Epiphyseal Growth Zones in Acute Lathyrism.* Uptake and Distribution of $^{35}SO_4$ in Cartilage and Bone. (31838)

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In the pathology of lathyritic growth zones, sulfomucopolysaccharides have drawn attention. Tracer studies on lathyritic epiphyseal cartilage have shown a depressed uptake of $^{35}SO_4$(1), and autoradiographic experiments have demonstrated that the shift in radioactivity from cells to intercellular substance normally observed with time was delayed (2,3).

Previously reported biochemical analyses showed that mucopolysaccharide concentrations decreased in epiphyseal cartilage and increased in metaphyseal bone of lathyritic rabbits(4). The present paper reports on radioactivity measurements on lathyritic growth zones, when $^{35}SO_4$ was given at different times in relation to the administration of the lathyrogen.

Materials and methods. Fifty-four albino rabbits weighing 200-250 g were divided into experimental and control groups, so that initial mean body weights were as close as possible.

A 6% sterile solution of aminoacetonitrile (AAN), Fluka AG, Switzerland, was made up and adjusted to pH 7.3-7.4 with NaOH. Experimental animals received a dosage of 15 mg per 100 g body weight per day, while control animals received a corresponding volume of physiological saline. Injections were given subcutaneously into the back of the neck.

Carrier-free radiosulfate in aqueous solution, Radiochemical Centre, Amersham, was diluted with 0.04% Na_2SO_4 to a final concentration of 1 mCi per ml. One-half ml of radiosulfate solution was injected intraperitoneally in all animals, either before (Experiment I), during (Experiment II) or after (Experiment III) the injections of AAN or saline. Animals were sacrificed by injecting 1.0-1.5 ml 6% Nembutal® intraperitoneally.

In Exp. I, animals were divided into two subgroups, A and B. Animals of this experiment were the same as those analyzed biochemically(4). Both groups were given $^{35}SO_4$ 24 hours before the first dosage of AAN or saline, and sacrificed on the day after the last dosage. Animals of Group A received 2, animals of Group B 4 dosages.

In Exp. II and III, 4 dosages of AAN or saline were administered. Radiosulfate was injected on the day of the third dosage in Exp. II, and on the day of the fourth dosage in Exp. III. All animals were sacrificed 2 days after the tracer injection.

Using a dissection microscope, epiphyseal cartilage and corresponding metaphyseal spongiosa were removed from proximal humerus and tibia, distal radius, ulna and femur of the 4 extremities. The growth zone was opened by a periosteal circumcision along the epiphyseal line, and cartilage gently scraped out. After longitudinal splitting of the shaft and sliding away the marrow, the spongious metaphysis plate was curetted out. After determination of wet weights, samples were dried to constant weight at room temperature over P_2O_5 in an Edwards Drier, at less than 0.5 mm Hg. Dried cartilage was crushed to a powder in a small glass grinder. Bone was defatted in ethanol (1 hr) and ether (1 hr) on a mechanical shaker, dried and powdered in a Wiley Mill using a 60-mesh sieve. Decalcification was not performed. Cartilage powder was homogenized for 5 minutes in 0.5 N NaOH (1 mg cartilage per ml NaOH) in a "VirTis 45" homogenizer running at "medium" speed, the glass being immersed in an ice bath.

The distal ulnar growth zone was used for histological examination. Undecalcified samples were fixed in a mixture of 8% cetylpyridinium chloride and 10% neutral formalin (5), paraffin-embedded, sectioned and stained with 0.05% toluidine blue pH 5 for 3 min-

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utes, processing control and experimental sections simultaneously.

For radioactivity measurements, the procedure described by Moltke(6) and Marckmann(7) was followed. One ml cartilage homogenate or approximately 10 mg bone powder were transferred to 100 ml Kjeldahl flasks, to which were added 0.5 ml 12.5% Na_2SO_4. Samples were digested with fuming nitric acid on a sandbath, precipitated with 1.5 ml 25% $BaCl_2$, filtered, dried, mounted on planchets and counted as infinitely thick layers with a G-M tube on an automatic sample changer. Statistical counting error was less than 2%. Samples were corrected for background, physical isotope decay and variations in animal body weight. Statistical evaluation of results was done according to Student's "t"-test.

Results. Experimental animals given 4 AAN-dosages showed clinical signs of lathyrism, such as a ruffled fur, drooping ears and a waddling gait. These symptoms did not appear until after the third or fourth AAN-dosage. Consequently, experimental animals of Group A did not appear lathyritic. However, weight increase was inhibited in all experimental animals.

Regardless of dosage, lathyritic cartilages were swollen, softened, and slipped easily off the metaphysis. In animals given 4 AAN-dosages, there was a reduction of metaphyseal plate height and irregular trabecular structure.

While cartilage water increased in all lathyritic animals, bone water was unchanged. Mean dry weight of lathyritic cartilage did not change, whereas a decrease in mean dry weight of lathyritic metaphyseal bone was noted, except in Group A(4).

