Calculations with Avogadro's number show that 6.7×10^{12} molecules of actinomycin D are bound to each sea urchin egg or unhatched embryo. Because this concentration is approximately equal to the concentration of actinomycin D in the volume of seawater displaced by an egg of *Arbacia punctulata* (*4*), no net accumulation within the space occupied by the eggs occurs. The drug is bound on first contact at a number of unknown sites on the outer surface of the egg or embryo. The fact that the concentration on the surface of cells is in equilibrium with the surrounding medium before hatching suggests that the binding is relatively weak. The autoradiographic studies show that no detectable radioactivity is trapped within cells before the membranes are removed at hatching. This conclusion is further strengthened by the inability of eggs to absorb sufficient actinomycin D after exposure for 4 hours before fertilization to prevent normal development to a highly differentiated, freely feeding stage.

The rate of protein synthesis during the first 4 hours of development in embryos treated with actinomycin was not significantly different from that in untreated controls (*2*); however, the effectiveness of the drug increased with subsequent development. Our results indicate that the lack of effect of actinomycin D on protein synthesis during early embryogenesis may be due to the inability of the drug to enter the cells. The arrest in development which occurs at the hatching stage in the presence of actinomycin D can best be explained by our observations that the embryo becomes vulnerable to actinomycin D only when the hatching process deprives it of its protective shell.

M. MICHAEL THALER
Department of Pediatrics,
University of California–
San Francisco Medical Center,
San Francisco 94122

MALCOLM C. L. COX
CLAUDE A. VILLEE
Department of Biological Chemistry,
Harvard Medical School,
Boston, Massachusetts 02115, and
Marine Biological Laboratory,
Woods Hole, Massachusetts 02543

References and Notes

1. J. Brachet and A. Ficq, *Compt. Rend.* **258**, 6258 (1964); F. DeVitry, *ibid.*, p. 4829; M. N. Goldstein, K. Hamm, E. Amrod, *Science* **151**, 1555 (1966).
2. P. R. Gross and G. H. Cousineau. *Exp. Cell Res.* **33**, 368 (1964); P. R. Gross, L. I. Malkin, W. A. Moyer, *Proc. Nat. Acad. Sci. U.S.* **51**, 407 (1964); R. B. Billiar, L. Zelewski, C. A. Villee, *Develop. Biol.* **13**, 282 (1966).
3. P. R. Gross, in *Current Topics in Developmental Biology*, A. A. Moscona and A. Monroy, Eds. (Academic Press, New York, 1967); A. Tyler, *Develop. Biol. Suppl.* **1**, 170 (1967).
4. E. B. Harvey, *The American Arbacia and Other Sea Urchins* (Princeton Univ. Press, Princeton, N.J., 1956).
5. We thank A. Pelger for technical assistance, and A. Schmid for help with the manuscript. Aided by PHS training grant HD-06.

9 January 1969; revised 20 March 1969

Soluble Sulfatase in Growing Bone of Rats

Abstract. *Soluble sulfatase has been found in epiphyseal, articular, and rib cartilages and in metaphysis and bone marrow of the rat. The greatest activity in young rats is in the metaphysis. Young rats had higher levels of activity in epiphyseal and articular cartilage and in the metaphysis than did the older rats.*

There has been growing attention in the recent literature to degradative changes in proteinpolysaccharides in calicifying cartilage, after the demonstration of a cartilage protease (*1*). Since there is a rapid turnover of radioactive sulfate in epiphyseal cartilage, an attempt was made to demonstrate the presence of sulfatase in this tissue. Soluble sulfatase has been found in many tissues of different animal species and in *Proteus vulgaris* (*2*) but has not hitherto been reported in cartilage and bone. The method used was adapted from Dodgson *et al.* (*2*) and Roy (*3*). We did not attempt to differentiate between soluble arylsulfatase A and B.

Epiphyseal, articular, and rib cartilages, bone marrow, and metaphysis were dissected from freshly killed Wistar rats 28 to 116 days old. The tissues were ground up separately with a mortar and pestle (5 ml of distilled water was added to each), alternately frozen and thawed three times, and kept cold overnight. The suspension was made up to 12 ml with distilled water and was centrifuged at 20,000 rev/min for 30 minutes. The precipitate was washed and recentrifuged at 30,000 rev/min for 30 minutes. Four volumes of acetone at $-20°C$ were added to the combined supernatants to precipitate the enzyme. The mixture was centrifuged at 25,000 rev/min for 45 minutes, and the supernatant was discarded. The precipitate was dried in a vacuum desiccator after the residual acetone was removed with a stream of air.

