HISTOCHEMICAL AND STRUCTURAL STUDY OF INFLAMMATORY GROWTHS OF THE PROSTATIC EPITHELIUM

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The prostatic epithelium of puppies and rabbits, in a focus of chronic aseptic inflammation produced by injection of coal tar, undergoes divergent differentiation: along protective and glandular lines. The character of the inflammatory growths of the prostatic epithelium indicates that it is an epithelium of epidermal type.

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The dynamics of growth of the prostatic epithelium has been described without histochemical analysis [1]. However, histochemical investigations of the pathologically changed prostate gland and of epithelial metaplasia of the organ has demonstrated correlations between biochemical processes in the tissues and structural changes [8-10].

It was therefore decided to make a histochemical and structural study of inflammatory growths of the prostatic epithelium.

EXPERIMENTAL METHOD

Under sterile conditions, using the operative access of Benjamin and co-workers [7], 0.1-0.2 ml coal tar was injected into the prostate gland of rabbits aged 9 months (series I). In the experiments of series II, coal tar was injected into the prostate of dogs aged one month. This is the first time that such experiments have been carried out on dogs. Altogether 33 rabbits and 30 dogs were used. Material was taken after 1, 3, 6, 8, 10, 12, 15, 22, 30, 60, and 90 days at the same time of day, and fixed in Carnoy's fluid, Becker's formol-calcium, and Peisakhovich's mixture. Histological sections, 5-6 μ in thickness, were stained with Mayer's hematoxylin–eosin, with iron hematoxylin and with azan by Heidenhain's method. Histochemical methods were used to study RNA, DNA (Brachet, Feulgen, Einarson reactions), glycogen and glycoproteins (PAS reaction by the McManus–Hotchkiss method), acid mucopolysaccharides (reaction with dialyzed iron by Hale's method, with toluidine blue and with fast cresyl violet [2] at different pH values), total protein (tetrazolium-coupling and ninhydrin-Schiff), acid and basic proteins (fast green, pH 2.2-8.2), and keratin (acid solution of basic brown by Shubich's method and reaction with performic acid–Schiff). The results of the histochemical tests were assessed after enzyme controls (diastase, testicular and streptococcal hyaluronidase, trypsin), and after methylation, dimethylation, acetylation, alkaline hydrolysis (0.02-0.2 N NaOH), treatment with sulfuric acid, blocking of aldehyde groups with hydroxylamine, and treatment with hot methanol and chloroform (1:1).

EXPERIMENTAL RESULTS

In the early stages of the experiment (1st-3rd days) three zones could be distinguished in the focus of inflammation in both series: zones of necrosis, of reactive changes, and an intact zone. The character of the reaction depended on the size of the drop of tar, the functional state of the organ, and the nearness of

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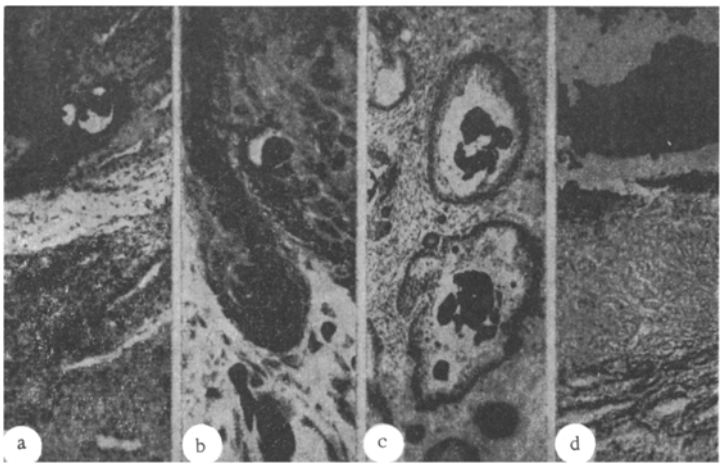

Fig. 1. Inflammatory growths of rabbit prostatic epithelium. a) Epithelial growth (stage 1 day; Heidenhain's iron-hematoxylin, 280 ×); b) appearance of glycogen in epithelium of terminal portion (stage 3 days; PAS; 140 ×); c) glandular differentiation of undifferentiated stratified layer (stage 10 days; PAS; 140 ×); d) keratinization of stratified squamous epithelium (stage 60 days; acid solution of basic brown).

the irritant to the epithelium. The content of RNA, DNA, and protein containing histidine, tryptophan, tyrosine, and amino groups was increased in the fibroblasts and cells of the single and double layers of epithelial cells (Fig. 1a) at the border with the necrotic tissues and tar. The content of acid proteins was higher than that of basic proteins. Fibroblasts synthetized mucopolysaccharides such as hyaluronic acid and chondroitin sulfates A and C intensively and secreted them into the space surrounding the regenerating epithelium, the terminal portions, and the efferent ducts in the zone of reactive changes.

However, by the end of the 3rd day, this zone was completely orthochromic, because of depolymerization of mucopolysaccharides. In dogs, depolymerization developed on the 1st day (Fig. 2a). The basement membrane gave stronger PAS and ninhydrin–Schiff reactions, because of breakdown of glycoproteins and liberation of active groups of carbohydrates and proteins. Depolymerization of the glycoproteins of the basement membrane increased its permeability, thus enabling energy-producing and structural materials (hyaluronic acid, glycogen) to be transported to the epithelium to provide for its growth. The basic conditions for proliferation of the epithelium of the terminal portions of the gland to begin in rabbits was the blocking of their secretory activity. On the 3rd-6th day of the experiment, the activated epithelium formed bands of downward growth and also grew into the lumen (apical growth). This downward and apical growth was largest in puppies (Fig. 2b).

