Investigations on the Influence of Aminoacetonitrile on Connective Tissue in the Hamster[1]

It is well established that the connective tissues of weanling rats undergo significant degenerative changes following the administration of *Lathyrus odoratus* seeds. The causative agent in these seeds is β-aminopropionitrile. Other aminonitriles and especially aminoacetonitrile act in an even stronger way[2]. The aminonitriles affect the mesenchymal tissues only, and the effect is most pronounced during certain periods of growth[3].

In adult animals connective tissue growth was studied in healing wounds.

Functional and morphological studies were carried out on hamsters because it is possible to do microscopic studies *in vivo* in the cheek pouch of these animals during the experiment.

Methods.—39 young adult hamsters weighing 80–120 g were used in the experiments. They were all kept on the same diet. Longitudinal incisions were made on the back of the animals, 5 cm in length and always at the same distance from the spine. The wounds were closed with interrupted nylon sutures.

Crystalline aminoacetonitrile sulfate (AAN) was dissolved in a physiological saline solution and neutralized with sodium bicarbonate.

The investigation was divided into two different experiments. In the *first experiment* 12 hamsters were given 10 mg of the AAN in 1 ml solution by daily subcutaneous injections. 12 hamsters served as controls and received 1 ml of physiological saline solution only. Three animals from each group were killed with a large dose of nembutal on the 3rd, 5th, 7th, and 9th day after starting the experiment.

In the *second experiment* 8 hamsters were given 20 mg of AAN by daily subcutaneous injections, while 7 served as controls. They were all killed on the 7th day after the incisions.

All animals were given 150 μC of ^{35}S-labelled sulfate (in physiological saline solution with 0·04% sodium sulfate) intraperitoneally 48 h before killing.

The mast cells of the cheek pouches were examined in each animal in nembutal anesthesia immediately before killing by a method previously described[4].

The tensile strength of the wounds was determined *in situ* and *in vivo* by the method described by SANDBLOM *et al.*[5]. The wound-areas were then carefully excised and examined for radioactivity by a method previously used[6].

In the first experiment the femur bones of all animals were freed from muscles and tendons and the two groups compared.

In the second experiment the thoracic and abdominal aortae were carefully dissected free from the surrounding tissue; the arteries were pooled and dried and the concentrations of hexosamine and hydroxyproline were determined. This part of the experiment was repeated in a new series giving a total of 15 animals in each of the two groups. The hexosamine content was determined by the ELSON and MORGAN method[7] as modified by DYRBYE[8], and the hydroxyproline content by MARTIN and AXELRODS modification[9] of NEUMAN and LOGANS procedure[10].

Results.—Tensile strength of the wounds: The values of the treated group in the first experiment were lower than the controls at the 0·03 level of significance. The decrease was even more pronounced in the second experiment ($p < 0{\cdot}001$) in which a larger number of animals received the double dose of AAN (Table I).

^{35}S-sulfate uptake of the wound tissue: The determination of radioactivity in the wounds showed no significant change in the uptake of ^{35}S-labelled sulfate during AAN administration. The values for the radioactivity in the second experiment are shown in Table I.

Mast cells observed *in vivo* in the cheek-pouches showed no morphological differences between the treated and the control groups. In both groups the mast cells were well-granulated and the number seemed to be similar in both groups.

The *femur bones* of the AAN-treated animals showed no change in the size, form or colour when compared with the controls.

The *hexosamine and hydroxyproline* concentration of the aortic tissue tended to show higher values in the AAN-treated group but the differences were not significant (Table II). The hexosamine:hydroxyproline ratio was the same in the two groups.

Discussion.—It is generally assumed that the changes in the tensile strength of healing wounds is caused first of all by changes in the amount and condition of collagen fibers[11]. Our results of tensile strength determinations

[1] Aided by grants from the Danish State Research Foundation, the Danish Rheumatism Association, and the Sigrid Juselius Stiftelse, Finland.

