Seeking Radionuclides, C. W. Mays, W. S. S. Jee, R. D. Lloyd, B. J. Stover, J. H. Dougherty, G. N. Taylor, Eds. (Univ. of Utah Press, Salt Lake City, 1969), p. 323.
8. H. Foreman, W. Moss, W. Langham, *Health Phys.* **2**, 326 (1960); C. R. Lagerquist, D. L. Bokowski, S. E. Hammond, D. B. Hylton, in *Proceedings of the 13th Annual Bioassay and Analytical Chemistry Meeting*, AEC Document Conference 671048 (1968), p. 103.
9. A. Catsch, *Int. J. Appl. Rad. Isotop.* **11**, 131 (1961).
10. J. F. Markley, *Int. J. Rad. Biol.* **7**, 405 (1963).
11. A. D. Bangham, M. M. Standish, J. C. Watkins, *J. Mol. Biol.* **13**, 238 (1965).
12. G. Gregoriadis and B. E. Ryman, *Biochem. J.* **129**, 123 (1972).
13. Y. E. Rahman and A. Lindenbaum, *Rad. Res.* **21**, 575 (1964).
14. A. Lindenbaum, C. Lund, M. Smoler, M. W. Rosenthal, in *Diagnosis and Treatment of Deposited Radionuclides*, H. A. Kornberg and W. D. Norwood, Eds. (Excerpta Medica, Amsterdam, 1968), p. 56.
15. A. Lindenbaum and W. Westfall, *Int. J. Appl. Rad. Isotop.* **16**, 545 (1965).
16. V. H. Smith, in *Hanford Biology Research Annual Report for 1958*, AEC Report HW-59500 (Hanford Atomic Products Operation, Richland, Washington, 1959), p. 63.
17. We have subsequently found that DTPA liposomes injected 2 hours or 5 days after preparation have the same effect in removal of Pu from mouse tissues.
18. A. Lindenbaum and M. A. Smyth, in *Organic Scintillators and Liquid Scintillation Counting*, D. L. Horrocks and C.-T. Peng, Eds. (Academic Press, New York, 1971), p. 951.
19. M. W. Rosenthal, E. Moretti, J. J. Russell, A. Lindenbaum, *Health Phys.* **22**, 743 (1972).
20. J. Schubert, J. F. Fried, M. W. Rosenthal, A. Lindenbaum, *Rad. Res.* **15**, 220 (1961).
21. We thank D. W. Baxter, S. Kalesperis, A. Lindenbaum, E. S. Moretti, G. Svihla, and B. J. Wright for assistance and helpful discussions. This work was supported by the U.S. Atomic Energy Commission.

17 November 1972; revised 30 January 1973 ■

Hyaluronate Inhibition of Chondrogenesis: Antagonism of Thyroxine, Growth Hormone, and Calcitonin

Abstract. *The formation of cartilage-like aggregates in high-density stationary cultures of trypsinized chick embryo precartilage cells is blocked by low concentrations of hyaluronate. Thyroxine (and triiodothyronine), growth hormone, calcitonin, and adenosine 3',5'-monophosphate prevented this inhibition by hyaluronate, whereas other hormones tested did not.*

Hyaluronate turnover is a prominent feature of the early development of limb, vertebral skeleton (*1*), and cornea in the chick embryo (*2*) and of the regeneration blastema of the amputated newt (*3*). Hyaluronate synthesis is generally associated with the morphogenetic phase of cell migration and proliferation in these systems, and its removal by the action of a hyaluronidase accompanies subsequent differentiation, such as cartilage deposition. It was hypothesized that hyaluronate may inhibit precartilage cell aggregation consequently delaying differentiation, and thus, that its turnover may be involved in the timing of events leading to tissue formation. In a test of this idea hyaluronate, in concentrations from 1 ng/ml to 500 μg/ml, was shown to prevent cartilage nodule formation in vitro from chick embryonic precartilage cells (*4*). This phenomenon could not be mimicked by other polyanions—chondroitin sulfate, chondroitin sulfate–protein, DNA, RNA, heparin—or by the monosaccharide components of hyaluronate, namely *N*-acetylglucosamine and glucuronate. Thus the control of hyaluronate turnover and of its interaction with mesenchymal cells might be an important feature of morphogenesis in the above-mentioned developmental systems. The antagonism of some growth-promoting hormones toward the inhibition of chondrogenesis in vitro by hyaluronate is described below.