Histological specimens from animals given 4 AAN-dosages demonstrated an increased proliferation of cells in the maturing zone, and the lack of a well-defined hypertrophic zone. Extracellular substance appeared smudged and in places fibrillar, but the intensity of metachromasia appeared unchanged from controls. The cartilage-metaphysis border was wavy and irregular, with cartilage cell rows protruding into the metaphysis. The trabeculae of the latter were thickened and

TABLE I. Uptake of $^{35}SO_4$ (Related to Dry Weight) in Epiphyseal Cartilage and Metaphyseal Bone of Control and Lathyritic Rabbits.

		3 days (A)	5 days (B)
Cartilage	Controls	2113 ± 207 (7)	†987 ± 92 (8)
	Experim	1943 ± 240§ (8)	†968 ± 56§ (8)
Bone	Controls	1335 ± 115	*971 ± 120
	Experim	930 ± 73†	§770 ± 45§
Ratio C/B	Controls	15.8 ± .6	‡10.5 ± .7
	Experim	20.4 ± 1.0†	‡13.2 ± 1.1§

Count rates of subgroups A and B of Exp I.

Figures represent mean values ± standard error of mean, and are expressed as counts per min per mg dry tissue (cartilage) or counts per min per 10 mg dried defatted tissue (bone).

3 days (A) = subgroup A = $^{35}SO_4$ given on day 0, AAN injected on days 1 and 2, animals sacrificed on day 3.

5 days (B) = subgroup B = $^{35}SO_4$ given on day 0, AAN injected on days 1-4, animals sacrificed on day 5.

Control animals saline-injected and sacrificed according to (A) and (B).

Figures in parentheses indicate number of animals, same for bone as for cartilage.

Ratio C/B indicates the proportion cartilage to bone count rates.

Significant alterations at 5%*, 1%† or 0.1%‡ levels of probability.

§ Indicates no significant change.

Symbols behind figures relate experimental to control values.

Symbols in front of 5-day figures relate these to 3-day figures.

distorted. They appeared to be discontinued from epiphyseal cartilage and to contain little or no metachromatic substance.

Radioactivity count rates of Exp. I were based either on dry weight (Table I), or on hexosamine content (Table II). In both groups, the activity of lathyritic cartilage was practically identical with that of control cartilage. In contrast, the activity of lathyritic metaphyses of Group A was significantly depressed, and experimental values of Group B were also at a lower level than control values. The changes were reflected in the radioactivity ratios indicated in Tables I and II, experimental values being significantly elevated or depressed, respectively.

In Exp. II and III (Table III), a significant depression of the $^{35}SO_4$-uptake in lathyritic cartilage and bone was observed. Radioactivity ratios were unchanged in these experiments. Count rates of lathyritic cartilage and bone of Exp. III were insignificantly

TABLE II. Uptake of $^{35}SO_4$ (Related to Hexosamine Content) in Epiphyseal Cartilage and Metaphyseal Bone of Control and Lathyritic Rabbits.

		3 days (A)	5 days (B)
Cartilage	Controls	37.4 ± 2.9	‡15.3 ± 1.7
	Experim	35.7 ± 4.3§	‡17.1 ± 1.0§
Bone	Controls	117.5 ± 13.4	§82.2 ± 10.5
	Experim	78.2 ± 6.5*	§53.1 ± 3.5*
Ratio B/C	Controls	3.2 ± .3	‡5.4 ± .4
	Experim	2.3 ± .1*	‡3.1 ± .2‡

Count rates of subgroups A and B of Exp I.
Figures represent mean values ± standard error of mean. Cartilage values are expressed as counts per min per μg hexosamine. In bone, hexosamine corresponding to uronic acid (UA-hex) was calculated as follows: carbazole value (μg per mg) × 179 : 194. Bone count rates are therefore expresed as counts per min per μg UA-hex.
Animals identical with those of Table I.
Ratio B/C indicates the proportion bone to cartilage count rates.
Symbols as in Table I.

lower than those of Exp. II.

Discussion. On the days of measurements in the present experiments, $^{35}SO_4$ would be incorporated almost exclusively in chondroitin sulfate(8). When comparing 2-day uptake with 3-day uptake of $^{35}SO_4$ in control animals (Tables III and I), it appears that during the decrease in cartilage activity from day 2 to day 3, metaphysis bone activity was increasing ($p<0.01$). This finding supports the view that metaphysis mucopolysaccharides are derived from epiphyseal cartilage(8). As radioactivity ratios indicate that a relatively higher activity persists in lathyritic cartilage (Table I), or, consequently, that the increase with time in lathyritic metaphysis activity is inhibited (Table II), it is deduced that the "transfer" of chondroitin sulfate from cartilage to bone is inhibited in lathyritic animals(2).

At the time of $^{35}SO_4$-administration in Exp. II (*viz.*, after 2 dosages of AAN), no alterations in mucopolysaccharide contents could be demonstrated(4), and experimental animals were clinically indiscernible from controls. As alterations in mucopolysaccharide contents(4) seem to develop concomitantly to the developing clinical symptoms, a depression of $^{35}SO_4$-uptake was to be expected in both Exp. II and III, and more so in Exp. III. As mentioned above, count rates of Exp. III were insignificantly lower than those of Exp. II.