[2] S. WAWZONEK, I. V. PONSETI, R. S. SHEPARD, and L. G. WIEDENMANN, Science *121*, 63 (1955).

[3] I. V. PONSETI, *Clinical Orthopaedics*, number 9 (Lippincott, Philadelphia), p. 131.

[4] O. WEGELIUS and G. HJELMMAN, Acta path. microbiol. scand. *36*, 304 (1955).

[5] P. SANDBLOM, P. PETERSEN, and A. MUREN, Acta chir. scand. *105*, 252 (1953).

[6] E. MOLTKE, Acta endocrinol. *25*, 179 (1957).

[7] L. A. ELSON and W. T. J. MORGAN, Biochem. J. *27*, 1824 (1933).

[8] M. O. DYRBYE, J. Gerontol. *14*, 32 (1959).

[9] C. J. MARTIN and A. E. AXELROD, Proc. Soc. exp. Biol. Med., N.Y. *83*, 461 (1953).

[10] R. E. NEUMAN and M. A. LOGAN, J. biol. Chem. *184*, 299 (1950).

[11] J. E. DUNPHY and K. N. UDUPA, New England J. Med. *253*, 847 (1955).

Table I

The tensile strength and the uptake of ^{35}S-labelled sulfate on the seventh day of wound healing in aminoacetonitrile poisoned hamsters

	Number of Animals	Tensile Strength	c.p.m./100 mg Wound tissue	Activity Ratio*
Controls	7	509 ± 29·4	78·8 ± 5·9	1·68 ± 0·15
Aminoacetonitrile (20 mg/daily)	8	163 ± 7·0	92·6 ± 14·4	1·39 ± 0·11
Significance		$P <$ 0·001	none	none

* Activity Ratio: $\frac{\text{c.p.m./mg of wound tissue}}{\text{c.p.m./mg of skin.}}$

Table II
The hexosamine and hydroxyproline concentrations in the aortae of aminoacetonitrile-treated hamsters as compared with control animals (mg/g dry tissue)

	Number of Animals	Hexosamine	Hydroxyproline	Hexosamine hydroxyproline ratio
AAN-treated animals .	8	3·6	18·7	0·23
	7	5·9	21·7	
Control animals . . .	7	3·3	12·3	0·23
	8	3·6	18·8	

agree with those of others[12] and may be regarded as the functional result of retarded maturation of fibroblasts influenced by lathyrogenic agents[13,14]. They are also in accordance with the experiment which indicates that aminopropionitrile reduces the incorporation of ^{14}C-glycine into collagen or ground substance[15].

The decrease in collagen content of healing wounds caused by AAN is probably caused neither by a change in the metabolism of the sulfomucopolysaccharides (Table I) nor in the total hexosamines. This is supported by others who found no change in the tissue hexosamine during such treatment[13] nor any change in the uptake of ^{35}S-sulfate in the tissues as determined by autoradiographic methods[16]. The lack of significant change in the hydroxyproline content of the aortic tissues is explained partly by the fact that the animals were adults and partly by the difficulties in dissecting the aortae free of adventitia and surrounding loose connective tissue.

Mast cell reactions to lathyrogenic agents have not previously been studied in living animals. The lack of morphological changes in the mast cells of the cheek-pouches in the AAN-treated group suggests that they are not the target cells.

O. WEGELIUS*, E. MOLTKE, and M. O. DYRBYE**

Connective Tissue Research Laboratory, University Institute of the Medical Anatomy, Copenhagen (Denmark), May 8, 1959.

