The shift occurs at a time when lens morphogenesis is essentially completed except for the laying down of many fibers. The shift cannot be correlated with any specific morphogenetic event since the essential features of the lens are formed by 8 days (*5*). It does occur at a time when the lens undergoes a slight and transitory increase in growth rate (*6*). The shift must result in initiation or accentuation of synthesis of a class of lens proteins, possibly monomers of one of the crystallins.

The work of Yamada (*7*) on regenerating amphibian lens points to the late appearance of γ-crystallin which is restricted to the fiber cells. If γ-crystallin is detectible before 11 days in the chick embryo lens (*8*, *9*) the polyribosomal shift possibly represents an increase in synthesis of γ-crystallin and not necessarily initiation of its synthesis. Also, it is not known whether the change in the distribution of polyribosomes between 11 and 12 days can be correlated with cytological maturation of lens cells which ultimately become filled with the crystallin proteins. Nuclei disappear during this process.

At 14 days many lens cell nuclei have not yet ceased to function. From the curve of polyribosome decay it is clear that at least two classes of messenger RNA's are being used by a relatively homogenous population of cells. One class has a half-life of about 3 hours and the other a half-life of longer than 30 hours. The presence of short-lived messenger RNA may be taken as evidence of nuclear function.

Even though short- and long-lived messenger RNA's coexist in the whole 14-day lens, which consists nearly entirely of cells that make crystallins, it is not yet clear that the two kinds of messenger RNA occur in the same cell at the same time. Either a cell uses short- and long-lived messenger RNA's continuously and concurrently, or it uses them sequentially, first translating short-lived messages and then translating long-lived messages. The transition from use of short- to use of long-lived messages would come at a time when nuclear function ceased. All proteins synthesized after that would be made on long-lived messenger RNA.

ROBERT B. SCOTT
EUGENE BELL
Department of Biology, Massachusetts Institute of Technology, Cambridge

References and Notes

1. T. D. Humphreys, S. Penman, E. Bell, *Biochem. Biophys. Res. Commun.* **17**, 618 (1964).
2. R. B. Scott and E. Bell, *Science* **145**, 711 (1964).
3. E. Bell, in *Natl. Cancer Institute Monograph No. 13*, W. J. Rutter, Ed. (U.S. Govt. Printing Office, Washington, 1964), p. 1.
4. C. Levinthal, A. Keynan, A. Higa, *Proc. Natl. Acad. Sci. U.S.* **48**, 1631 (1962).
5. C. Rabl, *Z. Wiss. Zool.* **65**, 257 (1899).
6. J. Schmalhausen, *Zbirnik Prats Biol. Instit.* **1**, 301 (1926), available at Marine Biological Laboratory, Woods Hole, Mass.
7. T. Yamada, personal communication.
8. G. ten Cate and W. J. Van Doorenmaalen, *Proc. Koninkl. Ned. Akad. Wetenschap.* **53**, 894 (1950).
9. H. Maisel and J. Langman, *J. Embryol. Exptl. Morphol.* **9**, 191 (1961).
10. K. Scherrer and J. E. Darnell, *Biochem. Biophys. Res. Commun.* **7**, 486 (1962).
11. J. Paul, *Cell and Tissue Culture* (Livingstone, Edinburgh, 1959), pp. 78–9.
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Heparin Enhancement of Factors Stimulating Bone Resorption in Tissue Culture

Abstract. *The addition of small amounts of a commercial heparin solution, or a synthetic polysaccharide-sulfuric ester similar in structure to heparin, to the medium in which bone tissue is cultured markedly enhances the amount of bone resorption obtained with suboptimal concentrations of parathyroid extract or other factors which stimulate bone resorption. It is suggested that heparin be considered a "cofactor" which stimulates bone resorption and which may play an important role in physiological or pathological resorption.*

In a previous study (*1*) it was shown that the extent of bone resorption obtained in our tissue culture system was dependent upon the amount of resorption-stimulating factor added to the medium as well as the concentration of oxygen in the gas phase. Known factors which stimulate bone resorption include parathyroid extract, crystalline vitamins A, D_2, and D_3, and dihydrotachysterol. The experiments described here indicate that the amount of bone destruction obtained with suboptimal concentrations of these factors may be markedly enhanced by the addition of heparin to the medium.