After 4 dosages of AAN, hexosamine and uronic acid concentrations decreased in epiphyseal cartilage and increased in metaphyseal bone(4). In lathyritic cartilage, the decreased mucopolysaccharide concentration and the depressed uptake of $^{35}SO_4$ observed in Exp. II and III point to an inhibition of the synthesis of chondroitin sulfate(1). In view hereof, a reduced activity of lathyritic cartilage in Exp. I should be expected, at least in Group B(1).

In lathyritic metaphyses, biochemical analyses(4) failed to show whether the observed increase in hexosamine and uronic acid was due to chondroitin sulfate or to hyaluronic acid. The depressed uptake of $^{35}SO_4$ (Table III) suggests that this increase was due to non-sulfated mucopolysaccharides.

Additional measurements on consecutive days would be necessary to estimate the significance of the unchanged radioactivity ratios in Exp. II and III.

The arrested weight increase of lathyritic animals should be taken into consideration when evaluating the results. A failure to grow will inhibit the uptake of $^{35}SO_4$(1), and a growth arrest may also delay the "transfer"

TABLE III. Two-day Uptake of $^{35}SO_4$ in Epiphyseal Cartilage and Metaphyseal Bone of Control and Lathyritic Rabbits.

	Controls	Exp II	Exp III
Cartilage	2544 ± 214 (12)	1316 ± 179* (4)	1037 ± 143‡ (7)
Bone	947 ± 58	536 ± 55‡	412 ± 57‡
Ratio C/B	26.7 ± 1.2	24.4 ± 1.4§	25.7 ± 2.2§

Count rates of Exp II and III.
Figures represent mean values ± standard error of mean, and are expressed as counts per min per mg dry tissue (cartilage) or counts per min per 10 mg dried defatted tissue (bone).
Exp II: AAN injected on days 1-4, $^{35}SO_4$ given on day 3, animals sacrificed on day 5.
Exp III: AAN injected on days 1-4, $^{35}SO_4$ given on day 4, animals sacrificed on day 6.
Control animals saline-injected and sacrificed accordingly.
Figures in parentheses indicate number of animals, same for bone as for cartilage.
Ratio C/B as in Table I.
Experimental values are significantly lower than control values at 5%* or 0.1%‡ levels of probability. § indicates no significant change.

of mucopolysaccharides from epiphyseal cartilage into the metaphysis.

Summary. Measurements of the uptake of $^{35}SO_4$ in epiphyseal cartilage and metaphyseal spongiosa of young rabbits given a short-term treatment with aminoacetonitrile (AAN) are reported. When $^{35}SO_4$ was administered previous to AAN, the results suggested an inhibited "transfer" of chondroitin sulfate from cartilage to bone. Injection of $^{35}SO_4$ during or after AAN-treatment resulted in a depressed uptake in the growth zones. The results are correlated to previously observed alterations in mucopolysaccharide concentrations. The arrested weight increase of lathyritic animals may affect the results.

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Specificity of a Neuraminidase Activity of Sendai Virus. (31839)

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Myxoviruses are known to liberate sialic acid from various substrates, such as glycoproteins, glycolipids and oligosaccharides. A constituent or a fraction bearing the enzymatic activity was first isolated from the Asian and PR8 strains of influenza A virus after disrupting the virus by trypsin(1). Trypsin was also used for disrupting Newcastle disease virus (NDV)(2).

When Sendai virus (Parainfluenza type 1) was disrupted with ether in the presence of emasol 1130 (polyoxyethylene sorbitan monolaurate). The resulting aqueous fraction had neuraminidase activity, hemagglutinability and S-antigenicity. The fraction which had neuraminidase activity was recovered from the supernatant of ultracentrifugation at 100,000 *g* for 120 minutes, though the fraction still contained the small hemagglutinins.

The present communication describes the comparison made between the neuraminidase activity of the intact Sendai virus grown in eggs or L cells and that of the neuraminidase fraction obtained by the disruption of the virus in relation to the pH optimum and the inhibition of antiserum. In addition to that, the comparison is also made among the influenza viruses, Sendai virus and NDV, and the specificity of the neuraminidase activity is discussed.

Materials and methods. Viruses and L cell culture: The Fushimi strain of Sendai virus was grown at 36°C in the allantoic sac of 10-day-old chick embryos and harvested 72 hours after inoculation of the stock virus. The same strain of virus was also grown in stationary cultures of L cells(3) in a medium containing 90 parts of YLE consisting of 0.5% lactalbumin hydrolysate (Difco) and 0.1% yeast extract (Difco) in Earle's solution with 0.45% glucose and 10 parts of bovine serum. Upon infection, the cultures were washed twice with Hanks' balanced salt solution (BSS), and inoculated with egg-grown virus at a multiplicity input of 100 in a maintenance solution which consisted of 98 parts of YLE and 2 parts of inactivated horse serum (MS). After 1 hour adsorption at 36°C, the cells were washed twice with BSS, refed with MS, and the incubation was continued up to 72 hours. The 1,500 ml