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UPTAKE *IN VIVO* OF [^{3}H]TESTOSTERONE BY THE INTERSTITIAL COMPARTMENT IN TESTES OF NORMAL ADULT MICE

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Although it is generally agreed that the Leydig cells are the predominant site of biosynthesis of testosterone, it has been suggested that androgen action upon these cells may be required for the maintenance of their steroid synthesizing enzymes (Blackburn, Chung, Bullock & Bardin, 1973). Parvinen, Hurme & Niem (1970) have shown that labelled testosterone, pregnenolone and progesterone administered intravenously can penetrate into the seminiferous tubules of rats within 2 to 5 min, but the radioactivity in the tubules was always significantly lower than in the interstitial tissue. Van Doorn, de Bruyn, Galjaard & van der Molen (1974) noted that [^{3}H]testosterone administered intravenously with Krebs–Ringer bicarbonate solution resulted primarily in interstitial localization for periods up to 1 hr in rabbits, but the delayed penetration into the tubules was not seen when the androgen was administered with blood as a medium. They concluded that androgens can pass from Leydig cells into the seminiferous epithelium and suggested that plasma proteins may influence the transport and uptake of these steroids by the tubules. In rabbits and rats, respectively, Galjaard, van Gaasbeek, de Bruyn & van der Molen (1970) and Cooke, De Jong, van der Molen & Rommerts (1970) have also demonstrated that androgens can pass from the interstitial compartment into the epithelium of the seminiferous tubules.

Investigations concerning androgen biosynthesis and the effect of androgens on the testis have been carried out predominantly on the individual (tubular or interstitial) compartments. Microdissection of the interstitial compartment from the seminiferous tubules makes possible separate analysis of these elements after experiments *in vivo* or *in vitro*. The purpose of our experiment was to localize the labelled androgen in the testis of intact adult mice within 60 min after intravenous administration of [^{3}H]testosterone.

Fourteen adult male white Swiss mice of approximately 30 g body wt were anaesthetized with an intraperitoneal injection of Nembutal (0·6 mg/10 g body weight) and were then given an injection into the external jugular vein of $15{\cdot}8\times10^{-5}$ mg [1,2,6,7-^{3}H]testosterone containing 0·05 mCi (sp. act. 85 to 105 Ci/mmol: New England Nuclear). The animals were killed by cervical dislocation 30 or 60 min after the injection of the isotope. The testes were removed, and transected across the narrow axis and immediately positioned,

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with the cut surface up, in minced liver heaped on a brass specimen holder. The specimen holder was then lowered into liquid propane at a rate slow enough to allow the tissue to freeze without cracking. Once frozen, the block of tissue was stored in liquid nitrogen until it was used for sectioning. Sections (4 μm) were cut in a Harris cryostat at $-40°C$ and then freeze-dried in a cryosorption pump at approximately 10^{-5} mmHg for 8 to 10 hr in the back chamber of the cryostat.

The method of Stumpf & Roth (1967) and Stumpf (1970) for autoradiography with steroid hormones was followed. In the dark room, the freeze-dried tissue sections were positioned on Teflon squares. With the red safe-light on, glass slides precoated with Kodak NTB-3 emulsion were pressed into contact with the Teflon squares and the tissue sections were thus dry-mounted onto the slides. The slides were stored at $-15°C$ for from 1 to 6 months in air-tight light-tight boxes containing a desiccant. To develop the autoradiograms, the boxes were allowed to reach room temperature before the slides were removed and developed in Kodak D-19 under red light conditions. The tissue sections were then stained with haematoxylin or methyl green–pyronin.

The localization of the [^{3}H]androgen 30 min after injection is shown in Pl. 1, Fig. 1. Although a small amount of the labelled steroid was detected within the seminiferous epithelium, the major amount of the radioactivity was found within the interstitial tissue. Similar localization was observed in autoradiograms from mice which were killed 60 min after injection. The isotope was localized over the cytoplasm of the interstitial cells at both times after injection (Pl. 1, Figs 1 and 2).

Desjardins, Ewing & Johnson (1971) have shown that completion of spermatogenesis in the hamster is dependent upon testosterone and Steinberger (1971) and Matsumoto & Yamado (1973) have stated that testosterone is necessary for reduction division of the primary spermatocyte in the rat. Berndtson, Desjardins & Ewing (1974) have shown that exogenous testosterone will maintain spermatogenesis in the rat, although, in the mouse, dihydrotestosterone is necessary to maintain complete spermatogenesis (C. Desjardins, personal communication). The results of our experiment indicate that the Leydig cells are also a target tissue for androgens. The fact that the majority of the isotope is localized within the interstitial area suggests the possibility that the Leydig cells sequester the androgen and, hence, limit its access to the tubular epithelium. Lacy, Fyson, Collins, Tsang & Pettitt (1973) state that [^{3}H]-testosterone administered intravenously to hypophysectomized rats passes rapidly into the seminiferous tubules and equilibrates in concentration with that of the interstitial area. The present experiment demonstrates that this does not occur in the intact mouse.