Zusammenfassung

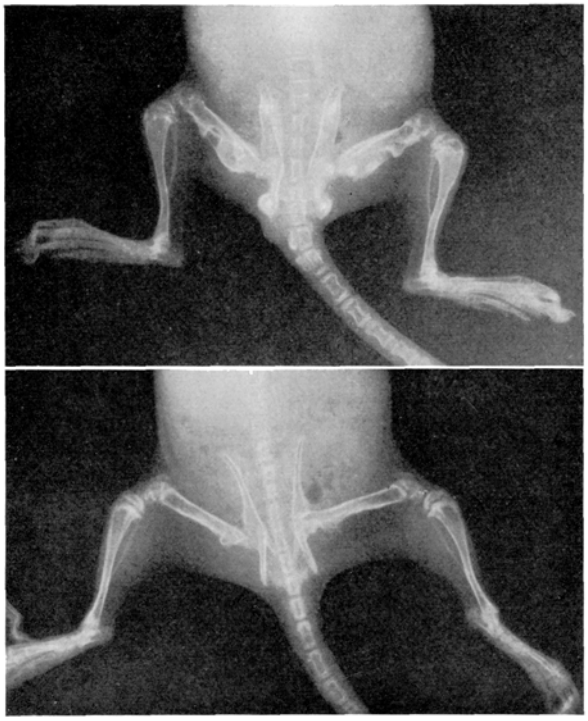
Aminoazetonitril entfaltet eine deutliche Wirkung am Bindegewebe des Goldhamsters. Diese Wirkung äussert sich in einer Verminderung der Zugfestigkeit von Wundgewebe. Die Mastzellen zeigten keine morphologischen Änderungen. Sowohl die Anreicherung von Radioschwefel im Wundgewebe wie die Konzentrationen von Hydroxyprolin und Hexosamin im Aortengewebe änderten sich nicht in signifikanter Weise.

[12] F. M. ENZINGER and E. D. WARNER, Lab. Invest. *6*, 251 (1957).
[13] J. E. MIELKE, J. J. LALICH, and D. M. ANGEVINE, Proc. Soc. exp. Biol. Med., N.Y. *94*, 673 (1957).
[14] J. V. HURLEY, E. STOREY, and K. N. HAM, Brit. J. exp. Path. *39*, 119 (1958).
[15] N. C. BRUEMMER, L. L. LALICH, and G. C. MUELLER, Proc. Soc. exp. Biol. Med., N.Y. *96*, 340 (1957).
[16] I. V. PONSETI, S. WAWZONEK, R. S. SHEPARD, T. C. EVANS, and G. STEARNS, Proc. Soc. exp. Biol. Med., N.Y. *92*, 366 (1956).

* Home Address: The IV Medical Clinic, Maria Hospital, University of Helsinki/Helsingfors (Finland).
** Fellow of the Helen Hay Whitney Foundation, New York (U.S.A.).

Iproniazid and Experimental Lathyrism[1]

There is evidence that the metabolically active radical of the β-aminopropionitrile is the amino group. It must not be substituted[2], and in the physiological detoxication a nontoxic cyanoacetic acid is formed[3]. On the contrary, the cyan group can be replaced with the SH-group[4].



Top: iproniazid-aggravated experimental lathyrism; Below: lathyrism produced by identical diet without iproniazid.

A hypothesis that the amino-oxidase is involved in the detoxication of β-aminopropionitrile was tested with rats (10 in each group) by adding iproniazid (Marsilid 'Roche') to the diet[5] (430 mg in 1000 g of food), which contained

[1] This work forms a part of a program supported by Sigrid Jusélius Foundation. Prof. C. WEGELIUS and Prof. O. JÄRVI have placed to our disposal the facilities for x-ray studies and Mr. I. LINDGREN, cand. med., has given much valuable advice.
[2] T. E. BACHHUBER, J. J. LALICH, D. M. ANGEVINE, E. D. SCHILLING, and F. M. STRONG, Proc. Soc. exp. Biol. Med., N.Y. *89*, 294 (1957).
[3] F. M. STRONG, J. J. LALICH, S. H. LIPTON, H. W. SIEWERT, and J. T. GARBUTT, *IV. Internationaler Kongress für Biochemie*, Communication 8–83, Wien 1958.
[4] W. DASLER, Proc. Soc. exp. Biol. Med., N.Y. *88*, 196 (1955).
[5] Modified from the diet previously described in Exper. *13*, 495 (1957) by L. KALLIOMÄKI, M. YLI-POHJA, and E. KULONEN.