

Androgen Target Cells in the Bursa of Fabricius of the Chick Embryo: Autoradiographic Localization (40387)

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The bursa of Fabricius is a lymphoid organ responsible for the humoral immunity in birds (1). Its involution as a consequence of the sexual maturity is still debated. While most studies show the inhibition of the bursal growth by steroid hormones (2), others suggest a more complicated pattern of steroid effects on the bursa of Fabricius (2-5). From a recent quantitative study, it appears that the involution of the bursa is a long term process which lasts throughout the whole life of the animal (6).

In the chick embryo steroid hormone treatment, specially with androgens, prevents the formation (7, 8) or the differentiation (9, 10) of the bursa of Fabricius, depending on the developmental stage when the treatment is started.

The involution of the bursa of Fabricius at sexual maturity and the inhibitory effects of androgen treatments in the chick embryo, led to the belief that male steroid hormones are responsible for the natural regression of the bursa of Fabricius in young chicken. However, Landreth and Glick (3) pointed out that this involution occurs before the beginning of the sexual maturation. In a recent study Norton and Wira (5) showed that treatment with large doses of steroid hormones (0.2-2.0 mg/egg) inhibits while treatment with low doses (20-200 µg/egg) stimulates the growth of the bursa of Fabricius. Since the bursa grows and differentiates in the presence of steroid hormones in the embryo, this suggests a growth stimulating effect of steroid hormones under certain conditions.

Whatever is the effect of steroids, specially androgens, on the development of the bursa of Fabricius, any physiological effect should

involve a steroid hormone receptor. The potential importance of androgen receptors for humoral immunity prompted us to search for and anatomically define androgen target sites in the bursa of Fabricius. The age of 15 days of incubation was considered since this time corresponds to an important step in the bursal development; that is, the formation of follicles in which the first lymphocytes differentiate. Testosterone was selected because it is a circulating male hormone in the embryo (11) and commonly used in experiments on the regression of the bursa (2, 5, 12).

Materials and methods. Chicken eggs of White Rock strain were incubated at 38° and the genetic sex was determined on day 4 of incubation (13, 14). On day 15 [1,2,6,7-³H]-testosterone (S.A.: 85 or 98.8 Ci/m mole) was injected intravenously in an extra-embryonic vessel: Four embryos, 2 males and 2 females, with 0.5 µg, and 3 embryos, 2 males and 1 female, with 0.2 µg each. In competition studies a 100 times higher dose of unlabeled testosterone (2 females and 1 male) or estradiol (2 females) was applied on the chorioallantoic membrane, 30 min prior to the injection of [³H]testosterone. In another experiment, 2 males and 1 female were injected with 0.25 µg each of [2,4,6,7-³H]-estradiol-17β (S.A. 102 Ci/m mole) in a similar fashion as done with [³H]testosterone. Following the injection of labeled hormone, embryos were incubated for 1 or 2 hr, then decapitated and dissected. The caudal part of the body, including the bursa of Fabricius, was mounted on a tissue holder, frozen in liquefied propane and stored in liquid nitrogen.

Three µm frozen sections were cut, mounted on precoated slides (Kodak NTB3 emulsion), kept in desiccated light proof boxes and stored at -15°. After 3 or 4 months exposure, slides were photographically processed and stained with methyl green pyronin. The thaw-mount autoradiographic technique

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has been described in detail (15).

Results. After [^3H]testosterone injection, 1 or 2 hr incubation, a concentration of radioactivity is observed in nuclei of certain mesenchymal cells of the bursa of Fabricius. The radioactively labeled cells are located in large number around the follicles, but also encountered throughout the mesenchyme (Fig. 1a). Since methyl green pyronin does not permit

distinction between mesenchymal cells and lymphopoietic stem cells, which are both present in the mesenchyme, a possible labeling of lymphopoietic stem cells cannot be excluded. However, in the bursal epithelium or in the follicles, no labeled cells are observed.

When embryos were pretreated with a 100-fold excess of unlabeled testosterone, nuclear

