TABLE I. Summary of Attempted Homologous Transplantation of X5563 Tumor.

Group	No. of attempted transmissions	No. of deaths within 30 days	No. of successful transmissions
Non-irrad.	24	5	2
300 r	12	7	1
600 r	21	11	5
Total	57	23	8

has been attempted on 57 occasions. This is summarized in Table I. Total body irradiation was administered 48 hours prior to transplantation. Several animals dying within 30 days manifested hemorrhagic tendencies. Eight of 34 surviving animals developed palpable tumors and electrophoretic plasma changes typical of X5563. From 3 mice in which transplantation was successful, we were able to transmit X5563 to other C3H mice. Four other animals were followed with serial electrophoretic patterns, and in these spontaneous regression of tumor was observed. Fig. 2 illustrates electrophoretic changes occurring in these animals. One animal died of unknown cause.

Discussion. That removal of palpable subcutaneous tumor results in return of electrophoretic pattern to near normal is evidence that source of abnormal globulin is tumor. That this occurs with only partial removal indicates that there is a quantitative relationship between amount of tumor and degree of electrophoretic abnormality.

Observations regarding homologous transplants and tumor regression illustrate genetics of transplantation as outlined by Snell(3). However, successful transplantation and subsequent regression of a tumor have been followed here for the first time without interval sacrifice of host. The number of homologous transplants has been inadequate to determine effect of irradiation, but based on other studies(4,5,6,7), enhanced success in homologous transplantation of X5563 should be expected with prior irradiation of host.

Summary. Experimental mouse plasma cell tumor, X5563, produces plasma gamma globulin alterations. Temporary disappearance of abnormal globulin occurs following partial removal of neoplasm. Homologous transplantation of tumor has been carried out. Because surviving tumor can be readily identified by changes in plasma proteins without animal sacrifice, it provides a new and useful tool for investigation of tumor immunity.

The authors appreciate technical assistance of Richard Torrey and Jovilla Mannard.

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Received April 22, 1959. P.S.E.B.M., 1959, v101.

Decrease of Enzymatic Synthesis of Hexosamine in Epiphyseal Plates of Aminoacetonitrile-Treated Rabbits.* (24939)

VITTORIO PEDRINI AND ANGIOLA PEDRINI-MILLE (Introduced by I. V. Ponseti)
Dept. of Orthopedic Surgery, College of Medicine, State University of Iowa, Iowa City

The most evident alterations of experimental lathyrism are in the epiphyseal plates of long bones that appear very widened and lack of cohesion and organization. The changes seem to be due to some defect in ground substance, but there is no agreement about the metabolic block produced by the lathyrogenic agents. An alteration in mucopolysaccharides was suggested some years ago by Ponseti and Shepard(1), and more recently by Pyörälä *et al.*(2). Castellani *et al.*

* Aided by grant from N.I.H.

(3,4) demonstrated a remarkable decrease of hexosamine, especially galactosamine, in the epiphyseal cartilage hydrolysates of aminoacetonitrile (AAN) treated rabbits. As hexosamine is a major component of mucopolysaccharides, we studied its enzymatic formation in epiphyseal plates of normal and lathyric animals. The synthesis of hexosamine is known to occur in normal growing cartilage of rabbits(5,6).

Methods. Fifteen-day-old rabbits were used. Animals from the same litter, about half were injected subcutaneously with AAN neutralized with Na_2CO_3 daily for 6 days (from 10th through 15th day of life) and killed at least 5 hours after last injection. The others were controls. All animals were killed by decapitation, the legs immediately dissected and put in ice. The epiphyseal cartilage, freed from muscles and periosteum, was excised from the proximal and distal ends of radius, ulna, humerus, tibia and femur and kept in test tube immersed in ice. After weighing, the tissue was homogenized in a Potter-Elvehjem glass homogenizer at 0°C. The homogenizing medium was 10^{-2} M glutamine and 10^{-2} M glucose-6-phosphate Na salt in 0.1 M citrate buffer pH 6.4. The concentration of homogenates was 2.5% since this value was most suitable for enzyme activity(7). Two ml portions of the homogenates were put into 2 Pyrex centrifuge tubes. In one the reaction was immediately blocked by boiling for 10 minutes in water bath and adding HCl to final concentration of 0.3 N. Under these conditions no appreciable hydrolysis of the mucopolysaccharides occurs. After centrifugation at 3000 rpm the supernatant liquid was transferred to a calibrated tube, the precipitate resuspended and washed twice with a little distilled water. The washings were added to the supernatant and the volume made to exactly 3 ml with distilled water. The other portion was incubated for 3 hours at 30°C and thereafter inactivated as described above. The hexosamine was determined on 2 ml portions of these solutions according to Schloss(8). Readings were made at 512 mμ with Beckman DU spectrophotometer taking as blanks the inactivated samples. In some experiments AAN (1 mg/ml) was added to the homogenizing medium of normal cartilage, to test whether or not under these conditions, there was inhibition of enzyme activity. In other experiments adenosintriphosphate (ATP) and uridintriphosphate (UTP) were added to the homogenates of lathyric cartilage to test whether or not the synthesis of hexosamine was enhanced by these compounds. To know if there was any difference in water content between normal and lathyric cartilage, some samples of tissue were dried in oven at 105°C to constant weight.