Proliferation of the epithelium took place on account of the basal cells of the acini and the epithelium of the afferent ducts and the prostatic urethra. Another source of proliferating epithelium in rabbits was the dedifferentiating cells of the secretory epithelium. Some cells in the acini of the rabbit prostate mature abortively and desquamate into the lumen. Destructive processes in puppies are less marked. The appearance of glycogen (not detectable histochemically in the normal secretory epithelium of the rabbit), and the increase in content of RNA and chromotropic mucopolysaccharides in the cytoplasm of the epithelial cells of the bands of downward growth and undifferentiated stratified layers were evidently associated with breakdown of protein-carbohydrate complexes in the epithelium itself and with the assimilation of these substances from connective tissue [4]. The irregular distribution of these substances in the acinar epithelium in rabbits demonstrates different levels of differentiation of cells in the same terminal portion. Parallel with the increase in RNA, there was also an increase in total protein. In the bands of downward growth in puppies, the increase in glycogen content was generalized in character, while in rabbits it was focal and concentrated in the apical zones of the cells (Fig.1b). The glycogen and RNA contents were inversely proportional. On the 8th-12th day in rabbits, a process of glandular metamorphosis began in the stratified undifferentiated layers not directly in contact with the irritant; either by redistribution of the cell material or by death

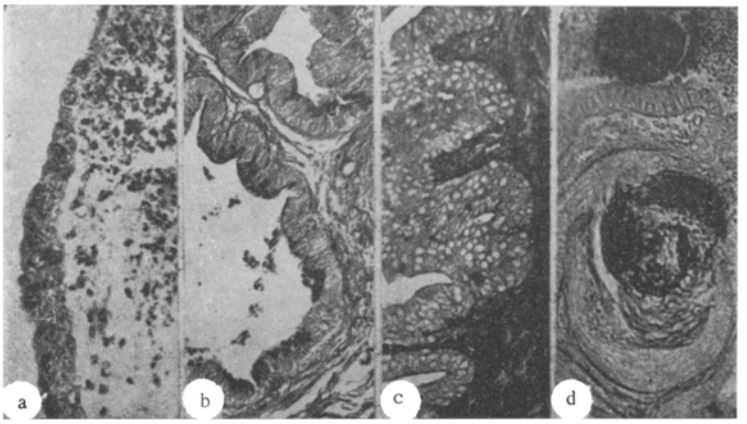

Fig. 2. Inflammatory growths of prostatic epithelium in puppies. a) Depolymerization of acid mucopolysaccharides in reactive zone (stage 1 day; toluidine blue, pH 4.6; 56 ×); b) bands of downward growth (stage 6 days; Brachet's methyl green–pyronine; 280 ×); c) elimination of tar drops by cysts (stage 12 days; toluidine blue, pH 4.6; 56 ×); d) layer of stratified squamous epithelium with atypical keratinization (stage 22 days, acid solution of basic brown).

of the central epithelial cells, a mozaic of cavities appeared (Fig. 1c). Similar processes were seen in the bands of downward growth. Secondary differentiation of the proliferating tissue was accompanied by a decrease in the content of RNA, hyaluronates, and glycogen and by the appearance of PAS-positive diastase-resistant material in the neighborhood of the cell borders and in the apical zones.

By the 20th-25th days, glandular differentiation was completed by the formation of new secretory terminal portions, resembling acini of the intact gland in their structure. In puppies, the organotypical complexes were converted into cysts and did not secrete.

Organotypical growth was accompanied by a decrease in the intensity of the PAS reaction of the basement membranes of the epithelial growths, and by a diffuse distribution of chromotropic mucopolysaccharides in the surrounding connective tissue (in dogs), while in rabbits, the metabolism of the connective tissue moved over toward synthesis of glycoproteins.

The stratified epithelial bands formed from epithelium of the open terminal portions or efferent ducts at the point of direct contact with large drops of tar or with foci of necrosis underwent structural changes of a different type. Cells of the basal layers were specialized for the cytoplasmic synthesis of RNA and acid mucopolysaccharides, their glycogen disappeared, and carbohydrate-protein complexes of the glycoprotein type, showing a high degree of polymerization and lower reactivity, appeared in the cells of the surface layers [11, 12]. These features explain their long existence and their structural organization into stratified squamous keratinizing and nonkeratinizing epithelium or epithelium of transitional type. Areas of keratinized epithelium were grouped into foci and they are described here for the first time in the rabbit prostate (Fig. 1d). Keratinized cells separated from the basal layers to form stratified structures resembling amyloid bodies.

Stratified basal-cell layers with hyperkeratinization were still present at the stage of 90 days of the experiment, but the other epithelial growth had degenerated. Stratified layers in puppies keratinized only in the most superficial layers (Fig. 2d). Tar drops were eliminated into the lumen of the efferent ducts or into the urethra and were partly absorbed. The unabsorbed tar fractions were encapsulated or eliminated by cysts lined with polymorphic epithelium (Fig. 2c). The process of elimination in puppies was complete by the 22nd and in rabbits by the 30th day of the experiment.

Analysis of this material reveals the close relationship between histochemical and structural differentiation of inflammatory growth of the prostatic epithelium, confirming results obtained by other workers [5, 6]. Differentiation of the prostatic epithelium in a focus of chronic aseptic inflammation follows divergent

lines: protective and glandular. Comparison of the character of inflammatory epithelial growths of the prostate and the implantation growth of this epithelium [3] suggests that it is an epithelium of epidermal type.

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