UPTAKE OF HORSERADISH PEROXIDASE BY THE TESTIS AND EPIDIDYMIS OF MICE

I. LIGHT-MICROSCOPIC STUDY

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Mancini, Vilar, Alvarez & Seiguer (1965) have demonstrated the passage through the blood–testis barrier of labelled serum proteins injected intravenously into rats. Horseradish peroxidase was earlier introduced as a tracer by Straus (1957a) and applied to the ultrastructural level by Karnovsky (1965) and Graham & Karnovsky (1966).

Sedar (1966a, b) reported, in two abstracts, that horseradish peroxidase injected intravenously into the hamster became localized in the epididymis and ductuli efferentes, and in the rete testis. Reddy & Svoboda (1967) observed that horseradish peroxidase injected intravenously into the rat was taken up by the cells of the testis. Recently, Fawcett, Leak & Heidger (1970), studying the permeability of the blood–testis barrier in guinea-pigs, reported the uptake of horseradish peroxidase injected interstitially in the testis.

The different results obtained by Fawcett *et al.* (1970) and Reddy & Svoboda (1967) on the uptake of horseradish peroxidase by the testis in different species, induced us to study the permeability of the blood–testis barrier and the epididymal tubules in the mouse.

In the present report, light microscopic observations on the absorption of intravenously injected peroxidase by testes and epididymides of normal mice are described. The ultrastructural aspect of this study is in progress.

Horseradish peroxidase (Sigma, type II), in a dose of 8 mg/mouse dissolved in 0.3 ml saline solution, was injected intravenously into the tails of twenty-five male mice (Rockland) varying from 25 to 30 g in weight. Animals were killed at 1, 15, 30, 60, 90 and 120 min, and 3, 6, 9, 12, 18 and 24 hr following injection. The test for peroxidase activity was performed as described by Graham & Karnovsky (1966) on 6- μ m frozen sections of testis and epididymis. Free base, 3,3'-diaminobenzidine, was used as the substrate for the peroxidase with peroxide in concentrations of 0.01% and 0.001%, the latter according to the method of Nakane & Pierce (1967).

The following controls were used: (1) animals injected with saline solution and tested for peroxidase activity, (2) uninjected animals to test for the presence of endogenous peroxidase, (3) animals injected with peroxidase but not tested for peroxidase activity.

The sections were observed unstained or counterstained with Light Green.

To decrease or to prevent non-specific adsorption of peroxidase, hypertonic sucrose was added to the washing fluids used after fixation. In order to confirm that the localization of peroxidase was not due to a diffusion artifact (Straus, 1967b), some experiments with fixation by perfusion were performed. Perfusion was started through the tail vein with 10 to 15 ml of saline warmed to 37° C until the kidney, liver and testis were pale. Perfusion with saline was followed immediately by perfusion with 10 ml 5% glutaraldehyde in 0.1 M-phosphate

TABLE 1
THE INTENSITY OF THE PEROXIDASE REACTION IN THE TESTIS
AND EPIDIDYMIS OF MICE INJECTED INTRAVENOUSLY WITH
HORSE RADISH PEROXIDASE

Time	Testis			Epididymis	
	Interstitial cells	Tubular wall	Germinal epithelium	Tubular wall	Epithelial cells
1 min	++	++	++	+	+++
5 min	++	++	++	++	+++
15 min	+++	+++	+++	++	++++
30 min	++	++	++	++	+++
60 min	++	+	+	++	+++
180 min	++	±	—	+	+
12 hr	++	—	—	±	—
24 hr	—	—	—	—	—

The degree in the intensity of the reaction was arbitrarily classified from — (negative reaction) to ++++ (maximum positive reaction).

buffer, pH 7.6. Spermatic veins were sectioned in order to allow drainage of the perfusion fluids.

The results are shown in Table 1. Perfusion experiments confirmed the results obtained by the standard methods of fixation. Horseradish peroxidase was detected in the testis and in the epididymis as early as 1 min after injection. The intensity of the peroxidase activity increased with time, reaching a maximum approximately 15 min after injection. The positive reaction persisted longer in the epididymis than in the testis but was completely negative in both tissues 24 hr after injection.

In the testis, apart from the lumina of the capillaries, a positive reaction was

EXPLANATION OF PLATE I

All sections were treated for peroxidase and unstained.

FIG. 1. Frozen section of mouse testis 1 min after intravenous injection of horseradish peroxidase. The reaction product is visible in the tubular wall and in the cells of the interstitial tissue. $\times 96$.

FIG. 2. Frozen section of mouse epididymis (same conditions as in Fig. 1). The reaction product is present in the tubular wall, in the intertubular tissue and in the spermatozoa in the tubular lumen. $\times 100$.

FIG. 3. Frozen section of mouse testis. 15 min after intravenous injection of horseradish peroxidase. The reaction product is present in the tubular wall and in most of the cells of the germinal epithelium. $\times 400$.

FIG. 4. Frozen section of mouse epididymis (same conditions as Fig. 3). The reaction product is present in the tubular wall and in the epithelial cells. $\times 400$.

PLATE 1

(Facing p. 300)

first observed in the cytoplasm of the interstitial cells. The reaction product then appeared in the tubular wall and in the germinal epithelium. Peroxidase uptake was not seen in all the tubules of the testis (Pl. 1, Fig. 1).

In the epididymis, results were similar to the ones observed in the testis, but a higher amount of uniformly distributed peroxidase was found (Pl. 1, Fig. 2). In both tissues, peroxidase was localized in the epithelium as small precipitates in the cell cytoplasm and in the intercellular spaces. By 1 min after injection, the basal cells of the germinal epithelium showed a reaction product, but by 15 min, peroxidase was also present in the cells lining the tubular lumen. In the epididymis, the spermatozoa lying in the tubular lumen showed a positive reaction within the first few minutes after injection. (Pl. 1, Figs 3 and 4). All controls were negative with the exception of the endogenous peroxidase of the blood cells present in the lumen of the vessels.

Our results confirm and amplify those reported by Reddy & Svoboda (1967) in the testis of rats. Fawcett *et al.* (1970) demonstrated the presence of peroxidase in the intercellular clefts between spermatogonia and Sertoli cells and as small pinocytosis vesicles in the peripheral cytoplasm of both cells. They did not observe any peroxidase-positive reaction in the epithelium deeper than the spermatogonia. They postulated that the peritubular myoid cell layer and the junctional specializations between adjacent Sertoli cells both play a rôle in the blood-testis barrier.

Our different results could be due to the route of injection and to the species used. We chose the intravenous route since this was expected to yield a more physiological distribution of the tracer. The possibility that the positive reaction observed could have been due to endogenous peroxidase was discounted since all the controls were negative.

The observation that not all the tubules of the testis are permeable to the tracer, suggests that an active blood-testis barrier is only present in certain areas. Further studies are required to determine whether these topographical differences are due to variations in the spermatogenic cycle or some other factors, such as vascularity or hormonal influence.

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