made to increase to a limited maximal concentration of saturation upon the oral administration of pyridoxine.

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Decrease in Hexosamine Content of Epiphyseal Plates in Experimental Lathyrism.* (24030)

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Lathyrism is caused by ingestion of excessive amounts of sweet pea seeds (Lathvrus odoratus). The most characteristic lesions are deformities of bones, enlargement of epiphyseal plates, dissecting aneurysm of the aorta, and loss of weight. Research in this field has increased greatly since Ponseti et al. (1-2) showed the possible relations between lathyrism and many human bone diseases of unknown etiology. The earlier literature on lathyrism has been reviewed recently by Selye(3). The disease can be reproduced experimentally by injection of beta-aminopropionitrile, the active principle of sweet pea, or by aminoacetonitrile which is still more active in reproducing the bone lesions. The nature of metabolic block produced by aminonitriles remains to be elucidated. Since the most evident lesions appear in ground substance of the epiphyseal plates, some interference with mucopolysaccharide or collagen metabolism can be supposed. For this reason we studied the content of hexosamine, a component of mucopolysaccharides, and hydroxyproline, characteristic of collagen, in normal and lathyric epiphyseal plates.

Methods. Twenty rabbits, 7-10 days old, were used. Ten were injected with amino-

acetonitrile sulfate,§ the others were controls. Each experimental animal received subcutaneously 10 mg of aminoacetonitrile sulfate neutralized with NaHCO₃, daily for 6 days. With this dosage the animals showed very severe skeletal lesions; the epiphyseal plates were very wide and distorted as described by Ponseti and Shepard(1). Longer treatment resulted in death of animals. After 6 days all rabbits were killed with ether; epiphyseal plates (about 200 mg each) were excised from proximal and distal ends of long bones (radius, ulna, tibia, femur) after removal of muscles and periosteum. The tissue was dried at 104°C to constant weight, then homogenized with 4N HCl in a Potter-Elvehjem homogenizer and refluxed at 100°C for 15 hours; a large excess of acid was used (500-600 times the quantity of tissue) to reduce humin formation. After hydrolysis, the acid solution was put in a vacuum desiccator with NaOH and CaCl₂ and the air evacuated. The dry residue was taken up in 25 ml H₂O. Hexosamine was determined by Schloss' method(4). Eastoe's method(5), using Dowex 50, was tried for a few hydrolysates, to separate glucosamine and galactosamine. Hydroxyproline was determined by the method of Neuman and Logan(6) according to suggestions of

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Wt).				
	Normal, mg	Lathyric, mg		
	89.4	57.9		
	84.1	55.0		
	104.1	55.6		
	89.7	59.3		
	85.3	53.8		
	84.0	58.5		
	84.7	58.4		
	89.0	56.7		
	89.2	57.2		
	88.2	57.0		
Avg	88.8	56.9		
Decrease	•	36%		

TABLE I. Content of Hexosamine in Epiphyseal Plate of Lathyric and Normal Rabbits (mg/g Dry Wt).

Martin and Axelrod(7). To obtain a clear solution, after adding paradimethylaminobenzaldehyde solution, we used 3 ml each of 5.3 N H₂SO₄ and 2.66% paradimethylaminobenzaldehyde instead of 4 ml of 4 N H₂SO₄ and 2 ml of 4% paradimethylaminobenzaldehyde, as called for in the Martin and Axelrod method.

Results. The results obtained in hexosamine analysis are reported in Table I. The values are expressed in mg of aminosugar for every g of dry tissue. The mean content of hexosamine in normal epiphyseal plate was 88.8 mg and in experimental tissue 56.9 mg, a decrease of 36%.

Some hydrolysates have been analyzed chromatographically on Dowex 50 to know the comparative content of galactosamine and glucosamine. As expected, the content of galactosamine was 9 times greater than that of glucosamine, which correlates with the fact that chondroitinsulfate is the principal mucopolysaccharide present in cartilaginous tissues.

Table II shows the content of hydroxyproline in normal and lathyric epiphyseal plates. Only small differences were found between experimental and normal cartilages.

