EFFECT OF ESTROGENS ON GROWTH AND DIFFERENTIATION OF THE SALIVARY GLANDS OF ALBINO RATS IN ORGAN CULTURE

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Salivary glands of albino rats in organ culture were investigated after the addition of 17β-estradiol to the medium. After addition of estrogens characteristic glandular tubes with secretion containing acid and neutral mucopolysaccharides were found in the zone of growth. Necrobiotic changes occurred in explants of the control group in the early stages of culture and no marked differentiation of the epithelium was observed. Addition of estrogens in a physiological dose led to differentiation of the epithelium of the salivary glands with the formation of the specific secretion and it improved the changes of survival of the cultured tissue.

KEY WORDS: 17β-estradiol; organ culture; salivary glands.

Attempts to grow salivary glands of adult animals in culture are often unsuccessful for after rats have reached sexual maturity their salivary glands lose their ability to survive for a long time in vitro [5, 6].

The object of this investigation was to study the action of estrogens in vitro on the ability of explanted pieces of the submandibular and parotid salivary glands of albino rats to survive and differentiate.

EXPERIMENTAL METHOD

The salivary glands were taken after decapitation of young sexually mature female rats weighing 120-140 g, transferred to Hanks' solution with antibiotics, cut up with scissors, and attempts were made to obtain intact lobules measuring 1 × 1 mm free from loose connective tissue. The material thus obtained was placed on specially treated millipore filters (pore size 0.45 μ) by the method suggested by Kastrikin [1]. Medium No. 199 was used with the addition of 20% bovine serum (hormones contained in the serum were disregarded), glucose (40 mg/100 ml medium), vitamin C (3 mg/100 ml medium), and antibiotics (penicillin and streptomycin, each in a dose of 10,000 units/100 ml medium). In the experimental series 0.5 μg 17β-estradiol was added to the medium. The gaseous phase consisted of 95% O_2 plus 5% CO_2. The medium was changed every 3 days. The period of culture was 1-10 days. Material for histological examination was taken every day, fixed with Brodskii's mixture, embedded in paraffin wax, and stained with hematoxylin-eosin; acid mucopolysaccharides were stained by Hale's method and neutral polysaccharides by the PAS-reaction.

EXPERIMENTAL RESULTS

Investigation of sections of an organ culture of the submandibular and parotid salivary glands of albino rats after 24 h in culture revealed identical changes in all explants of the control and experimental groups. They consisted of slight vacuolation of the cytoplasm, ill-defined pycnosis of the nuclei, and an increase in the secretion of acid mucopolysaccharide, which were found in granular tubules. On the 2nd-3rd day of culture the explant and the zone of growth could be clearly distinguished. At the same time differences were found between the control and experimental series. Necrobiotic changes have increased in explants grown without the addition of estradiol: numerous degenerating cells with vacuolated nuclei were

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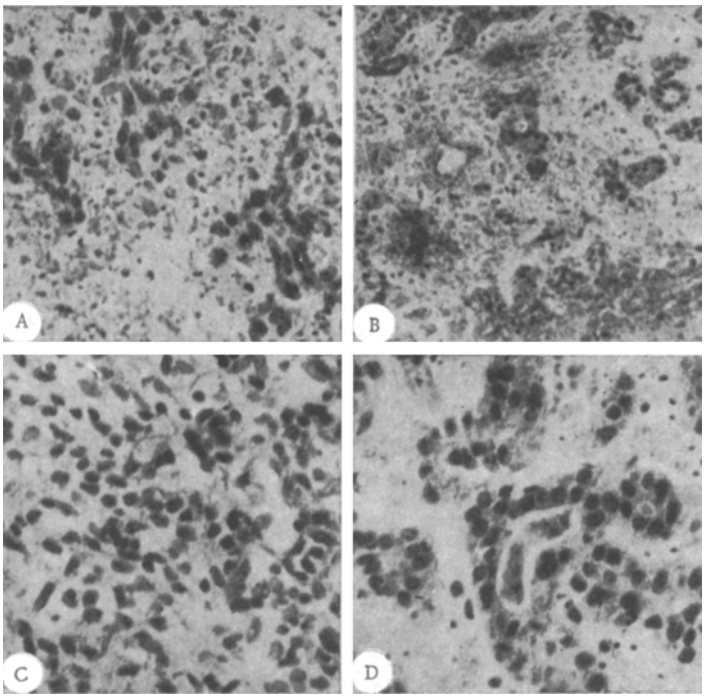

