Autoradiographic Studies of Sulfated Mucopolysaccharide Metabolism in Cartilage of Osteolathyric Rats and Chicks.* (24434)

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Experimental osteolathyrism is produced by feeding animals seeds of *Lathyrus odoratus*(1, 2) or injecting them with the toxic factor β-aminopropionitrile(3). One of the characteristic features of this syndrome is hypertrophy of cartilage(1,2) accompanied by disorganization of the cellular architecture(2,3,4) formation of clefts(4), slipping of articular surfaces (2,3,4,5) and detachment of tendinous and ligamentous insertions(2). These conditions have been theoretically ascribed to "defective formation or excessive destruction of chondroitin sulfate of ground substance"(2). Sulfur^{35} recovered chemically from skeleton, has been increased after 7 days(6) in animals when P^{32} of bone was also increased. At the same time, weight of femurs was increased while general body weight was smaller than that of controls(6). Autoradiographic studies made 20 hr after injection of tracer sulfate have shown "deposition of S^{35} in about equal amounts in experimental as well as in control animals"(7). On the other hand, histochemical studies involving use of periodic acid-Schiff, alcian blue and toluidine blue have led Menzies and Mills to conclude that when sweet pea seed was fed to rats, it produced in the aorta and bone, "an accumulation of chondroitin sulfate with the general breakdown of supporting structures such as collagen fibers" (8). Our experiments have been carried out on 2 groups of 6 weanling Sprague-Dawley rats fed a 50-50 mixture of complete diet and *Lathyrus odoratus* seed ground thin, for 10 and 16 days with the same number of controls fed the complete diet; also 6 one-day-old chicks which ate a 50-50 mixture of Purina Growing Mash and ground Lathyrus seed and were kept for 7 days at 35°C along with 6 controls on Growing Mash exclusively. Some rats after 16 days of lathyrus feeding and most chicks after 7 days showed symptoms of severe intoxication(5).

Methods. All rats and 3 chicks in each group were injected with 1 μc/g $S^{35}O_4$ subcutaneously, 2 hr prior to decapitation. The tibiae were demineralized with 5% nitric acid in 70% ethanol for 7 days, then dehydrated and embedded in paraffin. Sections of tibiae of chicks, which had not been injected with radiosulfate, were placed for 1 hr in a weak solution of $Ca^{45}Cl_2$(9), then thoroughly washed, dehydrated and prepared for autoradiography by the coating-inversion technic (10,11) along with similar sections from animals injected with S^{35}.

Results. In control rats (Fig. 1), the radiosulfate appeared incorporated by small and large cells except in calcifying cartilage next to the site of bone formation (Fig. 1). Cartilage cells were neatly arranged in columns parallel to the bone axis. A small amount of ground substance was visible between the columns of cells. The autoradiographic image was almost exclusively related to the chondrocytes, 2 hours after subcutaneous injection of tracer (Fig. 1).

The epiphyseal plate of the tibia in lathyric rats of 10 days, (Fig. 2) was already thicker than that of controls (Fig. 1). The lower border appeared jagged and columns of chondrocytes were irregularly arranged and dispersed in a more abundant matrix (Fig. 2). A comparable amount of radiosulfate seemed to have been retained by individual chondrocytes (Fig. 2). The radioactive material appeared also almost exclusively intracellular at 2 hrs. It seemed, however, that the increase in thickness of the plate was due to a larger amount of ground substance and also to an increase in number of cells capable of synthetizing radiosulfur (Fig. 2). The thickness of cartilage where no synthesis took place showed less change (Fig. 2).

