8. Reed, L. J., Muench, H., *Am. J. Pub. Health,* 1938, v27, 493.
9. Kligman, A., Daldridge, G. D., Rebell, G., Pillsbury, M. D., *Brit. Med. J.,* 1951, v35, 1285.

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Hexosamine and Hydroxyproline Content of Fracture Callus in Normal and Aminoacetonitrile Treated Rats.* (26847)

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Aminoacetonitrile (AAN) administered to adult rats with experimental fractures induces formation of a voluminous fibrocartilaginous callus which ossifies well during the second week of fracture and becomes very osteoporotic during the third week. Connective tissue cells and immature cartilaginous cells are extremely abundant in the callus. Histological and histochemical studies have indicated that the ground substance and collagen fibers are defective(1). We are reporting here studies on the hexosamine and hydroxyproline content of fracture callus of normal and AAN-treated animals obtained 5, 10, 15 and 21 days following the experimental fracture.

Methods. Forty Sprague Dawley male rats weighing from 150 to 155 g were used. Half of the animals received finely ground, laboratory Purina chow and the other half received the same diet containing 0.075% AAN. A fracture in the middle third of both tibiae was produced manually in every rat under anesthesia by bending the leg over the dull edge of a large knife held in a vise. The fractures were not immobilized.

Five experimental and 5 control animals were sacrificed with ether 5, 10, 15 and 21 days following the fracture. Both hind legs were immediately dissected and the fracture callus was collected and placed in dry ice. Also both femurs were obtained, but only the shaft was used, and placed in dry ice. The callus tissue and femurs were pooled and homogenized in acetone in a Virtis blendor, dried and the acetonic powder was used as a starting material for the analysis.

Chemical analysis: The acetonic powder was dried in an oven at 104°C at constant weight and was hydrolyzed in HCl 4N during 15 hours and refluxed at 100°C. After hydrolysis, the solution was dried in a flash evaporator. The residue was dissolved, filtered, and the volume made up with water to 25 ml. This solution was used for the analysis.

The hexosamine was determined with a modification(2) of Boas' method(3). The hydroxyproline was determined according to the method of Neumann and Logan(4) modified by Leach(5).

The hexosamine content in the femoral shaft (including marrow) varied from 5.8 to 7 γ/mg of dried weight and the hydroxyproline content in the femoral shaft varied from 14 to 18 γ/mg in both experimental and control animals. These values did not change markedly throughout the experiment.

Discussion. In the fracture callus of the control animals fed normal laboratory diet an increase of hexosamine was noted which reached its maximum 15 days following fracture. If it is assumed that hexosamine is an index of mucopolysaccharide content of the callus our results agree with the work of Aoike *et al.*(6) who found large increase of chondroitin sulfate in the 2-week-old fracture callus using S^{35} as index of the chondroitin sulfate synthesis. The increase of hexosamine in the callus of the AAN-treated animals is small as compared with the controls. In the 15-day-old callus, the hexosamine of the AAN-treated rats was half that found in the controls and hexosamine decreased very

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TABLE I. Hexosamine and Hydroxyproline Content in Fracture Callus, γ/mg Acetonic Powder Dried at 104°C.

Days after fracture	Hexosamine		Hydroxyproline	
	Exp.	Controls	Exp.	Control
5	20.5	18	30.4	25.3
10	23.4	36.2	24.2	22.9
15	23.8	55.3	27.2	33.4
20	9.3	27.0	16.5	17.5

sharply in the 20-day-old callus. A similar decrease in hexosamine was found in the epiphyseal plates of AAN-treated rabbits by Castellani and Castellani-Bisi(7). Pedrini and Pedrini-Mille also demonstrated that the synthesis of hexosamine in the epiphyseal plates of these animals was greatly decreased in comparison with the controls(8).

On the other hand, no significant differences were found in the hydroxyproline content of the fracture callus of normal and AAN-treated rats.

The values of hexosamine and hydroxyproline in the femoral shafts of normal and AAN-treated rats were similar throughout the experiment.

Summary. The content of hexosamine in the fracture callus of normal rats increased sharply during the first 2 weeks. Much less hexosamine was found in the fracture callus of AAN-treated rats. No significant difference was found in the content of hydroxyproline of the callus of normal and AAN-treated rats.

1. Ponseti, I. V., Aleu, F., *J. Bone and Joint Surg.*, 1958, v40A, 1093.

2. Bolognani, L., Coppi, G., Zambotti, V., *Boll. Soc. It. Biol. Sper.*, 1958, v34, 1950.

3. Boas, N. F., *J. Biol. Chem.*, 1953, v204, 553.

4. Neuman, R. E., Logan, M. A., *ibid.*, 1950, v184, 299.

5. Leach, A. A., *Biochem. J.*, 1960, v74, 70.

6. Aoike, I., Ishigama, H., Yoneda, T., Proc. 2nd United Nations Internat. Conf. on Peaceful Uses of Atomic Energy, Geneva, Sept., 1958, v24, P/1500, 164.

7. Castellani, A. A., Castellani-Bisi, C., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 318.

8. Pedrini, V., Pedrini-Mille, A., *ibid.*, 1959, v101, 358.

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Cholinesterases in Serum as Demonstrated by Starch Gel Electrophoresis.* (26848)

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The demonstration by Alles and Hawes(1) that cholinesterase of red blood cells differs from that of serum has led to our present understanding that 2 types of these enzymes exist, one possessing maximum hydrolytic activity for the 2-carbon acyl group and one for the 4-carbon chain. While other esterases can split some choline esters(2), the property of physostigmine in low concentration to inhibit cholinesterases in a reversible manner(3,4) distinguishes these enzymes from other esterases. In the serum of various species the 2 types of cholinesterases exist in varying proportions(5), predominantly the butyrylcholine-splitting enzyme in the human and the acetylcholine-splitting enzyme in the rabbit. Other authors(6,7) have reported the presence in serum of various butyrylcholine esterases.

In a study of serum esterases by starch gel electrophoresis, a number of bands were obtained, in addition to the expected cholinesterase band, which were active against choline esters and which possessed the property of being inhibited by physostigmine. The number of these bands varied with the species.

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