Table II records also collagen values obtained by multiplying the hydroxyproline by 7.46. These values represent total collagen, including insoluble collagen and acid- and alkali-soluble pre-collagens that are probably present in pre-osseus cartilage.

Discussion. Owing to the decrease of galactosamine in epiphyseal plates of young rabbits injected with amino-acetonitrile, we could suppose that this compound interferes in some way with the metabolism of mucopolysaccharides. It is interesting that Dasler(8) was able to prevent characteristic lesions caused by aminonitriles by including l-glutamine in the diet of the animals. Glutamine is a precursor of hexosamine according to results obtained by Leloir and Cardini(9) in Neurospora crassa. Our research with homogenates of epiphyseal plates(10) showed formation of hexosamine from glutamine and glucose-6phosphate. The enzyme essential to this synthesis, that may be called hexosamine synthetase, was found by us also in liver, callus of bone, rib cartilage and other tissues(11-12); its presence in liver was recently confirmed by Pogell(13,14).

The results reported here are not sufficient to prove a direct action of aminonitriles on mucopolysaccharide biosynthesis. Epiphyseal plates of lathyric animals are greatly widened and cell proliferation appears sharply increased(1); so a direct action of aminonitriles on protein synthesis, with a consequent change in ratio of mucopolysaccharides to proteins cannot be excluded. Study of this phase is planned.

Summary. The content of hexosamine and hydroxyproline in epiphyseal plates of lathyric and normal rabbits has been determined. A significant decrease (36%) of hexosamine was found in cartilage from experimental animals. Only small differences for hydroxyproline have been observed between experimental and control cartilage.

TABLE II. Content of Hydroxyproline in Epiphyscal Plate of Lathyric and Normal Rabbits (mg/g

	Normal		Lathyric		
	Hydroxy- proline	Collagen*	Hydroxy- proline	Collagen*	
		n	.g		
	19.6	146.2	19.8	147.7	
	19.7	147.0	20.1	149.9	
	18.4	137.3	19.9	148.4	
	22.7	169.3	17.0	126.8	
	20.7	154.4	19.8	147.7	
	21.0	156.7			
Avg	20.3	151.8	19.3	144.1	

* Hydroxyproline \times 7.46.

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Effect of Tranquilizers and Other Agents on Sexual Cycle of Mice.* (24031)

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Some agents which act on the central nervous system have been shown to affect the reproductive system of animals. Daily subcutaneous doses of reservine have been shown to interfere with estrus in mice(1) and to suppress ovulation and menstruation in monkeys (2). Chlorpromazine may delay ovulation and menstruation in women(3). Reservine, chlorpromazine, pentobarbital, morphine, atropine, dibenamine and SKF 501, given as single doses prior to ovulation, can all block release of ovulating hormone in rats(4,5). Our work shows that some of these and other centrally acting agents interfere with estrus cycles in mice when given more or less continuously by drug-diet administration.⁺

Methods. Vaginal smears were obtained by saline lavage once daily from ZBC mice[‡] before, during and after treatment. Only animals with regular estrus cycles before therapy were used. Mice were housed in individual Control diet was Purina laboratory cages. chow. Drugs were given by drug-diet administration ad libitum with all drugs except alcohol, which was administered in drinking water ad libitum. Drugs, other than alcohol, were carefully powdered and thoroughly mixed with control food in the desired concentrations. The number of estrous cycles originating during treatment was compared with the number of cycles originating during the previous control period over the same number of days, usually 20. Thus mice served as their own controls. Cycles originating during the first 3 days of treatment were excluded.

Results. Table I gives results obtained with the various agents used. Reserpine (0.00075 and 0.001%), chlorpromazine HCl (0.3%), promethazine HCl (0.3%), phenaglycodol (1.0%), meprobamate (3.0%), phenobarbital (0.2%), ethyl alcohol (20%), atropine SO₄ (0.1 and 0.2%) and SKF 501 (1.0%)§

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[‡] Mice were obtained from Dr. J. J. Bittner. ZBC mice are back cross $Z(C_3H)$ mice bred by mating AxZ F_1 hybrid females with Z males.

[§] SKF 501, or N-(9-fluorenyl)-N-ethyl-B-chloroethylamine hydrochloride, is an adrenolytic agent.