Testicular Involution Following Optic Enucleation

An Ultrastructural and Cytochemical Study

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Summary. The testes of adult male Syrian hamsters underwent involution within six weeks after optic enucleation. The diameter of the seminiferous tubules was 39% less than controls. Sertoli cells, spermatogonia, and primary spermatocytes were still present, but all steps of spermatids were completely absent from the involuted testes. Lipid droplets filled the Sertoli cell cytoplasm and often encroached upon the nucleus. Sertoli cells had sparse mitochondria and smooth endoplasmic reticulum, but Golgi cisternae were abundant. Typical Sertoli-Sertoli junctions attached contiguous Sertoli cells. With lanthanum tracers it was demonstrated that these junctions were impenetrable; therefore, the blood-testis barrier was deemed intact. Irregularly shaped protrusions often arose from the peritubular tissue and extended inward toward the seminiferous epithelium, often displacing the cytoplasm of the Sertoli cells and spermatogonia. The core of these protrusions consisted of irregular extensions of myoid cell cytoplasm surrounded by the myoid cells' basal lamina. External to the myoid cell basal lamina were bundles of collagen filaments with the basal lamina of the seminiferous epithelium forming the outermost layer of these protrusions. The apices of the Sertoli cells gave rise to numerous leaf-like processes that extended into and obliterated the lumen of the tubules. The Sertoli cell basal cytoplasm often contained phagocytized degenerating germ cells that appeared to give rise to the lipid droplets that filled the Sertoli cell cytoplasm. Acid phosphatase rich lysosome-like organelles were seen fusing with the degenerating germ cells and lipid droplets. The degenerating germ cells also were shown to contain acid phosphatase activity.

Key words. Testes (Syrian hamster) – Involution and optic enucleation – Ultrastructure – Cytochemistry.

Introduction

Darkness, operating via the pineal gland, induces testicular atrophy in hamsters (Hoffman and Reiter, 1965; Reiter, 1968; 1972). Short photoperiods, dark
exposure, and blinding are thought to stimulate the antigonadotropic action of the pineal and thereby inhibit testicular function. Because of its intense sensitivity to short photoperiods and darkness, the hamster has been used extensively as an experimental model in studies dealing with the interaction of the photoperiod and the pineal-gonadal axis (Reiter and Sorrentino, 1970).

Until recently the indole melatonin had been regarded as the primary gonad-inhibiting substance produced by the pineal (Wurtman et al., 1968). Recent evidence suggests that pineal polypeptides may have a greater effect on the pituitary-gonadal axis than the indoles (Reiter et al., 1976). Low molecular weight polypeptides taken from pineal extracts have been shown to be potent inhibitors of the release of pituitary gonadotrophins (Ebels et al., 1973; Moszkowska and Ebels, 1971; Orts et al., 1974; Vaughan et al., 1974). Regardless of whether the pineal hormone is a polypeptide or an indole, it has been conclusively demonstrated that serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels are reduced in hamsters exposed to darkness presumably by the pineal exerting its influence on the synthesis or release of the hypothalamic releasing hormones (Berndtson and Desjardins, 1974; Reiter, 1976).

Although the endocrine aspects of the pineal-mediated, dark-induced testicular involution have been extensively examined, the morphologically observable effects on the testis have received only cursory investigation. Numerous studies have shown that exposing animals to short photoperiods and darkness causes a drastic decrease in testicular weights (Hoffman et al., 1965; Hoffman and Reiter, 1965; Reiter, 1968; 1972). In light microscopic studies, it has been observed that the seminiferous tubules were greatly reduced in size and contained only Sertoli cells and spermatogonia in blinded hamsters (Reiter, 1968).

It was the purpose of the present investigation to examine the testes of blinded hamsters with light and electron microscopy, acid phosphatase cytochemistry, and lanthanum tracers and to provide a detailed account of dark-induced testicular involution relative to the general morphology, the blood-testis barrier, and the disposal of degenerating germ cells in blinded hamsters. This investigation is part of a study designed to increase our understanding of the effects of darkness on the reproductive system.

**Materials and Methods**

Male Syrian hamsters (*Mesocricetus auratus*) obtained from Simonsen laboratories Gilroy, California were used in these studies. Animals weighed 80–90 grams at the outset of the experiments and were housed six to a cage. They received food and water ad libitum. Twelve control animals were maintained in the laboratory under constant lighting. Twelve hamsters were blinded according to the method of Reiter and Hester (1966), which consisted of optic enucleation under ether anesthesia. Two months after the commencement of the experiment the controls and six blinded animals were perfused through the left ventricle with 5% glutaraldehyde in 0.1M collidine buffer (pH 7.4) under sodium pentobarbital anesthesia. Following perfusion 2mm blocks of the testes were post-fixed in 1% osmium tetroxide in 0.1 M collidine buffer. After osmication the tissue was immersed in 1% potassium ferrocyanide in 0.1m collidine buffer for 15 min. The potassium ferrocyanide treatment, a modification of the technique of Karnovsky (1971), enhanced contrast of the tissue. The tissue was then stained en bloc with uranyl acetate, dehydrated in graded series of ethanol, infiltrated with propylene oxide and embedded in Epon 812. Thin sections were stained with lead citrate and were examined using a Siemens 1-A electron microscope. For light microscopy, 4 mm cubes of fixed tissue were post-osmicated in 1% osmium tetroxide in 0.1M S-collidine buffer. The tissue was dehydrated in ethanol, passed through propylene