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The effect of antirheumatic drugs on the extractable collagen in lathyric chick embryos

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The lathyrogenic effect of 2·5 mg of β-aminopropionitrile in the chick embryo has been measured by the increase in the extractability of collagen from bones using M saline. Sodium salicylate (3 mg/embryo) and hydrocortisone (1·5 mg/embryo) applied 24 hr after the injection of β-aminopropionitrile decreased the elevated amount of soluble collagen. Phenylbutazone, chloroquine diphosphate and sodium aurothiosulphate were ineffective in this experimental system.

THE lathyrogenic substances induce in experimental animals a generalized disease of connective tissue characterized by the occurrence of skeletal deformities, aneurysm and hernias (Selye & Bois, 1957). Underlying the disease is an increase in the amount of soluble collagen present in tissues, apparently resulting from decrease of covalent interchain cross-links (Levene & Gross, 1959; Martin, Piez & Lewis, 1963). Recently it has been postulated that lathyrogens may exert their effect by inhibition of the amine oxidase necessary for deamination of lysyl side-chains of collagen participating in cross-link formation (Page & Benditt, 1967).

Some substances from the group of hormones, antirheumatic drugs and divalent cations are able to influence favourably the lesions of experimental lathyrism as manifested by decrease of the elevated level of soluble collagen and the return of morphological changes to normal (Selye & Bois, 1957; Ponsetti, 1959; Trnavský, Trnavská & others, 1965; Naber, Scott & Johnson, 1965). These chronic experiments were made mostly in rats. To ascertain the influence of anti-rheumatic drugs in an acute experiment on a more closed system we used the assay in chicken embryos. We followed the method based on the increase of extractability of chick embryos bones in M cold saline after the injection of lathyrogen (Gross, Levene & Orloff, 1960).

Experimental

MATERIALS AND METHODS

Fertilized eggs of the white Leghorn variety were injected via the chorioallantoic membrane with 2·5 mg of β-aminopropionitrile fumarate in 0·1 ml of sterile distilled water at 14 days of incubation. After 24 hr the test drugs were administered in the same way and in the same volume. Control embryos were injected with 0·1 ml of distilled water on the 14th and 15th day of incubation. Each experimental group consisted of 30–35 embryos divided in 5 samples. The experiment was finished after another 24 hr when the tibiae and femora were stripped of adjacent tissues, minced and homogenized in the cold. A small sample was taken for the estimation of total hydroxyproline. The main part of the homogenate was then extracted in 2 volumes (v/w) of cold M saline containing phosphate (ionic strength 0·02, pH 7·6) for 24 hr with shaking in the cold (2°). After centrifugation the extraction was continued for a further 24 hr. The

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supernatants from centrifugation (1 hr at 30,000 *g*) were pooled and assessed by the method of Stegemann (1958) for the hydroxyproline content after hydrolysis with 6N hydrochloric acid.

The antirheumatic drugs tested were: sodium salicylate, hydrocortisone soluble (Spofa), phenylbutazone (Spofa), chloroquine diphosphate (Resochin-Bayer), Sanocrysin (sodium aurothiosulphate, Ferrosan).

Results

In the first experiment sodium salicylate at a dose of 3 mg depressed significantly the elevated amount of cold M saline-soluble hydroxyproline in β-aminopropionitrile-treated embryos. Salicylate itself did not change significantly the bone extractability. Hydrocortisone was also effective in decreasing the increased bone extractability of the nitrile-treated embryos. But in normal chicken embryos it decreased the content of soluble hydroxyproline. Phenylbutazone, 2 mg/embryo, chloroquine diphosphate, 5 mg/embryo, and Sanocrysin, 1 mg/embryo, were without effect.

TABLE 1. EFFECT OF ANTIRHEUMATIC DRUGS ON SOLUBLE HYDROXYPROLINE IN LATHYRIC CHICKEN EMBRYOS

Drug (dose/embryo)	Total hydroxyproline μg/100 mg of dry tissue	M NaCl-soluble hydroxyproline μg/100 mg of dry tissue	P
Control	1,362 ± 137	70 ± 12	
Sodium salicylate 5 mg	1,683 ± 62	71 ± 3	
β-Aminopropionitrile 2·5 mg	1,717 ± 51	348 ± 27	
β-Aminopropionitrile 2·5 mg + sodium salicylate 3 mg	1,677 ± 160	253 ± 34	<0·01
Control	1,390 ± 79	46 ± 8	
Hydrocortisone 1·5 mg	1,530 ± 84	36 ± 11	
β-Aminopropionitrile 2·5 mg	1,405 ± 54	230 ± 10	
β-Aminopropionitrile 2·5 mg + hydrocortisone 1·5 mg	1,342 ± 122	184 ± 18	<0·01
Control	1,556 ± 63	57 ± 5	
Phenylbutazone 3 mg	1,932 ± 212	73 ± 4	
β-Aminopropionitrile 2·5 mg	1,861 ± 66	201 ± 13	
β-Aminopropionitrile 2·5 mg + phenylbutazone 2 mg	1,864 ± 11	202 ± 24	
Control	1,859 ± 77	61 ± 4	
Chloroquine 5 mg	1,796 ± 26	66 ± 8	
β-Aminopropionitrile 2·5 mg	1,775 ± 45	265 ± 22	
β-Aminopropionitrile 2·5 mg + chloroquine 5 mg	1,859 ± 125	290 ± 24	
Control	2,102 ± 143	62 ± 11	
Sanocrysin 1 mg	2,229 ± 208	81 ± 7	
β-Aminopropionitrile 2·5 mg	1,846 ± 266	331 ± 19	
β-Aminopropionitrile 2·5 mg + Sanocrysine 1 mg	1,914 ± 196	362 ± 25	

Discussion

Sodium salicylate and hydrocortisone proved to be effective in depressing the increased solubility of lathyric collagen. But at the time when the tested drugs were injected, β-aminopropionitrile had been acting for 24 hr, so we would not expect the M sodium chloride soluble hydroxyproline level to completely return to normal. Most of the antirheumatic drugs return increased levels of skin-soluble hydroxyproline in lathyric

rats to normal. Even in this acute experiment in chicken embryos, which differs from the chronic experiment in rats, two of the drugs were effective.

The mechanism of action of antirheumatic drugs in experimental lathyrism is difficult to explain. Hydrocortisone is known to decrease the content of soluble collagen fractions in tissues and to promote the conversion of soluble into insoluble collagen (Kivirikko, 1963; Kühn, Iwangoff & others, 1964). The combined inhibitory effect on biosynthesis and at the same time promotion of collagen maturation could be responsible for the influence of hydrocortisone in experimental lathyrism. Knowledge about the influence of salicylate on the metabolism of collagen is scarce. Preliminary observations have shown that sodium salicylate is capable of promoting the transformation of soluble into insoluble collagen (Trnavská, Trnavský & Kühn, 1968). The influence of antirheumatic drugs on the collagen defect in experimental lathyrism is probably a result of a more general metabolic effect. Whether the ameliorating effect of antirheumatic drugs, at least in chronic experiments, is a characteristic feature for this group of drugs remains to be solved.

This report provides further evidence that it may be possible to modify the lathyric changes. Besides hormones, divalent cations and antirheumatic drugs are able to influence the lathyric toxicity favourably to some extent.

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