According to our system of tissue culture, calvariae were removed aseptically from 4- to 5-day-old Swiss albino mice of the Webster strain (litters were pooled when more than 10 to 12 calvariae were needed); the occipital bone was removed, and the remaining portion (frontal bone and parietal bones) was attached to a rectangular coverslip by covering the tissue with a thin film of a mixture of chicken plasma and chick embryo extract (2 : 1). After clotting occurred, each coverslip was inserted into the "well" portion of a Leighton tube, covered with 2 ml of a supernatant fluid composed of heated horse serum (80 percent), Gey's balanced salt solution (10 percent), and 100 units each of penicillin and streptomycin in Gey's balanced salt solution (10 percent).

Water-soluble test substances were usually diluted in the balanced salt solution or in the serum, whereas fat-soluble substances were diluted in the horse serum component or first dissolved in a small volume of absolute alcohol before being diluted in the serum. The oxygen tension of the gas phase in the culture tubes was increased to 50 percent by introducing a pipette (leading from a tank containing a mixture of 50 percent oxygen and 50 percent nitrogen) into the bottom of each tube containing 2 ml of medium and raising gas bubbles until they completely filled the length of the tube and replaced all the air. The tubes were stoppered and then placed horizontally in a rotor at 37°C. The media were changed every 2 days, at which time the procedure for increasing oxygen tension was repeated. Microscopic

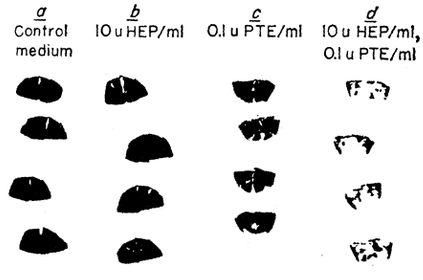

Fig. 1. Effect on bone resorption of heparin (*HEP*) in combination with a suboptimal concentration of parathyroid extract (*PTE*); the bone was cultured for 12 days and then stained by the Von Kossa reaction (*u*, units).

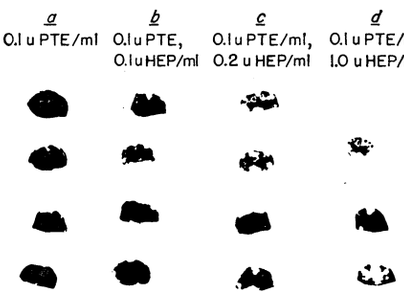

Fig. 2. Effect of different concentrations of heparin (*HEP*) when used in combination with a suboptimal concentration of parathyroid extract (*PTE*); the bone was cultured for 12 days and then stained by the Von Kossa reaction. Note the definite enhancement of resorption when 0.2 unit (*u*) of heparin per milliliter was used (*c*), and the more extensive resorption when 1.0 unit of heparin per milliliter was used (*d*).

observations were made daily on the living cultures (three to four specimens per group) and the extent of resorption observed was scored. The experiments were terminated between 7 and 14 days, at which time the bone samples, still attached to the coverslips, were removed from the tubes, fixed overnight in 10-percent neutral formalin, and then subjected to the Von Kossa reaction which stained the remaining mineral portion of the bone black. The stained calvariae of each experimental group were mounted on separate microscopic slides, and the groups to be compared were photographed together by means of transmitted light. In general, there was excellent correlation between the microscopic scoring of the living calvariae and the Von Kossa-treated bones.

Figure 1 shows that the addition to the medium of heparin sodium (Lilly) alone (10 units/ml) or parathyroid extract (Lilly) alone (0.1 unit/ml) was ineffective (Fig. 1, *b* and *c*), the stained bones being comparable to those in the unsupplemented control group (Fig. 1*a*). However, the combination of 10 units of heparin and 0.1 unit of parathyroid extract per milliliter resulted in extensive resorption (Fig. 1*d*), equivalent to that ordinarily obtained with 0.5 unit of parathyroid extract per milliliter alone. Although no clear-cut effect was demonstrable with parathyroid extract in combination with 0.1 unit of heparin (Fig. 2, *a* and *b*), definite enhancement was obtained with as little as 0.2 unit of heparin per milliliter (Fig. 2*c*). Very good enhancement was obtained with 1.0 unit of heparin per milliliter (Fig. 2*d*). The finding that a synthetic polysaccharide-sulfuric ester (Treburon) (*2*) similar in structure to heparin (*3*) was effective in the same dose range as heparin (0.01 to 0.1 mg/ml) rules out the possibility that the commercial heparin preparation contained contaminants responsible for the effect.

