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EFFECT OF ANTI-RHEUMATIC DRUGS ON EXPERIMENTAL LATHYRISM

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Abstract—A comparison was made of the properties of collagen isolated from the skins of rats treated with sodium salicylate, hydrocortisone, chloroquine and phenylbutazone respectively, and from skins of lathyric rats treated during the development of lathyrism with these anti-rheumatic drugs. The rate of reconstitution of fibrils from acid soluble collagen isolated from the lathyric group is inhibited. The dissolution of the collagen gel formed on incubation at 37° after subsequent cooling to 5° is increased. These manifestations of lathyrus toxicity did not occur in rats given either sodium salicylate, phenylbutazone and chloroquine at the same time or the lathyrogen. Administration of hydrocortisone with the lathyrogen affected the lathyric properties of collagen to a lesser extent.

THE ANTI-RHEUMATIC drugs are from the chemical and biological aspect very heterogenous. In an attempt to find common features the action of the clinically important anti-rheumatic drugs, sodium salicylate, hydrocortisone, chloroquine and phenylbutazone was studied in experimental lathyrism. The lathyrogens, beta-aminopropionitrile and other compounds of the aminonitrile group, inhibit the maturation of collagen by suppressing the formation of inter- and intramolecular cross-links.[1,2] Although the true mechanism of their interference has yet not been elucidated in all details, experimental lathyrism represents the best known and most reproducible model for the damage to the structural stability of collagen. Steven[3] presented evidence for an analogous impairment of the structural stability of collagen in articular tissues from patients with rheumatoid arthritis.

In previous papers[4,5] we found that all the drugs mentioned influence to the same extent lathyric changes such as increased solubility of collagen and urinary hydroxyproline excretion.[6] The aim of our study was to check whether acid soluble collagen isolated from the skins of rats treated with lathyrogenic diet and given simultaneously separate anti-rheumatic drugs show differences in character as compared to control or lathyric soluble collagen. As an indicator, the process of reconstitution of fibrils from acid soluble collagen was used. It is assumed that there is some analogy between this process and the fibrilogenesis of collagen *in vivo*. Further, we investigated the reversibility of heat gelation of collagen on cooling to 5°. Gross has shown that the gel formed at 37° from neutral salt soluble collagen isolated from the skins of lathyric animals dissolved to a greater extent than collagen isolated from control animals.[7]

MATERIALS AND METHODS

Rats of the Wistar strain with initial weights of 60 g were divided into 10 groups and treated for 3 weeks with anti-rheumatic drugs as follows: (1) control group (2)

lathyric group/seeds of Lathyrus odoratus in 60% concentration in diet (3) sodium salicylate 200 mg/kg i.p. (4) hydrocortisone 7·5 mg/kg s.c. (5) chloroquinediphosphate/ Resochin-Bayer/20 mg/kg intramuscularly (6) phenylbutazone 75 mg/kg s.c. (7) Lathyrus odoratus and sodium salicylate (8) Lathyrus odoratus and hydrocortisone (9) Lathyrus odoratus and chloroquine (10) Lathyrus odoratus and phenylbutazone. The antirheumatic drugs were administered daily (6 days a week).

Acid soluble collagen was isolated by the methods of Wood and Keech[8] and Jackson *et al.*[9] The process of reconstitution was investigated in phosphate buffer pH 7·2, ionic strength 0·25, at 30°. The recording of the turbidimetric curve started after an interval of 1 minute and was carried on for 60 min. The absorbance at infinite time A_∞ was measured after 4 hr. No further significant change took place after this period. For the objective evaluation we employed the value p = extent of precipitation, $p^t = A^t/A_\infty$, where A^t is the absorbance at the time t.[8] It is generally believed that the formation of collagen fibrils from the solution of a soluble collagen results from two processes: the nucleation process, during which the aggregation of soluble collagen particles causes the formation of nuclei, and the process of the growth of these nuclei into fibrils by successive reactions of further collagen molecules with the surface of the growing nucleus[8, 10–12]. When the formation of fibrils in collagen solution is registered turbidimetrically the nucleation process is represented by a so called lag phase, given by the coefficient K_L. The growth of nuclei is represented by the growth phase of the turbidimetrical curve given by the coefficient K_G. When the value of the coefficient K_L decreases, the lag phase of the curve, becomes elongated, and the nucleation process is slower. The formation of fibrils takes place at a slower rate because the nucleation process is decisive for the whole reconstitution of collagen fibrils. In our experiments the coefficient of the growth phase remained unchanged. The calculation of these cofficients has been described.[13]

The investigation of reversibility of heat gelation of collagen on cooling to 5° was made by the method of Gross.[7] We used, however, acid soluble collagen, while neutral-salt soluble collagen had been used by Gross, isolated from the separate test groups similarly as in the experiment made to investigate the reconstitution of collagen fibrils. A 0·1% solution of acid soluble collagen was incubated at 37° for 136 hr. The resulting gel was then cooled to 5° for 7 hr. The amount of dissolved collagen was estimated after centrifugation by the amount of hydroxyproline in supernatant.[14] The results were expressed as a percentage of solubility.