In rats which had been on the lathyric diet for 16 days, the epiphyseal plate was thicker

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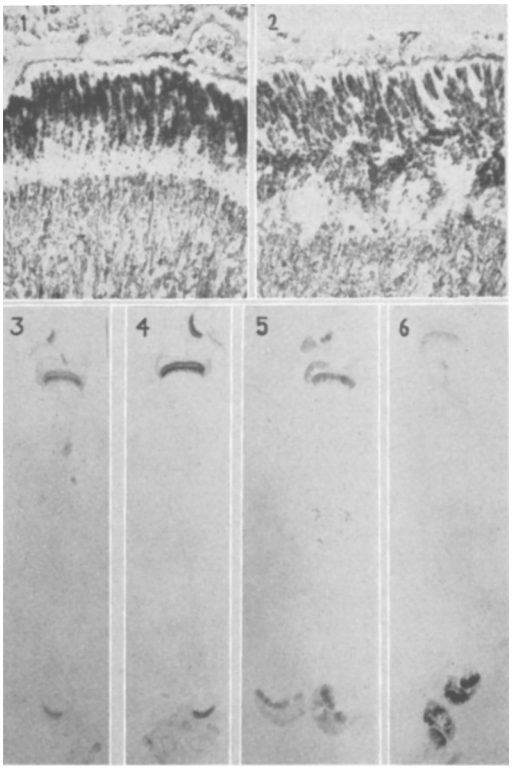

FIG. 1. Integrated, stained autoradiograph of epiphyseal plate of a normal young rat 2 hr after subcut. inj. of radiosulfate, × 26.

FIG. 2. Integrated, stained autoradiograph of epiphyseal plate of a young rat on lathyrogenic diet for 10 days and 2 hr after radiosulfate, × 26.

FIG. 3. Integrated, unstained autoradiograph of tibia of 7-day-old chick, 2 hr after radiosulfate, × 1.25.

FIG. 4. Integrated, unstained autoradiograph of tibia of 7-day-old chick on lathyrogenic diet, 2 hr after radiosulfate, × 1.25.

FIG. 5. Integrated, unstained autoradiograph of tibia of 7-day-old chick, 1 hr *in vitro* treatment with Ca^{45}, × 1.25.

FIG. 6. Integrated, unstained autoradiograph of tibia of 7-day-old chick on lathyrogenic diet, 1 hr *in vitro* treatment with Ca^{45}, × 1.25.

but there was also a proportional increase in amount of ground substance and of cells capable of picking up S^{35} *in vivo*. These results agree with the observations of Ponseti *et al.* (7) and possibly also with conclusions of Bauer *et al.*(6) in regard to overall sulfur content of the epiphyseal plate since the total number of cells capable of synthetizing sulfate is increased.

Furthermore, localized areas of sulfur uptake in connection with activated callus-like growth at the periosteum were also observed in our lathyric rat autoradiographs. These observations reinforce the opinion of Bauer *et al.*(6) in regard to overall sulfur content in the bone.

Hypertrophic changes observed in the rat were not apparent in the chick after 7 days (Figs. 3, 4, 5, 6). In birds, radioactive sulfur was found in similar locations in both controls (Fig. 3) and lathyric animals (Fig. 4). Autoradiographs have shown this material deposited mainly in a layer of small cells near the junction of articular and growing portions of cartilage and in a second layer of large cells preceding the mineralized portion (Figs. 3, 4). There seemed to be larger uptake at the faster growng extremity (Figs. 3,4) and also an equal or larger uptake by cartilage of lathyric chicks (Fig. 4) as compared to that of normal birds (Fig. 3).

These observations on radioactive sulfur uptake in the live animal are not informative in regard to residual content of the matrix which results from synthetic activity of the chondrocytes(12) but is also dependent on rate of turnover or utilization. *In vitro* uptake of Ca^{45} by demineralized sections of tissue has given results in previous problems(9,13,14) of skeletal physio-pathology. It is based on the demonstrated ability of chondroitin sulfate to act as a cation binder(15). With the present material, the upper extremity of the tibia in normal chicks (Fig. 5) showed a larger amount of Ca^{45} uptake in the growing zone of cartilage as compared to the articular portion. The lower extremity of bone seemed to contain slightly more than the upper extremity; the tibiae of lathyric chicks contained considerably less material capable of binding Ca^{45} *in vitro* (Fig. 6) and by opposition, the lower extremity contained a much larger amount (Fig. 6). It is noteworthy that in animals which have shown cleavage and slipping of the epiphysis(5), the upper extremity of the tibia was always involved while the lower extremity remained intact.

