SC 2190

Action of aminoacetonitrile on bone collagen in tissue culture

While lathyrogens produce marked alterations in the strength and structure of connective tissues[1,2], their direct action on these tissues has not been demonstrated. Since an increased extractability of collagen has been shown to parallel the symptoms of lathyrism[1] and since tissue hydroxyproline has been shown to reflect collagen content[1], we have used the extractability of hydroxyproline in neutral salt solutions as a means of determining any direct effect of aminoacetonitrile* on bone grown in roller-tube tissue cultures. Preliminary experiments indicated that aminoacetonitrile caused a significant increase in the amount of collagen extractable with 1 M NaCl from 5-day-mouse calvaria (minus the occipital bone) grown in our system under conditions which lead to the formation of new osteoid within 2 weeks. In the present study we have repeated this experiment in the presence of uniformly labelled L-[^{14}C]-proline in the supernatant fluid.

Each calvarium, attached to a coverslip by means of a chicken-plasma clot, was inserted into a Leighton tube containing 2 ml of Gey's balanced salt solution, chick-embryo extract and heated horse serum in a ratio of 6:2:2[4]. Radioactive proline (0.1 μC/ml) was added to the whole medium, the experimental media receiving 10 or 50 μg of aminoacetonitrile in addition. The media were replaced every 2 days. After 14 days the calvaria (wet wt., approx. 8 mg) were removed, rinsed in 0.16 M NaCl at room temperature and placed in 5 ml of 1 M NaCl (pH 7.4) at 4°. After 48 h the extract was separated from the residue and both were hydrolyzed at 120° for 3 h in 6 N HCl. Hydroxyproline[5] and [^{14}C]hydroxyproline[6] assays were carried out on the hydrolysates. Microscopic observations of the fibroblastic outgrowth did not reveal any cytotoxic action of the lathyrogen at these dose levels.

TABLE I

ACTION *in vitro* OF AMINOACETONITRILE ON THE EXTRACTABILITY OF BONE COLLAGEN*

Aminoacetonitrile	*% of total tissue hydroxyproline extracted by 1 M NaCl*		*Specific activity (counts/min/µg) of hydroxyproline*	
	Hydroxyproline	*[^{14}C]Hydroxyproline*	*1 M NaCl extract*	*Residue*
None	1.4 ± 0.2	3.4 ± 0.2	232 ± 40	92 ± 3
10 µg aminoacetonitrile/ml	5.4 ± 0.1	14.8 ± 1.1	241 ± 9	81 ± 4
50 µg aminoacetonitrile/ml	10.9 ± 1.7	40.1 ± 3.0	233 ± 5	42 ± 4

* Each value is the average obtained from 4 samples followed by the standard error.

The percentage of total tissue hydroxyproline extractable with 1 M NaCl from the calvaria was markedly elevated by aminoacetonitrile treatment (Table I). This effect was more pronounced at the higher dose. An even greater effect was observed on the extractability of [^{14}C]hydroxyproline indicating that newly synthesized collagen was more susceptible to the action of aminoacetonitrile than was the old collagen. Interestingly the specific activity of hydroxyproline in the extracts in 1 M NaCl was the same in the control as in the aminoacetonitrile-treated samples while the specific activity of hydroxyproline in the residue was markedly reduced in these samples.

* The aminoacetonitrile hydrogen sulfate was supplied by Abbott Laboratories.

These results indicate that aminoacetonitrile can act directly on bone in tissue culture to cause an alteration in the extractability of collagen similar to that observed *in vivo*. Since the hydroxyproline in the salt extracts was labeled equally in the control and aminoacetonitrile samples, while the specific activity of hydroxyproline in the residue was depressed by aminoacetonitrile, it seems likely that the latter exerts its action by blocking the normal maturation process whereby newly synthesized collagen is converted to an insoluble matrix[7]. A similar mechanism has been proposed to account for the action *in vivo* of another lathyrogen β-aminopropionitrile[8–10].

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Macromolecular properties and biological activity of heparin
II. Further electrophoretic studies

In a previous publication on the electrophoresis of heparin[1], it was shown that the number of components observed and their relative concentrations were strongly dependent on the ionic strength. This phenomenon was attributed to the formation of reaction boundaries. In the same publication it was also shown that a correlation existed between the anticoagulant activity and the relative amount of a new intermediate peak formed when heparin was subjected to electrophoresis in the presence of streptomycin. It is the object of this work to examine further the reaction-boundary hypothesis and to use the data available on the heparin–streptomycin complex to attempt further purification of heparin.

All electrophoresis runs were performed in a Spinco Model H Electrophoresis–Diffusion Apparatus. Analytical runs were made in either the 2-ml or the 11-ml cell depending on the amount of material available. The preparative runs were performed in the 80-ml preparative cell using the automatic sampling attachment of the instrument to withdraw fraction.

Analytical moving-boundary electrophoresis on a commercial heparin from pig