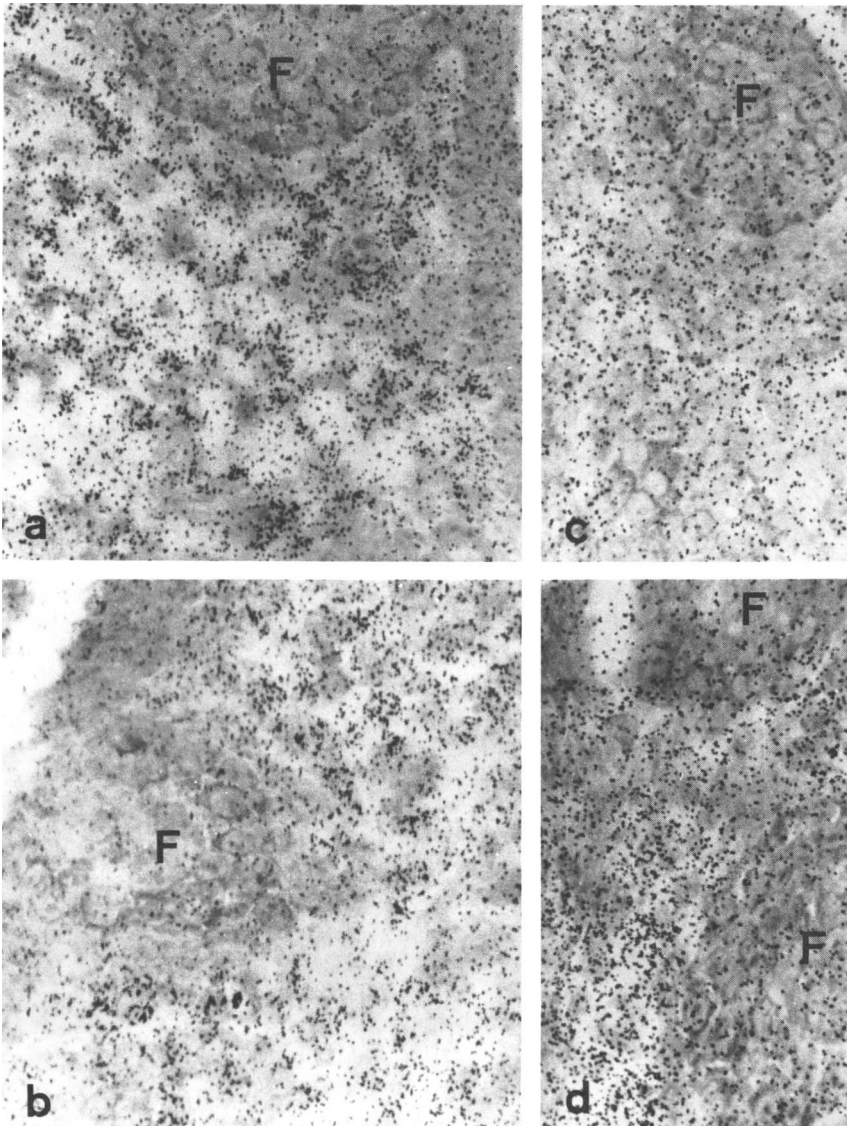


FIG. 1. Bursa of Fabricius of 15-day-old chick embryos shows concentration of radioactivity in nuclei of mesenchymal cells but not in follicles (F) 1 hr after injection of [^3H]testosterone alone (Fig. 1a, exposure time 120 days). Pretreatment with estradiol does not abolish the nuclear labeling (Fig. 1b, exposure time 120 days) while pretreatment with testosterone competes with [^3H]testosterone (Fig. 1c, exposure time 120 days). After [^3H]testosterone injection no labeled nuclei are observed (Fig. 1d, exposure time 150 days). Magnification 200 \times .

labeling after tritiated testosterone is abolished (Fig. 1c). In contrast, pretreatment with estradiol does not eliminate the nuclear labeling (Fig. 1b) observed after [^3H]testosterone injection. When embryos were injected with [^3H]estradiol no nuclear concentration of radioactivity is observed in the bursa of Fabricius, even after longer exposure times (Fig. 1d), while a nuclear labeling exists in certain cells of the cloacal area on the same sections (16).

Discussion. The autoradiographic results for the first time demonstrate concentration of radioactivity after injection of [^3H]testosterone in nuclei of mesenchymal cells of the bursa of Fabricius. This indicates, in conjunction with the competition study, that these cells contain receptors for androgen. Whether testosterone, or androgenic metabolite(s) of it, binds to the nuclei remains to be clarified. The presence of estrogenic metabolites is unlikely since no nuclear labeling occurs after [^3H]estradiol injection, and unlabeled estradiol does not abolish nuclear concentration of radioactivity after [^3H]testosterone injection.

Since nonandrogen steroids display stimulating, regressive or both effects on the bursa of Fabricius (5, 12), the specificity of the receptors remains uncertain. Additional studies with active steroids, such as progesterone or cortisol, will be necessary to further establish specificity of the binding sites for androgens. It is noteworthy that testosterone, or its metabolites, which concentrates in nuclei of mesenchyme cells of the bursa, is active at lower doses, when compared with estradiol (5) which does not concentrate in nuclei of bursal cells under the conditions of the experiment. This observation may help to explain why a hormone can be simulating or regressing, depending on the dose.

Whether mesenchymal cells alone or also lymphopoietic stem cells are target sites for androgens requires further clarification. While the selectivity of the androgen specific sites and the large number of labeled cells suggest that mesenchymal cells are primarily involved, the possibility of other cell involvement cannot be excluded. The use of another stain compatible with the autoradiographic technique, or of interspecific chimeras (17, 18) might give the definitive answer to this question.

The role of the bursa of Fabricius in humoral immunity (1) emphasizes the importance of androgen receptors for the development and functioning of the avian immune system. The involvement of steroid hormones in immunological mechanisms is further supported by the localization of [^3H]estradiol in reticular cells of the rat thymus (19) and by the presence of cytoplasmic binding sites for various steroids in the bursa of Fabricius of the young chicken (20).

Summary. After intravenous injection of [^3H]testosterone in 15-day-old chick embryo nuclear concentration of radioactivity is observed in mesenchymal cells of the bursa of Fabricius. In competition experiment with excess of unlabeled testosterone, the concentration of radioactivity is abolished, but not with unlabeled estradiol. When [^3H]estradiol is injected alone no nuclear concentration of radioactivity is observed in the bursa of Fabricius. The results demonstrate the presence of target cells for androgen in the bursa of Fabricius and suggest involvement of androgens in the development and functioning of the immune system.

Note added in proof: While this paper was in press G. H. Michel and E. E. Baulieu (J. Ster. Biochem., **9**, 832, 1978) and T. Noumura (J. Ster. Biochem., **9**, 890, 1978) reported in an abstract the presence of an androgen receptor in the cytosol of the bursa of Fabricius in embryos of chick, quail, and duck.

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