These facts are interpreted as showing a difference in metabolism and growth activity of the 2 extremities of the tibia. Even if lathyric animals have a normal or slightly exaggerated uptake of sulfate probably incorporated as chondroitin sulfate(16,17), the

turnover or loss of that substance must be greater at the upper extremity of bone so that the residual content is less than that of controls after 7 days. On the other hand, the lower extremity, with a much slower growth potential, seems to have accumulated more cation binder than that of the normal chick.

In previous studies, the hypertrophic epiphyseal plate of rickets(9,14) and fluorosis (13) has shown an accumulation of cation binder, while with impaired synthesis, the epiphyseal plate of magnesium deficient rats has shown a lower residual content(18).

Summary. In lathyric rats and chicks, the *in vivo* sulfate fixation per individual cell was equal or slightly higher than that of normal animals. Since there were more active cells in the hypertrophic plate of the lathyric rat, the overall sulfur upake by cartilage was apparently greater. In 7 day lathyric chicks, the plate was not hypertrophied but the sulfate uptake seemed slightly greater. *In vitro* Ca^{45} uptake by demineralized cartilage of the upper extremity of the tibia was considerably less than that of the lower extremity and also less than that of controls, indicating a more rapid utilization or turnover.†

† PROC. SOC. EXP. BIOL. AND MED. (v98, 318) contains a report by Castellani and Castellani-Bisi showing that in a mixture of epiphyseal plates from osteolathyric rats, hexosamine was significantly decreased (36%) while hydroxyproline remained practically unchanged.

1. Geiger, B. J., Steenbock, H., Parsons, H. T., *J. Nutrit.*, 1933, v6, 427.

2. Ponseti, I. V., Shepard, R. S., *J. Bone Joint Surg.*, 1954, v36A, 1031.

3. Selye, H., *Rev. Can. de Biol.*, 1957, v16, 1.

4. Selye, H., Bois, P., *Endocrinology*, 1957, v60, 507.

5. Bélanger, L. F., submitted to *J. Bone Joint Surg.*

6. Bauer, G. C. H., Carlsson, A., Lindquist, B., *Acta Path. Bact. Scand.*, 1955, v37, 407.

7. Ponseti, I. V., Wawzonek, S., Shepard, R. S., Evans, T. C., Stearns, G., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 366.

8. Menzies, D. W., Mills, K. W., *J. Path. Biol.*, 1957, v73, 223.

9. Bélanger, L. F., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 150.

10. Bélanger, L. F., Leblond, C. P., *Endocrinology*, 1946, v39, 8.

11. Bélanger, L. F., *Nature*, 1952, v170, 625.

12. Bélanger, L. F., *Can. J. Biochem. Physiol.*, 1954, v32, 161.

13. Bélanger, L. F., Visek, W. J., Lotz, W. C., Comar, C. L., *J. Biophys. Biochem. Cytol.*, 1957, v4, 559.

14. Bélanger, L. F., Migicovsky, B. B., *J. Exp. Med.*, 1958, v107, 821.

15. Neuman, W. F., Boyd, E. S., Feldman, A., 1952 Tr. 4th Conf. Metabolic Interrelat., Josiah Macy, N. Y.

16. Dziewiatkowski, D. D., *J. Biol. Chem.*, 1951, v189, 187.

17. Dziewiatkowski, D. D., di Ferranti, N., Bronner, F., Okinaka, G., *J. Exp. Med.*, 1957, v106, 509.

18. Bélanger, L. F., *J. Histochem. Cytochem.*, 1958, v6, 146.

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Factors Influencing Isoimmunization in Rabbit. Effect of Early Postnatal Immunization on Specific Isoantibody Response.* (24435)

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Although our knowledge of blood groups, incompatibility, and hemolytic disease has increased considerably in recent years, the fact that only about 5% of Rh-negative women become immunized during pregnancies in which the necessary preconditions for Rh incompatibility exist has not been fully explained(1). Mitchison, Brambell(1) and

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