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COLLAGEN FRACTIONS IN LATHYRITIC RATS

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Abstract—The effect of β-aminopropionitrile ("Lathyrism") on collagen was studied. Albino rats were fed with a diet containing 73 per cent sweet peas, and the bone changes resulting were ascertained by x-ray studies. The collagen fractions were prepared from skin and bone by extraction with disodium phosphate solution, acid citrate solution, and by gelatinization.

The alkali-soluble collagen was 5 times greater in cases of lathyrism. The solubility of non-collagenous proteins was also increased. The total collagen content was not altered.

USING the electron microscope, Follis and Tousimis[1] have demonstrated that fibrous collagen is deposited more slowly in the cartilage of lathyritic rats than in control rats. The hydroxyproline content is unaltered, but a disturbance in the synthesis of collagen is indicated by other observations: there is a delay in the formation of mature fibres in subcutaneous abscesses,[2] as well as in the formation of hydroxyproline both in croton oil pouches[3] and in growing animals;[3] and there is a decrease in the tensile strength of collagen in healing wounds.[4, 5] On the other hand, it has been claimed that the collagen content in skin is normal.[6] These statements lead us to study collagen fractions of biological interest.

MATERIALS AND METHODS

The selected rats (Wistar) weighed from 30 g to 44 g initially. They were separated into two groups of ten animals each, the first being fed on the sweet pea diet, the second on the control diet.[4] These diets contained approximately 73 per cent of *Lathyrus odoratus* seeds and of *Pisum sativum* peas, respectively. The animals were part of a larger experiment on diets, and five rats from each group received iproniazid, which aggravates lathyrism,[7] while the remainder received methionine sulphoxide, which does not. The diet lasted 22 days, at the end of which time the rats weighed from 38 to 69 g in the lathyritic group, and from 49 to 79 g in the control group. The development of lathyrism was then established by individual x-ray examinations.

The hair and subcutaneous fat having been removed, the skins were cut up finely with scissors, extracted with ether, and air-dried. For the extraction, four samples were formed in each group by combining two or three skins (giving samples weighing 1·06–2·62 g and 2·34–5·42 g, in lathyritic and control groups respectively). Some thigh and shin bones were also extracted by a similar technique.

The extraction fluids were those of Harkness *et al.*,[8] and a Microid flask-shaker (Griffin and Tatlock Ltd.) was used. The whole extraction was carried out at 2–4 ° for 8–22 hr, using 10 parts of saturated disodium phosphate solution to one part of

the sample (v/w): it was repeated five times, and the supernatant extracts were separated at each stage by centrifugation for 30 min at 2500 rev/min. The extracts were combined, filtered through Jena Glass Filter G3, and saturated disodium phosphate solution was added to make a total volume of 250 ml: the resulting solution was dialysed for four days against running tap water (10–14°), in cellophane bags. The precipitate was separated by centrifugation for one hr at 2500 rev/min, and was suspended in 25 ml of 0·08 N NaOH.

The residue was extracted again by a similar technique, using 0·25 M citrate buffer, pH 3·8, and the collagen soluble in acid citrate was precipitated by dialysis, and collected.

The insoluble collagen was then obtained by autoclaving the residue twice with 100 ml distilled water for 1½ hr at 1 atm, the fluids being filtered while still hot. Autoclaving a third time did not add appreciably to the extracted nitrogenous material. A residue of about 20–25 per cent of the original weight remained.

The total soluble protein and the citrate-soluble collagen were calculated from nitrogen content (estimated by Kjeldahl combustion and distillation). The analysis of the other collagen fractions was based on the determination of hydroxyproline.[9] Both carbazole[10] and orcinol[11] reactions were used for the determination of the uronic acid.

RESULTS AND DISCUSSION

The results are collected in Table 1. There is no difference between the analyses of uronic acid from different bone samples: the significance of this is still uncertain. But

TABLE 1. SKIN FRACTIONS IN EXPERIMENTAL LATHYRISM OF RAT (PER CENT OF AIR-DRIED SAMPLE)

Group	N	Soluble in saturated disodium phosphate solution					Acid-soluble collagen	Insoluble collagen
		Protein	Uronic acid		Hydroxyproline (×7·15)			
			Carb.	Orc.	Total	Precip.		
Lathyrus	4	27·7	0·20	0·16	3·15	1·07	0·48	19·4
Control	4	18·9	0·14	0·14	0·62	0·30	0·18	19·0
t		2·75	2·56	—	6·87	5·1	1·8	—
P		<0·05	<0·05	—	<0·001	<0·01	—	—

the difference in alkali-soluble collagen from healthy or lathyritic bone can be confirmed ($P<0·05$). When the hydroxyproline was determined in the disodium phosphate extract by accident without prior hydrolysis, the values so obtained were also greater for lathyritic materials ($P<0·001$ for skin; $P<0·01$ for bones). The proportion of this "directly acting" hydroxyproline of the total hydroxyproline was clearly less in cases of lathyrism.

On dialysis, a smaller proportion of the hydroxyproline soluble in disodium phosphate was precipitated in the lathyritic than in the control samples; and when this precipitate was suspended in pH 3·8 citrate buffer, after storage at 5°, it was found

that only the lathyritic samples were still soluble, which suggests differences in precipitation properties.

The recent reports on the solubilization of collagen in rats treated with acetonitrile[12], as well as on the increased solubility in M NaCl of collagen from chick embryos treated with aminopropionitrile[13,14], are in good agreement with the observations cited in this paper.

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