The existence of a blood–testis barrier which isolates the developing germinal cells of the seminiferous epithelium from blood-borne factors has been well established. Dym & Fawcett (1970) have described in detail the anatomical aspects of the tubular wall and Setchell, Voglmayr & Waites (1969) have demonstrated some physiological significance of the blood–testis barrier by comparing rete testis fluid with testicular venous blood and lymph in rams and rats. The interstitial localization observed in our autoradiograms offers

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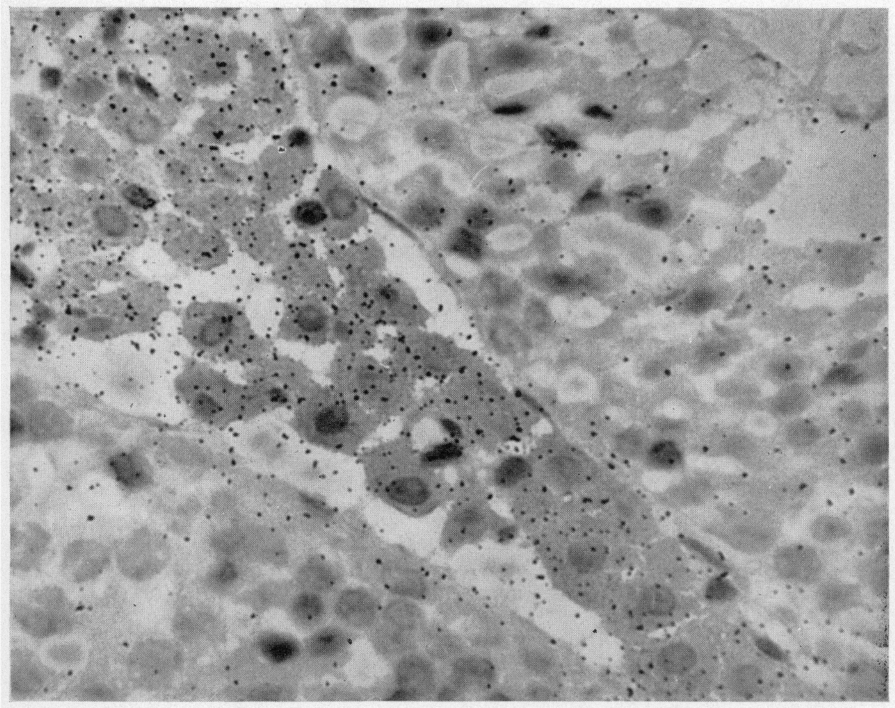

FIG. 1. Testis of a mouse killed 30 min after injection of [1,2,6,7-^{3}H]testosterone, showing interstitial and cytoplasmic localization. The autoradiogram was exposed for 3 months and stained with haematoxylin. ×250.

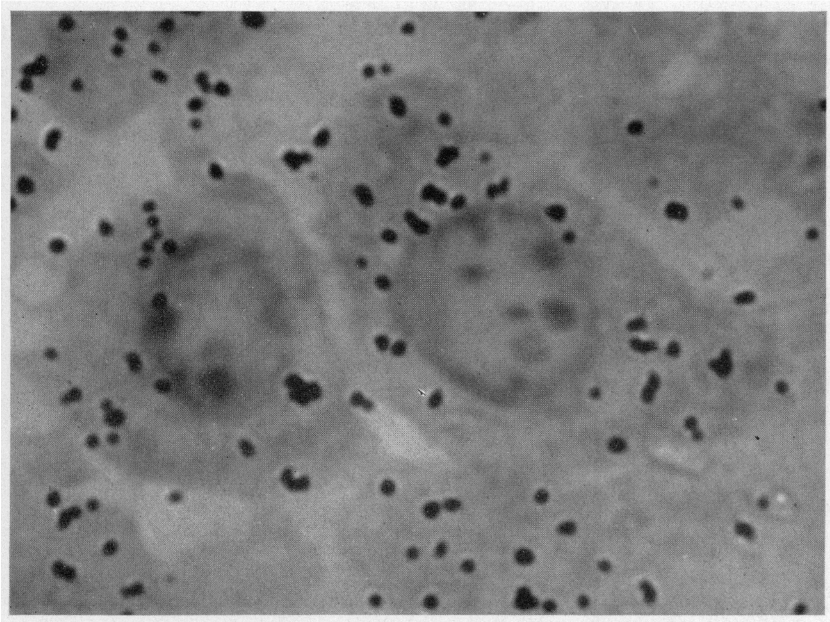

FIG. 2. Testis of a mouse killed 60 min after injection of [1,2,6,7-^{3}H]testosterone, showing cytoplasmic localization of the steroid. The autoradiogram was exposed for 6 months and stained with methyl green–pyronin. ×750.

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further evidence of a physiological barrier. The presence of a small amount of isotope within the tubules at both time intervals indicates that the barrier does not completely exclude androgen in the mouse testis. The fact that such a small amount of label was found within the seminiferous tubules suggests that the interstitial cells may have a greater attraction for the androgen in the form in which it was injected.

From the work of Jensen & DeSombre (1973), it is known that steroid hormones act at the nuclear level to induce RNA synthesis. The present study shows predominantly cytoplasmic localization of the labelled hormone both 30 and 60 min after injection. Kasai, Mizutani & Matsumoto (1973) have also shown a predominance of cytoplasmic localization of radioactivity in the mouse testis, and Aoki (1968) has shown that the agranular endoplasmic reticulum in the cytoplasm of the interstitial cells can be the site of storage of androgens. The cytoplasmic localization demonstrated here could, therefore, be a result of any or all of three possibilities. The steroid may be acting on the cytoplasmic enzymes of the Leydig cell, or it may just be sequestered and stored in the cytoplasm, or the exogenous androgen may be taken up and altered by the androgen-synthesizing enzymes in the Leydig cells.

Our hypothesis is that the interstitial cells in the mouse may be more receptive to testosterone than the seminiferous epithelium, resulting in uptake of the exogenous testosterone by the interstitial compartment of the testis. Once in the interstitial cell cytoplasm, this steroid may then be altered to form a metabolite which may more readily enter the seminiferous tubules to act on spermatogenesis. Preliminary evidence obtained in our laboratory suggests that the tubules begin to fill with the isotope between 1 and 2 hr after injection. We are currently investigating this further.

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