Epiphyseal Growth Zones in Acute Lathyrism.* Determinations of Sodium, Potassium and Chloride. (31903)

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In pathological conditions of connective tissues affecting water and mucopolysaccharides, alterations in electrolyte composition may be expected. Quantitative changes in water and mucopolysaccharides in lathyritic growth zones of rabbit bones have been reported(1), and the occurrence of electrolyte changes in lathyritic rat epiphyseal cartilage has earlier been demonstrated(2).

To elucidate further biochemical changes in lathyritic rabbit growth zones, determinations of sodium, potassium and chloride on epiphyseal cartilage and metaphyseal bone were carried out.

Materials and methods. A 6% sterile solution of the lathyrogen aminoacetonitrile (AAN), Fluka AG, Switzerland, was made up and adjusted to pH 7.3-7.4 with NaOH.

Fifteen albino rabbits in their third week of life were divided into 8 experimental and 7 control animals. Experimental animals were given 15 mg AAN per 100 g body weight per day through 4 days, while control animals received a corresponding volume of physiological saline.

On the day after the last injection, animals were anesthetized with 0.1-0.2 ml 6% Nembutal® intraperitoneally. Through a transverse incision on the anterior side of the neck, blood samples were drawn from the exposed external jugular veins into heparinized tubes, immediately centrifuged, and plasma pipetted off. Animals were then sacrificed by an intraperitoneal injection of 1.0-1.5 ml Nembutal®.

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. By circumcising periosteum along the epiphyseal line, the growth zone was opened and cartilage gently scraped out. After longitudinal splitting of the shaft and removal of 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 powder in a small glass grinder. Bone was defatted in ethanol (1 hr) and ether (1 hr) in a mechanical shaker, dried and powdered in a Wiley Mill fitted with a 60-mesh sieve. Decalcification was not performed.

Analyses. Tissue. About 10 mg dried cartilage or dried defatted bone powder were treated overnight with 2 ml concentrated HNO_3 and 0.5 ml 30% H_2O_2. After neutralization with 0.4 N NH_4OH and dilution to 25 ml with water, sodium and potassium were determined using a Beckman flame photometer(3).

Chloride analyses were carried out on 10-20 mg dried cartilage and 19-20 mg dried defatted bone powder. Samples were hydrolyzed with 1.5 ml 0.6 N KOH at 70°C for 30 min. After cooling, 1.5 ml 4% $ZnSO_4$, 7 H_2O in 0.2 N HNO_3 was added to precipitate protein. After shaking and centrifugation for 15 minutes at 3500 rpm, 2 ml aliquots of the supernatant were titrated in 50% acetic acid with 20 mM $AgNO_3$ by electrometric titration(4,3).

Plasma. Sodium and potassium concentrations were determined on 200 µl plasma with a Baird flame photometer. For chloride analysis, 200 µl plasma was titrated in acetic solution with $AgNO_3$ by electrometric titration.

Tissue concentrations were calculated as mEq per 100 g dried (defatted) tissue and as mEq per liter tissue water, and plasma concentrations as mEq per liter. Statistical evaluation of results was done according to Student's "t"-test.

Results. Experimental animals presented

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TABLE I. Water, Sodium, Potassium and Chloride Concentrations of Epiphyseal Cartilage and Metaphyseal Bone of Control and Lathyritic Rabbits.

		g per 100 g dry tissue	mEq per 100 g dry tissue			mEq per liter tissue water		
		H_2O	Na	K	Cl	Na	K	Cl
Cartilage	Controls (7)	477 ± 14	80 ± 1.6	44 ± 1.3	27 ± .3 (6)	168 ± 5.1	93 ± 3.5	56 ± .8
	Exp (8)	697 ± 18†	94 ± 2.4†	35 ± 1.3†	49 ± 1.6†	136 ± 3.6†	50 ± 1.6†	71 ± 1.4†
Bone	Controls (7)	211 ± 8	21 ± 1.4	15 ± .4	10 ± .5	101 ± 4.3	71 ± 1.6	47 ± 1.6
	Exp (8)	198 ± 4‡	26 ± 1.5*	11 ± .2†	12 ± .4‡	130 ± 6.2†	58 ± 1.3†	58 ± 2.0†

Figures are mean values ± s.e.m.
Dry tissue = dried cartilage and dried defatted bone.
Figures in parentheses indicate number of animals. In one control animal, no cartilage tissue was available for chloride determination.
Experimental animals were injected subcutaneously with AAN 15 mg per 100 g body weight per day through 4 days.
Control animals were saline-injected. All animals were sacrificed on day after last injection.
Experimental values differ significantly from control values at 5%(*) or 0.1%(†) levels of probability. (‡) indicates no significant change.

symptoms typical of lathyrism, such as drooping ears, a ruffled fur and a waddling gait. Besides, weight increase of lathyritic animals was inhibited. Lathyritic epiphyseal cartilage was swollen and transformed into a jelly-like, evidently hemorrhagic substance, and lathyritic metaphyseal bone showed a reduced height of the bone plate with irregular structure of bone trabeculae.