The dried samples were dissolved in 0.6 ml of distilled water at 37°C for 1 hour, and 0.6 ml of 0.5*M* sodium acetate–acetic acid buffer (*p*H 5.3) was added. To 0.6 ml of this mixture was added 0.6 ml of 0.5*M* acetate buffer (*p*H 5.3) containing 21 mg of purified *p*-nitrocatechol sulfate to make a final substrate concentration of 0.063*M*. After incubation for 1 hour at 37°C, 3 ml of alkaline quinol was added to develop a red color (*3*). The absorbancy of the red solution was determined at 515 nm. The *p*-nitrocatechol present was read from a standard curve and reported in micrograms per milliliter. Blanks were run containing *p*-nitrocatechol sulfate, but no enzyme; control samples, containing enzyme that had been boiled for 3 minutes before addition of *p*-nitrocatechol sulfate, were also run. All boiled controls showed negligible activity. The activity of sulfatase in the metaphysis (showing the highest activity) was determined to be linear with time during the hour's incubation.

Soluble *p*-nitrocatechol sulfatase activity was found in all of the tissues tested (Table 1). There was considerable variation in the level from animal to animal, but nevertheless, in the case of epiphyseal and articular cartilage and of the metaphysis, the younger

Table 1. *p*-Nitrocatechol sulfatase activity in growing rat bone. Results are expressed as the mean ± standard deviation, in micrograms of nitrocatechol released per milligram of wet weight per hour. Figures in parentheses are the number of animals.

Tissue	Age of rats (days)		*P**
	28–35	76–111	
Epiphyseal cartilage	6.95 ± 3.90 (5)	1.78 ± 0.44 (7)	.009
Articular cartilage	4.22 ± 2.01 (4)	1.22 ± .28 (4)	.04
Costal cartilage	1.08 ± 0.29 (3)	0.76 ± .15 (4)	.17
Metaphysis	12.26 ± 4.25 (5)	6.23 ± 2.64 (6)	.03
Bone marrow	7.82 ± 3.16 (4)	9.25 ± 2.77 (4)	.58

* Statistical significance between young and old groups.

group of animals had significantly higher levels of activity than the older group.

Inasmuch as sulfatase is known to be present in some leukocytes (*4*), and because some blood was present in the tissues used, we were concerned whether the sulfatase present was due to contamination with blood. The metaphysis, which also contains appreciable amounts of myeloid tissue and blood, showed greater activity per unit weight than bone marrow alone, in the case of the younger animals. Thus the presence of high sulfatase levels in the metaphysis could not be attributed solely to the presence of marrow. The epiphyseal, articular, and costal cartilages had relatively little blood, and it is not likely that this would affect the results appreciably.

The mechanisms by which sulfatase acts in growing bone is not known. Yamagata *et al.* (*5*) found that sulfatase by itself does not have any effect on chondroitin sulfate. In preliminary experiments, we have found no release of sulfate from proteinpolysaccharide incubated with sulfatase. Saito *et al.* (*5, 6*) have shown that a release of sulfate from chondroitin sulfate prepared from cartilage does occur if the chondroitin sulfate is degraded with other enzymes prior to treatment with sulfatase.

The presence of sulfatase as well as a protease and perhaps other degradative enzymes in the growing region of bone, and particularly the metaphysis, suggests that desulfation of the sulfated glycosaminoglycan part of the proteinpolysaccharide may occur here, but the function of the sulfatase in this region has not yet been demonstrated.

ALBERT HIRSCHMAN
MILDRED HIRSCHMAN
Department of Anatomy, Downstate Medical Center, State University of New York, Brooklyn 11203

References and Notes

1. D. D. Dziewiatkowski, *Clin. Orthop.* **35**, 189 (1964).
2. K. S. Dodgson, B. Spencer, J. Thomas, *Biochem. J.* **53**, 452 (1953); ———, *ibid.* **59**, 29 (1955); K. S. Dodgson, B. Spencer, C. H. Wynn, *ibid.* **62**, 500 (1956).
3. A. B. Roy, *ibid.* **53**, 12 (1953).
4. J. H. Austin and M. Bischel, *Blood* **17**, 216 (1961); L. M. Dzialoszynski and J. G. Szulzycka, *Clin. Chim. Acta* **15**, 381 (1967).
5. T. Yamagata, H. Saito, O. Habuchi, S. Suzuki, *J. Biol. Chem.* **243** (7), 1523 (1968).
6. H. Saito, T. Yamagata, S. Suzuki, *ibid.*, p. 1536.
7. Supported by grant AM05922 from the National Institute of Arthritis and Metabolic Diseases and contract AT (30-1) 2960 with AEC.