Table 1. Effect of hormone concentration on the prevention of hyaluronate (1 μg/ml) inhibition of chondrogenesis. Five to ten replicate dishes were used in each group.

Hyaluronate	Hormone	Aggregates/dish (mean ± S.D.)
None		18 ± 3
1 μg/ml		1 ± 1
	Thyroxine	
1 μg/ml	0.0001 μ*M*	5 ± 4
	0.01 μ*M*	16 ± 3
	0.1 μ*M*	16 ± 3
	1.0 μ*M*	23 ± 6
	Growth hormone	
1 μg/ml	0.001 μg/ml	8 ± 3
	0.01 μg/ml	15 ± 6
	0.1 μg/ml	14 ± 2
	1.0 μg/ml	10 ± 2
	Calcitonin	
1 μg/ml	0.001 μg/ml	0 ± 0
	0.1 μg/ml	14 ± 8
	1.0 μg/ml	13 ± 6

Stationary cultures of trypsinized 5-day (stage 26) chick embryo somite cells were used as described (*4*). Each dish (60-mm diameter) contained 2.5×10^6 to 5.0×10^6 cells, an amount which resulted in a cell density greater than a confluent monolayer and thus facilitated immediate cell-cell contact and the production of three-dimensional cartilage-like aggregates (*5*). The medium used was Ham's F12 containing fetal calf serum (10 percent), bovine serum albumin (1 percent), ascorbate (50 μg/ml), penicillin (100 units/ml) and streptomycin (100 μg/ml).

In the first series of experiments cultures were established in the presence of 0.1 μg of hyaluronate (*6*) with and without varying concentrations of hormones. Both hyaluronate and hormones were added to the medium before the trypsinized cell suspension was added. Thyroxine (and triiodothyronine), growth hormone, and calcitonin were consistently active in preventing the hyaluronate inhibition of cartilage nodule formation. Hydrocortisone was also active sporadically but only at the highest concentration tested, that is, 50 μ*M*. The following hormones were tested at two to four different concentrations in the ranges indicated in parentheses and found to be inactive: epinephrine, glucagon, and insulin (2.5 to 25 μ*M*); testosterone, progesterone, dexamethasone, and prednisolone (2.5 to 50 μ*M*); prolactin and adrenocorticotropic hormone (ACTH) (0.1 to 5 μg/ml). Thyroid stimulating hormone (0.4 unit/ml) was also inactive. Table 1 gives actual numbers of aggregates obtained in experiments with several concentrations of the active hormones. The ability of one of these hormones, thyroxine (1 μ*M*), to prevent the hyaluronate inhibition was tested at varying concentrations of polysaccharide and were effective up to 50 μg/ml under the conditions used (Table 2).

In various noncartilaginous tissues thyroxine (*7*), growth hormone (*8*), and calcitonin (*9*) have been shown to stimulate the production of cyclic AMP. If cyclic AMP mediates the action of these hormones in vivo it might be expected to duplicate their action in vitro as has been found to be the case for many other cell or tissue responses to hormones. Concentrations of cyclic AMP as low as 1 μ*M* were effective in preventing the hyaluronate inhibition. Dibutyryl cyclic AMP or theophylline

Table 2. Effect of hyaluronate concentration on chondrogenesis in the presence of thyroxine. Five replicate dishes were used in each group.

