of tritiated thymidine.

*Materials and methods.* Crossbred rabbits weighing between 330 g and 800 g were used for the experiments described below. Trowell Type 11 tissue culture chambers(6) were adapted for use in a 38°C incubator(4). Each aluminium chamber (internal volume 192 ml) was sealed with a glass lid and contained a stage which in turn carried a 10 ml flat fused silica dish. In the silica dish, 8-20 cultures were grown in fluid media, usually composed of 2 parts autogenous blood serum (from animals that provided the explants) and one part glucose saline (0.6% glucose, 0.7% sodium chloride). As the chambers were sealed it was possible to use any desired gas phase, in this case 95% oxygen and 5% carbon dioxide.

Explants were produced by cutting sagittal slices approximately 2 mm thick from the proximal tibial epiphyses. Each explant thus contained articular cartilage, residual anlage cartilage (the remaining primitive cartilage that originally formed the model of the bone), bone of the secondary ossification center, growth cartilage (ultimately the epiphyseal cartilage plate) with its adjacent fibrous perichondral ring, and a narrow zone of metaphyseal bone.

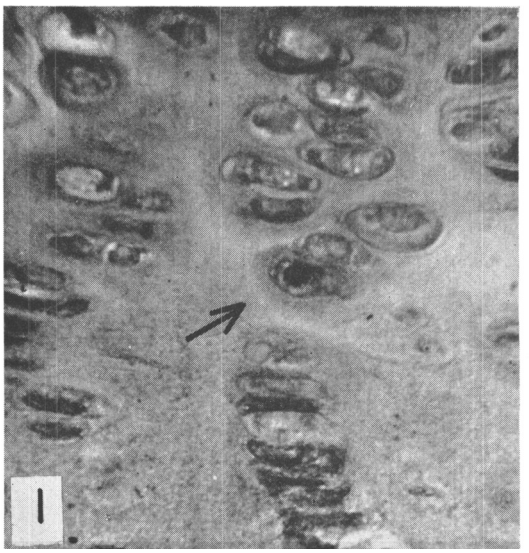
Immediately before the chambers were sealed 0.5 $\mu$c to 1 $\mu$c of tritiated thymidine was added to the medium. Following appropriate treatment and incubation periods, the explants were fixed in 10% neutral formal saline, decalcified with formic acid, embedded in paraffin wax and sectioned in the sagittal plane. After autoradiographs had been prepared by the Pelc stripping film technique(3) the sections were counter-stained with Leishman-Giemsa.

By the method described above, 484 explants were obtained from untreated rabbits and cultured, in batches of 8-20 per chamber. Individual explants were removed at intervals varying from one-half hour to 48 hours. By these control experiments, the pattern of cell division was determined in the tissues.

In studies of the *in vivo* action of growth hormone, 6 rabbits weighing 340 g to 500 g were given a commercial preparation by injection.* The first animal received a single dose of 5 international units and was killed 15 hours later. The remaining 5 animals were given multiple injections of the hormone at 24-hour intervals. Of them, 2 were given 2 injections of 5 i.u. each and were killed 36 hours after the first injection. The fourth, fifth, and sixth animals were given 3 injections (the daily dose being constant for each animal but being 5, 10, or 15 i.u. for the 3 animals, respectively) and were killed 72 hours after the first injection. All animals were killed by exsanguination after being anaesthetized with carbon dioxide (25-50%) and oxygen. A total of 60 explants were obtained from their proximal tibial epiphyses. The explants were then cultured for one-half hour to 48 hours in media containing 1 $\mu$c of tritiated thymidine.

In *in vitro* studies of the hormone's action, explants from 2 normal rabbits were cultured in medium containing 2.5 units (1 mg/5.5 ml

* "Antuitrin Growth", Parke Davis and Co., Stains Rd., Hounslow, Middlesex, England.



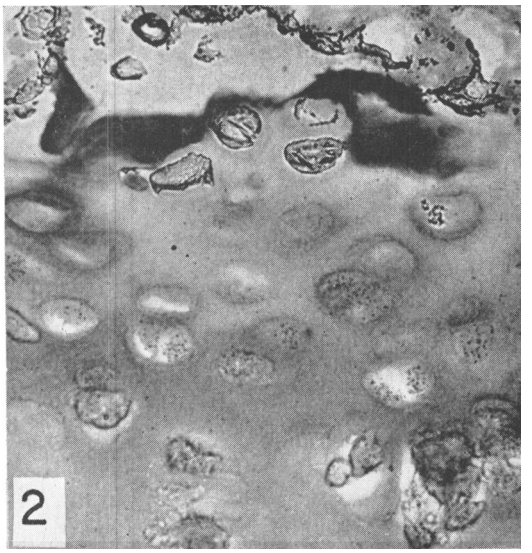




FIG. 1. Radioautograph of germinal zone of growth cartilage of an untreated control, magnification × 600. A single cell shows heavy labelling, as indicated by the arrow.