25 March 1969

Testicular Lactate Dehydrogenase Isozyme: Cyclic Appearance in Bats

Abstract. *In male bats* Tadarida brasiliensis *spermatogenesis occurs only during winter and spring. Two additional lactate dehydrogenase isozymes appear in testes containing mature spermatozoa. This type of isozymic change implies cyclic activation of genes and affords valuable markers for study of the factors involved.*

An additional isozyme of lactate dehydrogenase (LDH) (L-lactate: nicotinamide-adenine dinucleotide oxidoreductase, E.C. 1.1.1.27) occurs in homogenates of mature testes from many species (*1*). This isozyme, designated LDH-X, appears at the time of sexual maturation and is a tetramer of polypeptides (C) different from the A (M, muscle type) and B (H, heart type) monomers composing the five isozymes common to most tissues. Evidence obtained in a study of pigeon testes clearly indicated that the synthesis of the additional polypeptide is controlled by a third genetic locus (*2*). The LDH-X isozyme is the predominant LDH fraction in spermatozoa and has some peculiar catalytic properties suggesting a very specialized role (*3*).

The tissue specificity and close relationship of LDH-X with spermatogenesis raise the question of whether this isozyme shows a cyclic appearance in those species with a seasonal pattern of sexual activity. Males from some species of bats follow a seasonal sexual cycle. Copulation takes place only during a defined period of the year. The season and length of this period vary from one species to another and it also varies from one region to another within the same species (*4*).

To study the correlation between sexual activity and testicular LDH, we investigated histological and isozymic changes in testis of *Tadarida brasiliensis*, a species of bats widely distributed in the American continent, from the United States to Argentina and Chile (*4, 5*). Females are monoestrous and fecundation occurs only at the beginning of spring (*4*).

Throughout a period of 2 years, 133 adult males were collected at two different sites in the province of Córdoba (Argentina). Histological sections of testes did not show spermiogenic activity in animals obtained between the end of spring (November to December in Argentina) and early autumn (March to April). Testes were atrophic (average weight, 9.6 mg); seminiferous tubules had only Sertoli cells and quiescent spermatogonia. Spermatogenesis started during the fall (April to May); there were frequent mitoses and numerous spermatocytes, but no spermatozoa. Animals caught in winter and the beginning of spring (June through October) exhibited enlarged testes (average weight, 37 mg) with reduction of interstitial tissue and wider tubules showing the complete sequence of gametogenesis, including mature spermatozoa. Epididymides were full of sperm in specimens taken in September and October.

In 63 females captured during the same period, pregnancy was observed only from October to December. No pregnant animals were obtained at any other time.

This sexual cycle of *Tadarida brasiliensis* in Argentina is similar to that reported by Sherman for *T. cynocephala* in Florida and by Davis *et al.* for *T. brasiliensis* in Texas (*6*).

Animals were killed with ether, and heart, liver, kidney, stomach, intestine, skeletal muscle from several regions, uterus, testis, and epididymis were removed immediately and processed at once or stored at −20°C for a few days. Results were the same with fresh or frozen organs. Tissues were ground in a Potter-Elvejhem homogenizer with distilled water. Extracts were separated by centrifugation at 20,000*g* for 20 minutes at 4°C. Supernatants were subjected to electrophoresis on starch gel (*7*) made with 12 g of hydrolyzed starch for each 100 ml of 0.008*M* citrate-phosphate buffer, *p*H 7.0. The electrode vessels contained 0.15*M* citrate-phosphate, *p*H 7.0. A voltage gradient of 6 volt/cm was applied for 14 hours at 4°C. After electrophoresis, the gels were stained for identification of lactate dehydrogenase by the method of Zinkham *et al.* (*1*).

In most tissues, five lactate dehydrogenase isozymes were observed. Their relative distribution was similar to that observed in preparations of the same tissues from other species. The isozyme closest to the anode, LDH-1, predominates in heart- and breast-muscle extracts; LDH-5, closest to the cathode, is the dominant band in liver and leg muscle. Inactive testis showed only LDH-1, LDH-2, and LDH-3. In many

16 MAY 1969