Evidence for protein absorption from the lumen of the seminiferous tubule and rete of the rat testis*

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Summary. As luminal fluid moves from the seminiferous tubule and enters the rete testis, its protein concentration declines from approximately 6 mg/ml to 1 mg/ml. It was therefore suggested that protein is either 1) utilized by the spermatozoa, 2) transported across the epithelium of the terminal segment of the seminiferous tubule, the tubuli recti or rete testis, or 3) absorbed and degraded by the epithelium. Horseradish peroxidase (HRP), a protein marker, was microperfused into single seminiferous tubules or perfused directly into the rete. After fixation, the HRP was localized histochemically and the tissue observed under the light- and electron microscope. HRP was taken up via pinocytotic vesicles into the cytoplasm of the Sertoli cells and germ cells but did not permeate extracellularly beyond the tight junctions. Similar results were obtained in the cells lining the terminal segment and the tubuli recti. The rete epithelium showed uptake of HRP into coated and non-coated vesicles, while some cells additionally revealed diffuse cytoplasmic distribution of HRP. The terminal segment, tubuli recti, and rete testis may be important routes by which proteins may leave the testicular fluid either to be degraded or to enter the blood.

Key words: Rat — Testis — Protein — Endocytosis — Microperfusion

Seminiferous tubule fluid (STF) and rete testis fluid (RTF) are known to contain specific proteins different from those of blood plasma (Kormano et al. 1971; Koskimies and Kormano 1973; Setchell 1974) and among those

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so far isolated Androgen Binding Protein (Hansson et al. 1975) and an inhibitor of acrosomal proteinase (Suominen and Setchell 1972) are the best studied. Preliminary studies (Setchell et al. 1978) have demonstrated that as STF enters the rete testis there is a reduction in protein concentration and this decline does not appear to be due to dilution since sperm concentration remains unchanged. It has been suggested that the terminal segment of the seminiferous tubule or the tubuli recti may be responsible for absorption of the protein. Furthermore, anatomical studies by Leeson (1962) have suggested that the rete may also be transporting material across its epithelium. Therefore, the present study was undertaken to determine where in the testis protein uptake occurs. A combination of micropuncture, microperfusion, and histochemical and electron microscopic techniques was employed to answer this question.

Materials and methods

Adult white male Sprague Dawley rats (364–483 g; Hilltop, Philadelphia) were housed in the University Vivarium under a 12 h light: 12 h dark cycle and had free access to food and water. For studies undertaken in Cambridge, U.K., white Porton-Wistar rats were obtained from the Institute colony and housed under similar conditions.

Horseradish peroxidase (Type VI) was purchased from Sigma Chemical Company (St. Louis, Mo.), bovine serum albumin from Armour Pharmaceutical Company (Eastbourne, U.K.) and Folin-Coicalteau reagent from British Drug Houses (Poole, U.K.).

Seminiferous tubule fluid (STF), rete testis fluid (RTF) and extracellular fluid (often referred to as interstitial fluid) surrounding the seminiferous tubules were collected by micropuncture techniques as previously described (Hinton et al. 1979). Blood was collected from the inferior vena cava into a heparinized syringe. All samples were centrifuged and the supernatants estimated for protein as described below.

Estimation of protein

For the estimation of protein in testicular fluids and blood plasma, Hartree's modification of the Lowry method (Hartree 1972) was modified to accommodate small sample volumes. The assay was carried out in small glass tubes (50 mm length, 6.5 mm o.d., thoroughly cleaned) by use of bovine serum albumin (standard ranged from 0.3 to 10 mg/ml) as standard. Micropuncture sample volumes used for protein estimation were from 0.1 to 1.0 \( \mu l \). By means of semi-microcuvettes, all samples were read at 650 nm on a Gilford Spectrophotometer. Samples were assayed in duplicate or triplicate.

Microinjection and microperfusion of horseradish peroxidase (HRP)

A series of preliminary experiments were performed microinjectioning 30% HRP dissolved in either 0.154 M NaCl or artificial STF (ASTF: NaCl, 108 mM; KCl, 10 mM; CaCl\(_2\), 1 mM; MgCl\(_2\), 1 mM; KHCO\(_3\), 40 mM; inositol, 2 mM; mannitol, 45 mM; Hepes buffer, 5 mM; 330–350 mOsm/kg water, pH 7.3) into single seminiferous tubules of anesthetized rats (sodium pentobarbitone, 50 mg/kg; see also Pilsworth et al. 1981). The animals were allowed to recover and 18 h later the injected testis was removed, immersed into Karnovsky's fixative and prepared for histological observation as described below.

From these experiments it was found that 18 h was too long to record protein movement and isolation of the original injected tubule proved difficult. Therefore a microperfusion experiment was prepared as described by Hinton and Setchell (1978). Rats were prepared for micropuncture. A small incision was made through the tunica albuginea to expose a very small portion of a single seminiferous tubule. Then, HRP (1–5% in either saline or ASTF) was microperfused into the seminiferous tubule at a rate of 2.7 \( \mu l/min \) for 10–15 sec in pulses.