Hyaluronate (μg/ml)	Mean No. of aggregates per dish	
	1 μ*M* thyroxine	No thyroxine
0	12	20
0.001	27	1
0.1	18	3
10	25	1
50	22	2
100	5	1

at 10 μM also had this effect. However, adenosine triphosphate, adenosine diphosphate, AMP, cyclic guanosine monophosphate and cyclic uridine monophosphate were also at least partially effective in reversing the inhibition. Consequently any conclusions as to the involvement of cyclic AMP will have to await measurement of endogenous levels in the presence of hyaluronate and hormones.

The effect of thyroxine in preventing the inhibition by hyaluronate of chondrogenesis was predicted as a result of the following observations: (i) correlations between the action of thyroxine and the turnover of hyaluronate in several tissues, such as metamorphosing tadpole backskin (*10*), chick embryo cornea (*2, 11*), skin of hypothyroid rats (*12*); (ii) the enhancement of chondrogenesis in vitro by thyroxine (*13*); (iii) the necessity of thyroxine for correct skeletal maturation in vivo (*14*). Growth hormone also is necessary for skeletal maturation and stimulates chondroitin sulfate synthesis by chondroblasts in vitro (*15*). Thus it may be that these substances overcome the hyaluronate inhibition by direct stimulation of matrix synthesis. Alternatively the hormones and hyaluronate may act in an antagonistic fashion at some common site at the cell surface, for example, the adenylate cyclase system, indirectly modifying rates of chondroitin sulfate (*16*) and collagen synthesis (*17*) or levels of hyaluronidase activity (*18*).

BRYAN P. TOOLE

Developmental Biology Laboratory, Departments of Medicine and *Biological Chemistry, Harvard Medical School,* and *Massachusetts General Hospital, Boston 02114*

References and Notes

1. B. P. Toole, *Develop. Biol.* **29**, 321 (1972).
2. ——— and R. L. Trelstad, *ibid.* **26**, 28 (1971).
3. B. P. Toole and J. Gross, *ibid.* **25**, 57 (1971).
4. B. P. Toole, G. Jackson, J. Gross, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 1384 (1972).
5. B. B. Lavietes, *Develop. Biol.* **21**, 584 (1970); L. P. Schacter, *Exp. Cell Res.* **63**, 19 (1970).
6. Hyaluronate was obtained from Biotrics, Inc. (Arlington, Mass.) and was donated by Dr. Endre Balazs. Proactin (natural ovine), growth hormone (natural human), calcitonin (synthetic salmon), and ACTH (synthetic human) were generous gifts of Dr. John Potts and Dr. Hugh Niall. All other hormones used and adenosine 3′-5′-monophosphate (cyclic AMP), dibutyryl cyclic AMP, and theophylline were obtained from Sigma Chemical Co. (St. Louis, Mo.).
7. G. S. Levey and S. E. Epstein, *Biochem. Biophys. Res. Commun.* **33**, 990 (1968).
8. J. Moskowitz and J. N. Fain, *J. Biol. Chem.* **245**, 1101 (1970).
9. F. Murad, H. B. Brewer, M. Vaughan, *Proc. Nat. Acad. Sci. U.S.A.* **65**, 446 (1970).
10. M. J. Lipson, R. A. Cerskus, J. E. Silbert, *Develop. Biol.* **25**, 198 (1971).
11. A. J. Coulombre and J. L. Coulombre, *Exp. Eye Res.* **3**, 105 (1964).
12. S. Schiller, G. A. Slover, A. Dorfman, *Biochim. Biophys. Acta* **58**, 27 (1962).
13. J. M. Pawelek, *Develop. Biol.* **19**, 52 (1969).
14. H. A. Sissons, in *The Biochemistry and Physiology of Bone*, G. H. Bourne, Ed. (Academic Press, New York, 1971), vol. 3, p. 145.
15. S. Meier and M. Solursh, *Gen. Comp. Endocrinol.* **18**, 89 (1972).
16. J. F. Goggins, G. S. Johnson, I. Pastan, *J. Biol. Chem.* **247**, 5759 (1972).
17. A. W. Hsie, C. Jones, T. T. Puck, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1648 (1971).
18. E. Harper and B. P. Toole, *J. Biol. Chem.*, in press.
19. I thank Mrs. Geraldine Jackson for technical assistance and Dr. Jerome Gross for support and helpful discussion. This is publication No. 589 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities. Supported by grants from the National Foundation–March of Dimes (GRBS-229) and NIH (AM 3564).