TABLE I. Hexosamine Synthesized in 3 Hours at 30°C by Epiphyseal Plate Homogenates.

	μg/g of fresh tissue	
	Normal	Lathyric
	1105	159
	1193	282
	1250	239
	1265	346
	1295	361
	1325	346
	1205	316
	1265	376
	1160	241
Avg	1229	296
Decrease		−75.92%

Results. Values obtained for enzymatic synthesis of hexosamine from glutamine and glucose-6-phosphate in normal and lathyric cartilage are shown in Table I. They are expressed as μg of aminosugar synthesized in 3 hours at 30°C per gram of fresh tissue. In 9 experiments here reported, average value for normal cartilage was 1229 μg, and for lathyric cartilage 296 μg/g of fresh tissue, showing average decrease of 75.92%.

In experiments where AAN was added *in vitro* to normal cartilage, we were not able to observe any effect upon synthesis of hexosamine. The synthesis was not enhanced when ATP and UTP were added *in vitro* to lathyric cartilage homogenates.

Dry weights did not show any significant difference between normal and lathyric cartilage.

Discussion. Our results and those of Castellani *et al.*(3,4) suggest that alterations in ground substance of the epiphyseal plate in AAN induced lathyrism are due to a defect

in metabolism of mucopolysaccharides.

The enzymatic reaction we studied (glutamine + glucose-6-phosphate → glutamate + hexosamine-6-phosphate) was first demonstrated by Leloir and Cardini in *Neurospora crassa*(9). The involved enzyme, called hexosamine synthetase, occurred in the epiphyseal plates of rabbits(5,6), in bone callus of experimental healing fractures(10), and to a minor extent in other tissues(6,11). Pogell recently confirmed its occurrence in rat liver (12,13). The aminosugar formed was incorporated in a complex, probably chondroitinsulphate(7). Little is known about this enzyme. Several compounds were tested which showed no coenzyme activity(9,12). In some of our experiments, when ATP and UTP were added *in vitro* to the lathyric cartilage homogenates, we could not obtain evidence that these compounds act as limiting factors for the synthesis. The importance of hexosamine synthetase in relation to metabolism of mucopolysaccharides and mechanism of bone formation was emphasized by Zambotti(14).

The nature of the action of AAN upon synthesis of hexosamine in the experimental animals remains to be elucidated. As we obtained no evidence of any effect when AAN was added *in vitro* to normal cartilage, we cannot suppose a direct inhibition of the enzyme. We do not know if the AAN acts upon the existing enzyme by some product of its metabolism, by inhibition of enzyme formation, or by destruction of the same. Castellani and Castellani-Bisi(3) reported that the amount of total collagen, as determined by hydroxyproline content, is the same in normal and lathyric epiphyseal plates. This finding was confirmed by Follis and Tousimis(15) who also, in a study with the electron microscope, were able to demonstrate that the collagen extracted from epiphyseal plates of AAN treated animals is not organized in fibrils, as it normally is. We do not know how the collagen organizes itself into fibrils *in vivo*, but there is evidence(16,17) of a linkage between collagen and mucopolysaccharides. An alteration in quantity or quality of the latter could perhaps explain lack of formation of collagen fibrils.

Summary. Enzymatic synthesis of hexosamine from glutamine and glucose-6-phosphate in epiphyseal plates of normal and lathyric rabbits has been studied. In the cartilage from experimental animals a decrease of 75.92% of this synthesis was found. AAN added *in vitro* did not have any inhibitory effect upon formation of hexosamine.

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Received November 6, 1958. P.S.E.B.M., 1959, v101.