RESULTS AND DISCUSSION

Wood found that *in vitro* addition of semicarbazide to solution of neutral-salt soluble collagen, inhibited the formation of collagen fibrils.[15] We found, similarly, that with acid soluble collagen isolated from the lathyric group of rats the formation of fibrils takes place at a slower rate as can be seen in the first, lag phase of the turbidimetric curve (see Table 1). This table also illustrates the ability of reconstitution of acid soluble collagen isolated from experimental groups of rats treated with antirheumatic drugs alone or in combination with the lathyrogenic diet. Only the values for the coefficient K_L are presented as the growth rate and hence coefficient K_G remained practically unchanged. A marked shift towards normal occured in acid soluble collagen isolated from the group given Lathyrus odoratus seeds and sodium

salicylate, a lesser effect was recorded in the group given Lathyrus diet with chloroquine, and no change in lathyric behaviour was seen in the group receiving Lathyrus odoratus seeds and hydrocortisone. The rate of reconstitution of acid soluble collagen isolated from the group given Lathyrus odoratus seeds and phenylbutazone is even higher than that found in the control group. Phenylbutazone, as such, appears to

TABLE 1. LAG PHASE OF TURBIDIMETRIC CURVES CHARACTERIZING THE FIBRILS FORMATION FROM SOLUTION OF ACID SOLUBLE COLLAGEN ISOLATED FROM RATS TREATED WITH LATHYROGENIC DIET AND ANTI-RHEUMATIC DRUGS

Treatment	$K_L.10^{-2}$
Control	8·64
Sodium salicylate	6·48
Chloroquine	3·99
Phenylbutazone	31·10
Hydrocortisone	7·56
Lathyrus	1·94
Lathyrus + sodium salicylate	5·99
Lathyrus + chloroquine	2·16
Lathyrus + phenylbutazone	16·20
Lathyrus + hydrocortisone	1·40

Concentration of collagen solution—0·1%, phosphate buffer pH 7·2 ionic strength 0·25, temperature 30°. K_L—coefficient characterizing lag phase of the turbidimetric curve.

speed up the formation of fibrils. On the other hand chloroquine administered to control animals inhibits the formation of fibrils from collagen solution. Interesting is this respect are the observations of Holzmann and Coll who found after administration of chloroquine an elevation of soluble collagen fractions in skin of rats, similarly to lathyric animals.[16] Nevertheless chloroquine in lathyric animals normalized the lathyric changes. Sodium salicylate and hydrocortisone given to control animals had no effect on the rate of reconstitution of fibrils. In all groups the average final amount of collagen reconstituted into fibrils was 90 per cent of the total collagen.

In agreement with the results of Gross, we also found an increase in the solubility of lathyric collagen on cooling to 5° after incubation at 37° during 136 hr (Table 2).

TABLE 2. REVERSIBILITY OF HEAT GELATION OF COLLAGEN ON COOLING

Treatment	Percentage solubility
Control	0·4
Sodium salicylate	0·2
Chloroquine	8·5
Phenylbutazone	0·9
Hydrocortisone	1·6
Lathyrus	10·0
Lathyrus + sodium salicylate	8·0
Lathyrus + chloroquine	7·6
Lathyrus + phenylbutazone	1·0
Lathyrus + hydrocortisone	9·0

Concentration of collagen solution—0·1%, incubation at 37° for 136 hr, subsequent cooling at 5° for 7 hr.

Return to normal was demonstrated in the group treated with Lathyrus odoratus and phenylbutazone. A marked reversal of the increased lathyric solubility occurred also in the groups treated with Lathyrus odoratus and sodium salicylate, and Lathyrus odoratus and chloroquine, while in the group given Lathyrus odoratus and hydrocortisone the effect was poor.

It appears that collagen isolated from skin of lathyric animals treated with anti-rheumatic drugs form more readily fibrils *in vitro* and are less soluble than collagen isolated from untreated lathyric animals. These experiments using material modified *in vivo* completed previous observations on the influence of anti-rheumatic drugs on morphological changes and neutral-salt solubility in animals with experimental lathyrism.[17] The toxic effect of lathyrogens can be also modified by hormones and by the administration of calcium and copper.[18–20] The mechanism of action of these different agents in experimental lathyrism remains to be elucidated.

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