20 November 1972; revised 18 January 1973 ■

Blood Cells Preserved in a Mummy 2000 Years Old

Abstract. *Structures resembling red blood cells have been seen in mummies, but have been considered by some to be artifacts or molds. The finding of these structures, admixed with white blood cells, in the blood vessels of a mummified American Indian, confirms the original interpretation of preserved red blood cells.*

Although the tissues of a number of mummies have been examined microscopically, there have been no convincing demonstrations of the preservation of red or white blood cells (erythrocytes or leukocytes). Ruffer (*1*) noted his failure to identify erythrocytes in studying hundreds of Egyptian mummies. Wilder (*2*) claimed to have found red blood cells in the nasal cavities of a Basket Maker mummy but did not include a description or photomicrograph in his report. In 1927, Williams (*3*) found erythrocyte-like structures in a pair of Peruvian mummies dating to about A.D. 700. While showing the characteristic biconcavity of erythrocytes, these structures were extravascular, in skeletal muscle, and were 8 or 9 μm in diameter, about twice the size of red blood cells in sections of fresh tissue.

Sandison (*4*) considered the structures reported by Wilder and Williams to be fungi, because of their variation in size and extravascular location. In studying an Egyptian mummy, Sandison (*5*) discovered erythrocyte-like bodies in the thyroid gland. These bodies were 3.25 μm in diameter and had the staining characteristics of red blood cells. Unfortunately, his report includes neither a photomicrograph nor a description of the exact location of these structures in relation to blood vessels. In examining the skin of two 2600-year-old Egyptian mummies, Giacometti and Chiarelli (*6*) saw erythrocyte-like structures in spaces considered to be blood vessels.

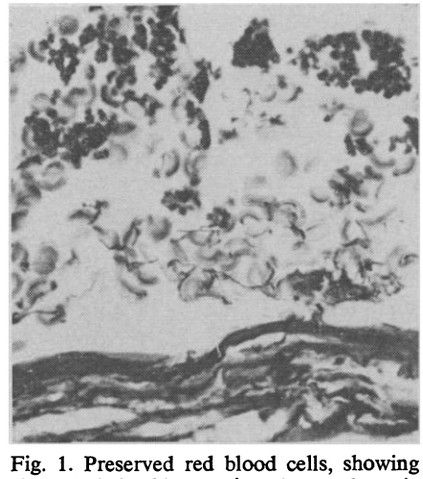

Fig. 1. Preserved red blood cells, showing characteristic biconcavity, in a thoracic vein. The dark granules are autolyzed leukocytes. Hematoxylin and eosin stain (× 500).

Blood in tissue experimentally desiccated and rehydrated appears as a homogeneous eosinophilic mass showing, in areas, preservation of the circular outlines of the erythrocytes (*7*). Biconcavity of the erythrocytes is poorly preserved. The polymorphonuclear leukocytes appear as small clusters of basophilic granules. This appearance is similar to that of autolyzed leukocytes in freshly processed tissue.

This report demonstrates the preservation of blood cells in the vessels of a 2000-year-old mummy, the desiccated body of a 9-year-old American Indian boy found in Salts Cave, Kentucky. Examination of the mummy permitted identification of the internal organs and of the diet of the individual (*8*). No cause of death was determined on gross examination. Portions of the heart, lung, kidney, thoracic tissue, and intestine were examined microscopically. All the tissues were dark brown, dry, and fragile. Only the lung showed gross structure suggestive of the normal architecture. Rehydration was accomplished by Ruffer's technique of immersion of the tissue in a solution of 50 parts of water, 30 parts of absolute