Fig. 1. Submandibular salivary gland of rat in organ culture after addition of 17 -estradiol: A) zone of growth with formation of bands of epithelial cells (third day in culture, hematoxylin-eosin; 320×); B) formation of bands and glandular tubules (third day in culture); PAS reaction, 250×) ; C) continuous growth (hematoxylin-eosin; 320×); D) appearance of secretion in lumen of newly formed glandular tubules (7th day in culture; Hale's stain, nuclei stained with carmine; 320×).

present, the PAS-reaction was more intense, and extensive areas of necrosis appeared by the 5th-6th day. Isolated intact acini and cells were hypertrophied in places to form zones of growth, consisting of undifferentiated basophilic cells and long bands, separated by clear traces. Only a narrow band of tissue at the periphery and the efferent ducts still remained viable after 10 days in culture. Addition of estradiol preserved the viability of the salivary gland explant at all periods of culture (Fig. 1). Cells of the terminal segment were enlarged, their nuclei were rather paler and located on the basement membrane, and the lumen of the glandular tubules was hard to distinguish. These changes were particularly marked in the salivary tubules of the parotid gland. It was impossible to distinguish the salivary tubules in explants aged 7-10 days. Acid and neutral mucopolysaccharides had accumulated in them and were distributed diffusely. The granular tubules remained unchanged and were clearly distinguishable in all explants.

Among the zones of growth observed in both the control and experimental groups, four types could be distinguished.

Type I: growth of the loose connective tissue was predominant, with isolated cells with a large nucleus arranged in it. Acid mucopolysaccharides were found in the cytoplasm of these cells.

Type II: epithelial cells were more numerous in the zone of growth. They formed complexes and bands of different shapes and sizes, with clear spaces between them. Acid and neutral polysaccharides were found in the cytoplasm of the cells, a characteristic feature reflecting early differentiation of the epithelial cells.

Type III: well formed tubules with characteristic dichotomous branching were found in the zone of growth. Depending on the time of culture the character of the secretion contained in the lumen of the tubules varied. In the early stages it contained mainly neutral mucopolysaccharides, but toward the 7th day acid sulfonated mucopolysaccharides began to appear in the secretion.

Type IV: the epithelial complexes had a tendency toward continuous growth, to form areas consisting of cells marked by considerable polymorphism. No histochemical evidence of secretion could be observed.

Explants of the control group were characterized by zones of growth of types I and II, which appeared on the third day in culture. On the addition of 17β-estradiol to the nutrient medium early signs of differentiation and predominance of the type III zones of growth could be seen as early as on the second day. No clearly marked differences in the character of growth could be seen between explants from the parotid and submandibular salivary glands.

The experiments thus showed that epithelial cells of all parts of the salivary glands in culture remain capable of growth and proliferation, with the characteristic adaptation of the glandular cells to new physiological conditions.

In the control series morphological differences between the epithelial cells of the secretary portions and efferent ducts tended to disappear and the growing epithelium became less differentiated in character. On the addition of estradiol the characteristic glandular tubules containing specific secretion appeared in the zone of growth in the earlier stages; according to Grobstein [4], this feature can be used as a marker of differentiation.

The results suggest that estradiol may have two effects: first, by its influence on the various aspects of metabolism it may raise the membrane potential, increase RNA synthesis [2], and thus improve the viability of the tissues; second, considering the possible hormonal dependence of the salivary glands, the addition of estradiol in nontoxic concentrations can be presumed to induce differentiation of the salivary glands, with the appearance of the characteristic secretion, just as is observed in the mammary gland [3, 7], which maintains its differentiation in vitro with milk formation only on the addition of hormones.

LITERATURE CITED

1. N. F. Kastrikin, Tsitologiya, 15, No. 5, 625 (1973).
2. P. Clegg and A. Clegg, Hormones, Cells, and Organisms, Stanford Univ. Press (1969).
3. C. Ceriani, G. Contesso, and B. Nataf, Cancer Res., 32, 2190 (1972).
4. C. Grobstein, Exp. Cell Res., 10, 424 (1956).
5. F. Jacoby and C. Leenson, J. Anat. (London), 93, 201 (1959).
6. D. Lucas and E. Peakman, Exp. Cell. Res., 60, 262 (1970).
7. D. V. Singh, J. Nat. Cancer Inst., 48, 1107 (1972).