FIG. 2. Under the influence of parenterally administered growth hormone, virtually all cells in this field of the germinal zone show labelling with tritiated thymidine. A shorter exposure time accounts for the decreased number of grains per individual cell.

medium) of growth hormone.† No isotope was added to the media initially. After 12 hours the chambers were opened and 1 $\mu$c of tritiated thymidine was added to the culture media. Explants were then removed at intervals varying from one to 48 hours.

*Results.* In tissue culture certain vital processes appeared to be slowed down. As a result a clear pattern of the sites of dividing cells emerged. In rabbits of the weight and age used, a thick layer of cartilage surrounds the incompletely formed secondary ossification center of the proximal tibial epiphysis. On the side of the center toward the joint 2 curved lines (each a single cell wide) of generatively active cells occur, *viz.*: a more superficial one occurs in the "articular" cartilage (*cf.* the generative or mitotic annulus of Harris(1,4), and a second line lies deeper in the cartilage, *i.e.*, in the remains of the "anlage" cartilage which has not yet been removed by expansion of the ossification center. Within the first zone of the epiphyseal growth cartilage, *i.e.*, the germinal zone, comparatively few cells (about 5%) take up tritiated thymidine (Fig. 1). In approximately the proximal one-half of the columnar region of the growth cartilage the cells showed generative activity. These findings were confirmed in separate experiments using colchicine and electron microscopy(4,5).

Under the influence of growth hormone *in vivo* no marked change in the appearance of the tissues could be detected either macroscopically or by conventional histological methods. In contrast, the autoradiographs made from this material showed a marked change from the controls, the most striking appearance being in the germinal zone. Whereas in the controls only 5% of the cells were generatively active, under the influence of growth hormone *in vivo* 25-50% of all germinal zone cells took up tritiated thymidine (Fig. 2). In some areas 30 or more adjacent cells were labelled. A similar but less marked increase in activity occurred in the anlage and articular cartilage. Where previously 6 or 7 inactive cells separated the active cells in each row, in treated animals the row of active cells became almost continuous and several cells wide. No marked change was detected in the proliferative zone itself.

In view of the marked activity that oc-

† "Antuitrin Growth," supplied especially prepared without preservative, Parke Davis & Co., Detroit, Mich.

curred when the hormone was administered to the animal before its tissues were explanted, some change at least was anticipated in normal tissues cultured *in vitro* in the presence of growth hormone. However, no increase in generative activity whatsoever was detected in the latter preparations. The picture was the same as that found in control cultures, in which there had been no contact with added growth hormone at any stage.‡

*Discussion.* It is noteworthy that bovine growth hormone, when administered to rabbits *in vivo,* produced a marked increase of chondrocyte proliferation in the epiphyses. On the other hand, when administered to normal tissue explants *in vitro* this hormone produced no change in the tissues. It is known that certain hormones, *e.g.,* insulin, thyroxine, and sex hormones, can influence tissues in *in vitro* culture(2). Although the difference in response cannot be explained at present, some suggestions deserve consideration. (1) It may be that the bovine growth hormone has to be modified within the animal's body before it can mimic the effect of endogenous rabbit growth hormone. (2) It may be that growth hormone does not exert its effect directly upon the cartilage cells themselves, but acts *via* an intermediate mechanism—enzymatic, hormonal, or enhancement of the nutrition or vascular supply of the region. (3) It is, of course, necessary to consider the possibility that the culture conditions were not sufficiently favorable for growth hormone to exert its action to a detectable degree.

Nevertheless, when administered to the intact animal the growth hormone produced a remarkable increase in proliferative activity in the 3 areas mentioned. If the cells concerned are not in fact the target organ for growth hormone they are at least the cells through which the hormone produces its effect on longitudinal growth of the bone and articular cartilage. The germinal zone is the stem cell area of the growth cartilage. By influencing this area the effect on growth can be expected to be far greater than it would be if the chief action were on the active population of the proliferative zone. One new active cell in the germinal zone can give rise to a whole new column of active cells in the proliferative zone. In both the anlage and the articular cartilage the active cell region plays the dual role of the stem cells as well as the normal active populations; thus, in each it corresponds to both germinal and proliferative zones of the epiphyseal growth cartilage.

*Summary.* Two experiments using added growth hormone in rabbits have been described. When supplied to explants of normal rabbit epiphysis, cultured *in vitro,* the hormone produced no detectable effects. However, when given to the animal *in vivo* before its tissues were explanted, the hormone produced a marked increase in chondrogenetic activity demonstrable in the cultured explants by autoradiographs of tritiated thymidine incorporation. The most marked effect occurred in the germinal zone of the epiphyseal growth cartilage and the corresponding cells of the articular and anlage cartilage.

‡ A comparable observation was made when Vit. C was excluded from the diets of a group of guinea pigs submitted to the culture techniques described here. The usual changes of scurvy were detected in the growth cartilage but neither in articular nor in growth cartilage did the pattern of cellular generative activity change as a result of the scorbutic diet.

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