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*).

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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).

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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.

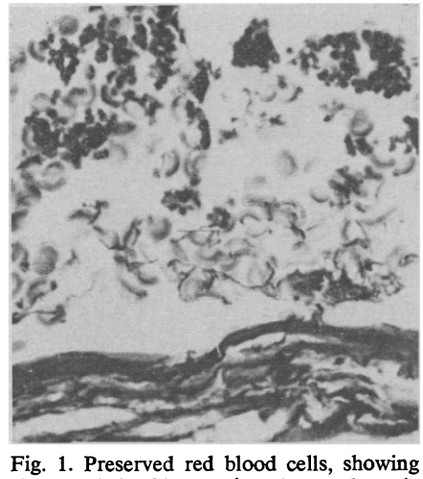

Fig. 1. Preserved red blood cells, showing characteristic biconcavity, in a thoracic vein. The dark granules are autolyzed leukocytes. Hematoxylin and eosin stain (× 500).

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

alcohol, and 20 parts of 5 percent sodium carbonate in water (*1*). One third of the solution was removed and replaced by absolute alcohol for three successive days, and the tissue was fixed in absolute alcohol for 3 days. The specimens were then embedded in paraffin and sectioned on a Spencer rotary microtome. Staining techniques were those given in the Armed Forces Institute of Pathology manual (*9*) and the phosphotungstic acid–hematoxylin method of Lieb (*10*). A section of congested human lung was used as a control for the staining characteristics of the erythrocytes. The mixed cell agglutination reaction (MCAR) (*11*) was utilized in an attempt to identify the blood group of the red cells.

There was no evidence of pathological processes on microscopic examination. However, examination of the tissues from the thorax revealed the preservation of several large blood vessels, identified as arteries and veins by connective and elastic tissue stains. Within the veins were small groups of cells showing the typical biconcave morphology of erythrocytes (Fig. 1). These cells showed a somewhat scaphoid shape on edge. The cells stained only faintly with Masson trichrome and phosphotungstic acid–hematoxylin stains but did not reveal any differences in staining characteristics from fresh red blood cells. Two fungal stains were used; a periodic acid–Schiff stain was negative, while a number of the cells did take a methenamine silver stain to a moderate degree. The sections were tested for A, B, and H(O) isoantigens by the MCAR, and all reactions were negative. Scattered among the red cells were clumps of fragmented basophilic material, representing nuclear fragments of autolyzed polymorphonuclear leukocytes.

The intravascular location, typical shape, admixture of autolyzed white blood cells, and staining characteristics are all consistent with the interpretation that the structures are preserved red blood cells. The negative MCAR probably represents loss of antigenicity over a 2000-year period, but may be due to washing out of the antigen by the rehydration process. The only other inconsistency was the ability of some of the cells to take the silver stain for a fungus. This finding, in view of the other evidence, must be interpreted as a variation in the staining characteristics of red blood cells with mummification and rehydration. Desiccated and rehydrated fungi retain a marked avidity for this stain.

These cells are identical in appearance with those described by Giacometti and Chiarelli (*6*) and by Williams (*3*). Williams had interpreted his sections as indicating an area of hemorrhage, and the present finding confirms that diagnosis (*12*).

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References and Notes

1. M. A. Ruffer, *Studies in the Paleopathology of Egypt* (Univ. of Chicago Press, Chicago, 1921).
2. H. H. Wilder, *Amer. Anthropol.* **6**, 1 (1904).
3. H. U. Williams, *Arch. Pathol.* **4**, 26 (1927).
4. A. T. Sandison, in *Science in Archeology*, D. Brothwell and E. Higgs, Eds. (Praeger, New York, 1970), p. 494.
5. ———, *Stain Technol.* **30**, 277 (1955).
6. L. Giacometti and B. Chiarelli, *Arch. Dermatol.* **97**, 712 (1968).
7. M. R. Zimmerman, *Amer. J. Phys. Anthropol.* **37**, 271 (1972).
8. L. M. Robbins, *Amer. Antiquity* **36**, 200 (1971).
9. L. Luna, *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology* (McGraw-Hill, New York, 1968).
10. E. Lieb, *Arch. Pathol.* **45**, 599 (1948).
11. S. Kovanik, I. Davidsohn, R. Stejskal, *ibid.* **86**, 12 (1968).
12. I thank Dr. Louise Robbins, formerly of the Department of Anthropology, University of Kentucky, for supplying the tissue used in this study, and Dr. Israel Davidsohn of the Chicago Medical School for performing the MCAR studies. This report was presented in a symposium on skeletal biology at the meeting of the American Association of Physical Anthropologists, Lawrence, Kansas, 13 April 1972.

27 November 1972

Cyclic AMP Phosphodiesterase in Cloned Astrocytoma Cells: Norepinephrine Induces a Specific Enzyme Form

Abstract. *The soluble supernatant fraction of homogenates of cloned rat astrocytoma cells (line C-2A) was subjected to polyacrylamide gel electrophoresis. Two peaks of adenosine 3′,5′-monophosphate phosphodiesterase activity were found, corresponding to peaks I and IV of a similarly prepared homogenate of rat brain. Incubating cells with norepinephrine (0.3 millimolar) caused about a threefold increase in the activity of peak IV but no change in peak I. This increase was completely inhibited by prior incubation with propranolol (0.1 millimolar), a beta-adrenergic blocking agent, or with cyclohexamine (40 micromolar), a protein synthesis inhibitor. Induction of a specific phosphodiesterase form by norepinephrine suggests another feedback control mechanism whereby an organism can prevent the effects of excessive sympathetic activity.*

Adenosine 3′,5′-monophosphate (cyclic AMP) phosphodiesterase, the enzyme that catalyzes the hydrolysis of cyclic AMP (*1*), exists in several molecular forms (*2*, *3*). For example, using polyacrylamide gel electrophoresis we found six distinct forms of cyclic AMP phosphodiesterase in the soluble supernatant fraction of rat cerebellar homogenates (*3*). These different molecular forms of phosphodiesterase found in brain may reside in specific types of cells. Thus, we found four peaks of phosphodiesterase activity in cerebrum (designated I to IV according to their order of emergence from the electrophoresis column). However, we found only two peaks of activity in a C-2A astrocytoma cell line. These peaks had the same electrophoretic mobilities and other characteristics similar to those of peaks I and IV of rat cerebrum, and will be designated peaks I and IV. In each of two cloned neuroblastoma cell lines (N1E and N18), we found only one peak of activity corresponding to peak III of rat cerebrum (*4*).

As part of a series of studies to determine the role of the sympathetic nervous system and other endogenous factors in controlling the intracellular concentration of cyclic nucleotides (*5*, *6*), we report here on the effect of norepinephrine on the two forms of cyclic AMP phosphodiesterase in cloned C-2A rat astrocytoma cells.

The C-2A rat astrocytoma cell line was derived by cloning rat brain glial tumors induced by the intravenous injection of *N*-nitrosomethylurea (*7*). The cells were grown to confluency in Falcon plastic tissue culture flasks in air supplemented with 10 percent CO_2; the medium was Dulbecco's modification of Eagle's medium, supplemented with 10 percent fetal calf serum, penicillin G (50 unit/ml) and streptomycin sulfate (10 μg/ml). At the initiation of each experiment, the medium was removed and replaced with fresh medium containing the