Other acid mucopolysaccharides were tested to determine whether they also would enhance the effectiveness of parathyroid extract. Preliminary studies indicate that neither hyaluronic acid nor chondroitin sulfate are effective. On the basis of experiments in which a five- to tenfold enhancing effect could be demonstrated with heparin in combination with suboptimal concentrations of other factors which stimulate bone resorption (such as crystalline vitamins A or D_2), it is suggested that heparin be considered a bone-resorption-stimulating "cofactor." Although the mechanism of this action of heparin is not yet known, it is conceivable that the mast cell, the only known source of heparin in tissues, might play an important role in physiological or pathological bone resorption. Along this line it should be noted that rats maintained for 15 weeks on a diet low in calcium and vitamin D and high in phosphorus developed a combination of moderate rickets, osteoporosis, and osteitis fibrosa as well as a marked increase in bone mast cells (*4*). Furthermore, in rabbits kept on a high fat diet and injected repeatedly over a long period with heparin, there were frequent occurrences of spontaneous fractures in various bones (*5*). Whether patients on long-term heparin therapy develop an unusual amount of rarefying bone disease remains to be seen.

PAUL GOLDHABER

Department of Oral Histopathology and Periodontology, Harvard School of Dental Medicine, Boston, Massachusetts

References and Notes

1. P. Goldhaber, in *Mechanisms of Hard Tissue Destruction*, R. F. Sognnaes, Ed. (AAAS, Washington, D.C., 1963), p. 609.
2. Kindly provided by Hoffman-La Roche Inc., Nutley 10, N.J.
3. C. N. Mangieri, R. Engelberg, L. O. Randall, *J. Pharmacol. Exptl. Therap.* **102**, 156 (1951).
4. M. R. Urist and F. C. McLean, *Arch. Pathol.* **63**, 239 (1957).
5. T. Gillman, in *Connective Tissue*, R. E. Tunbridge, Ed. (Blackwell, Oxford, 1957), p. 33.
6. Supported by USPHS grant D-1298 and a Career Development Award. I thank Miss Gunta Cirulis and Miss Lidija Trencis for their technical assistance.

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Simian Virus 40: Isolation of Two Plaque Types

Abstract. *Two different morphological plaque types of simian virus 40 were isolated from three of six strains. Increased uptake of neutral red gave one type of plaque a uniform red appearance, whereas the center of the other remained unstained. The viruses showed stability of plaque morphology on passage in tissue culture; cross neutralization studies identified both as simian virus 40.*

During an investigation of the infectivity of simian virus 40 (SV40) by the plaque technique two plaques of differing morphology were noted. The first plaques obtained were similar in appearance to those described by Stinebaugh and Melnick (*1*), although somewhat larger, but, in later attempts to plaque virus at different passage levels, a larger "red" mutant plaque was observed.

The strain of cells used in our study was the BS-C-1 continuous passage line of grivet monkey kidney cells (*2*), grown in medium 199 containing fetal bovine serum (10 percent), and maintained in medium 199 with the serum concentration reduced to 2 percent. The strain of SV40, designated VA 45-54, was obtained from M. Hilleman.

The cells were seeded in plastic petri dishes (60 mm) and were incubated in an atmosphere containing a mixture of 5 percent CO_2 and 95 percent air until a monolayer had formed. Dilutions of the virus were made in medium 199 containing fetal bovine serum (5 percent), and 0.4 ml of each dilution was inoculated onto each monolayer after the removal of the growth medium. A 2-hour absorption period at 37°C sufficed to obtain the maximum number of plaques. The inoculum was then removed, and 5 ml of medium 199 containing fetal bovine serum (5 percent) and purified agar (1.5 percent) (*3*) was added. When the agar had solidified, 4 ml of medium 199 containing fetal bovine serum (5 percent) was added as an overlay, and the plates were incubated at 37°C for 13 days in the CO_2 atmosphere already described. The liquid overlay was then removed, and 4 ml of medium 199 containing neutral red (1:10,000) was added, and the plates were incubated overnight. The neutral red overlay was then removed, and the number