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Concentration of Soluble and Insoluble Collagen in Testes of Penicillamine-Treated Rats (37831)

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Nimni and Bavetta (1) reported that administration of penicillamine (β,β-dimethylcysteine) results in a marked increase in the soluble collagen of skin and a sharp drop in insoluble collagen. The different modes of action of penicillamine were studied by Deshmukh and Nimni (2). They postulated that the inhibition of cross-linking, caused by penicillamine *in vivo* and *in vitro*, involves a reversible interaction with the aldehydes present in tropocollagen to form a thiazolidine-type complex.

Lack of information on changes induced in testicular collagen by penicillamine prompted us to investigate the concentration of soluble and insoluble collagen in albuginea and glandular tissue (seminiferous tubules and interstitial connective tissue) of rats.

Material and Methods. D-Penicillamine was administered orally to 30 adult male Wistar rats weighing 60–80 g. Each animal received 40 mg of penicillamine/day for 15 days. Since penicillamine induced a pyridoxine deficiency (3), presumably by formation of a thiazolidine complex which makes this vitamin partially unavailable (4), a supplement of 2 mg of vitamin B_6 was administered daily. Another 30 male adult Wistar rats of similar weight were used as controls. Both groups were weighed daily. At the end of the experimental period, controls and treated animals were sacrificed under ether anesthesia. Testes were removed quickly and the albuginea separated from the mass of glandular tissue. The albuginea and the glandular tissue from the testes of 15 control and 15 treated animals were pooled separately. Each pool was fractionated for soluble and total collagen. The experiment was then repeated with an additional 15 control and 15 treated rats. The values presented are the means from these two experiments.

The extraction of soluble collagen was performed according to the method of Nimni (5). Two neutrosoluble collagens (extracted in 0.15 and 0.5 *M* NaCl) and a citrosoluble collagen (extracted in 0.5 *M* sodium citrate buffer, pH 3.6) were obtained. Total collagen was extracted by the method of Neuman and Logan (6). The difference between total collagen and the sum of the three fractions of soluble collagens was taken as the amount of insoluble collagen present. Each of the collagen fractions was hydrolyzed in 6 *N* HCl for 24 hr at 105°. The hydrolysates were evaporated under vacuum and neutralized. The values of hydroxyproline were determined by the method of Prockop and Udenfriend (7), and collagen content was obtained by multiplying these values for hydroxyproline by the factor 7.46. Soluble collagens were expressed as milligrams per 100 grams of wet tissue and insoluble collagen as percent of wet tissue.

Results and Discussion. Throughout the experiment, normal growth was observed in both treated and control animals. Figure 1 shows that the values for soluble collagens in the albuginea are higher than in glandular tissue. Collagen fractions in both structures

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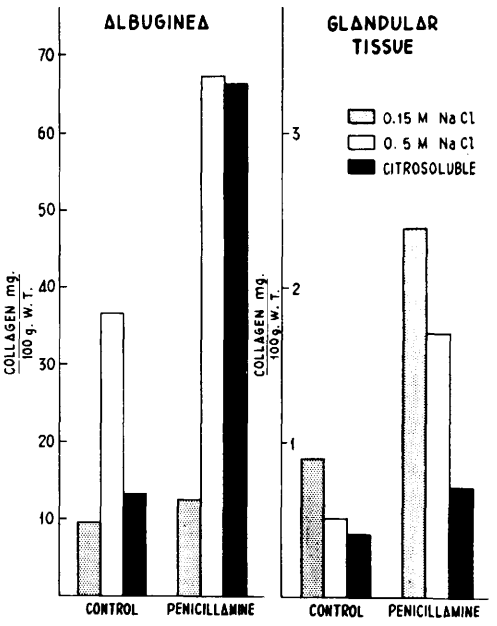

FIG. 1. Soluble collagens of albuginea and glandular tissue in control and penicillamine-treated rat testis. W. T. = wet tissue.

of penicillamine-treated rats as compared to untreated controls are increased. In the albuginea of treated rat testis, the fraction containing the highest value of soluble collagen was the one extracted in 0.5 *M* NaCl, whereas the lowest was the one extracted with 0.15 *M* NaCl. In the glandular tissue of treated rat testis, the highest soluble collagen content was found in the fraction extracted in 0.15 *M* NaCl, and the lowest was found in the citrosoluble fraction. Untreated control rats showed similar relationships.

These results demonstrate that in the testis, soluble collagen responds to penicillamine treatment as skin collagen does (1). Current studies by our group show that a similar increase of soluble collagen takes place in artificial unilateral cryptorchidism. Also, an increase in soluble collagen was reported in normal prepuberal rats (8).

Insoluble collagen found in albuginea and glandular tissue of treated and control rats are shown in Fig. 2. A significant decrease of insoluble collagen in the glandular tissue of penicillamine-treated rats was found, a similar result was reported for rat skin (1), while in the albuginea the amount of this collagen showed no change. In the glandular tissue, the decrease of insoluble collagen may be explained by the inhibition of the soluble collagen aggregation to form the insoluble fibrous material or by the rapid turnover of this tissue, as it was reported for rat skin (2).

Collagen is one of the main components of the seminiferous tubular wall, and electronmicroscopic studies of this wall show alternative layers of amorphous and fibrillar material and fibroblast-like cells. The role of the tubular wall as a supporting structure of the germinal cell line and as an anatomical and physicochemical entity related to the seminiferous tubule blood barrier is known (10–12).

Consequently, further studies are needed in order to demonstrate the usefulness of penicillamine as a tool able to modify the seminiferous tubule wall and study the consequences in tubular permeability.

Summary. The albuginea and glandular tissue of rat testes from animals treated daily for 15 days with 40 mg of penicillamine contained more soluble collagen (in the neutral and citrosoluble fractions) than

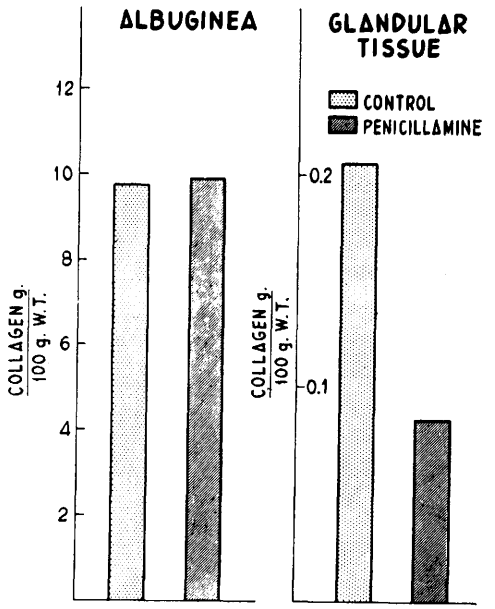

FIG. 2. Insoluble collagen of albuginea and glandular tissue in control and penicillamine-treated rat testis. W. T. = wet tissue.

tissues from the untreated controls. Insoluble collagen in glandular tissue of the penicillamine-treated rat testes was only 40% of the untreated controls, while in albuginea there was approximately the same amount of insoluble collagen in treated and untreated animals.

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