From Table I it appears that a large increase in water content was observed in lathyritic cartilage, whereas no significant change was demonstrated in lathyritic metaphyseal bone. As in previous experiments (1), mean wet weight of cartilage increased in lathyritic animals, while mean dry weight was unchanged. In lathyritic metaphyseal bone, mean wet as well as dry weights decreased.

On a dry weight base (Table I), sodium and chloride increased in lathyritic cartilage and metaphyseal bone. The rise in bone chloride was not significant, however. When expressed as mEq per liter tissue water (Table I), sodium decreased in cartilage and increased in bone, while chloride increased in both tissues. A decrease in potassium was noted in cartilage as well as in bone.

In lathyritic plasma samples (Table II), an increase in sodium and chloride was demonstrated, while potassium decreased insignificantly.

Discussion. Lathyrogenic agents are known to produce vascular lesions(5), and vascular damage is likely in animals given 4 AAN-injections, as, besides in cartilage, hemorrhages were evident also in muscles and under the periosteum. Plasma exudation, caused by increased vascular permeability, would increase cartilage water. Further, a tissue dilution with plasma would bring about an increase in sodium and chloride and a decrease in potassium, expressed on a dry weight base. However, if water increase was due only to plasma exudation, more profound alterations in sodium, potassium and chloride relative to dry weight should be expected.

TABLE II. Plasma Sodium, Potassium and Chloride of Control and Lathyritic Rabbits.

	mEq per liter		
	Na	K	Cl
Controls	140 ± .9 (7)	4.1 ± .2 (6)	97 ± .6 (7)
Exp (8)	144 ± .7*	3.4 ± .3‡	101 ± .9†

Figures are mean values ± s.e.m.
Animals are identical with those of Table I. Potassium value of hemolyzed plasma in one control animal is excluded.
Experimental values differ significantly from control values at 5%(*) or 1%(†) levels of probability. (‡) indicates no significant change.

Consequently, additional factors must affect the water content. A comparison of plasma, cartilage and bone control values indicates that a certain fraction of sodium and potassium (counter)ions must exist in an osmotically inactive form in the tissues(6). Acid mucopolysaccharides are, at least in part, responsible for this inactivation(7). As far as counterions attached to polyanions are osmotically active(8), they will attract water from the surroundings(9). The decrease in cation concentration in lathyritic cartilage water might therefore signify that a relatively larger part of small ions were present in an osmotically active form, thus "binding" more water.

In metaphyseal bone, as in cartilage, the alterations in cation and chloride concentrations based on dry weight are consistent with a slight exudation of plasma. The water content, however, was not significantly changed. This observation, together with the increased concentration of sodium in tissue water, point to a higher degree of osmotic inactivation of cations.

Because of the high amount of hexosamine in relation to uronic acid in plasma, a plasma exudation into cartilage offers an explanation of the lesser decrease of hexosamine compared to that of uronic acid(1). The content of dry matter in plasma would also tend to compensate the loss of cartilage dry matter due to the decrease in mucopolysaccharide concentration, thus possibly explaining the unchanged mean dry weight of lathyritic cartilage. A slight plasma exudation into metaphyseal bone may further account for the increase in "excess" hexosamine observed(1).

Any deduction of alterations in plasma concentrations from alterations in tissue composition, or *vice versa,* does not seem justified. Rather, the increase in sodium and the trend towards lowered potassium may arouse the suspicion of a corticosteroid effect, and thus cast doubt on the "purity" of the syndrome produced in the present lathyritic model.

Summary. Determinations of sodium, potassium and chloride in epiphyseal cartilage, metaphyseal bone and plasma of young rabbits suffering from acute lathyrism are reported. A mechanism is proposed for the increased hydration of lathyritic cartilage and unchanged water content of lathyritic metaphyses, *viz.*, a combination of plasma exudation and a change in the osmotic binding capacity of the tissues. The effects of plasma exudation are correlated to some of the previously described alterations in lathyritic cartilage and bone. Changes in plasma electrolytes may suggest a possible interplay of a corticosteroid effect in the lathyritic model employed.

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