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Enzyme histochemistry of mouse epididymis: effects of castration and androgen replacement therapy

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Fisiologia. — *Enzyme histochemistry of mouse epididymis: effects of castration and androgen replacement therapy* (*). Nota di MARIA DI MEGLIO, SILVIA FASANO e RAKESH K. RASTOGI, presentata (**) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — L'influenza della castrazione e del 5 α-diidrotestosterone sull'attività di diversi sistemi enzimatici è stata studiata istochimicamente nell'epididimo del topo. In generale era possibile dimostrare la presenza di tutti i dodici enzimi nei tre segmenti epididimali. L'intensità della reazione istochimica per tutti gli enzimi, eccetto la NADH–diaforasi, variava in maggiore o minore misura nel *caput*, *corpus* e *cauda* dell'epididimo di animali intatti.

Tutti i dodici enzimi studiati in questo lavoro rispondevano al trattamento con androgeni. I risultati mostrano, inoltre che vi sono delle differenze nella risposta dello stesso enzima nei diversi segmenti dell'epididimo alla castrazione e alla terapia restaurativa con androgeni. Questo fatto prova ulteriormente che l'epididimo non è uniforme, ma mostra attività differenti nelle varie regioni.

INTRODUCTION

It has been widely recognized that the epididymis plays a vital role in the physiology of reproduction in mammals. In fact it has been shown or suggested that the epididymis provides essential elements for the functional maturation of spermatozoa which pass through it after leaving the testis in an immature state (Hamilton, 1975; Orgebin–Crist *et al.*, 1975). A wide array of reports has shown that (*a*) the epididymis has both secretory as well as absorptive functions, (*b*) the structural and functional integrity of the epididymis is androgen-dependent, and (*c*) the epididymis receives androgens from the testis and from the general circulation, and also has its own steroid function (Nicander, 1970; Halmilton, 1971; Prasad *et al.*, 1972, 1973; Jones, 1974, 1977; Ganjam and Amann, 1976; Brooks, 1976 a, b, Diøseland *et al.*, 1976; Rastogi *et al.*, 1976; Nag *et al.*, 1977; Back *et al.*, 1977; Rastogi, 1979).

The histochemical occurrence of several enzymes and dehydrogenases has been reported in the epididymis of several mammalian species: rat (Martan and Risley, 1963; Moniem and Glover, 1972); mouse (Allen and Slater, 1957, 1958; Martan and Allen, 1964), rabbit (Linnetz and Amann, 1968; Moniem and Glover, 1972); ram and hamster (Moniem and Glover, 1972); bull (Rollinson, 1955; Roussel and Stallcup, 1967) and man (Montagna, 1952).

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The present study was thus undertaken in order to demonstrate histochemically the activity of several enzyme systems (mainly hydrolytic, glycolytic and those concerned with steroid biosynthesis) in the mouse epididymis with reference to the androgen–status of the animal (Table I).

TABLE I.

Enzyme systems studied.

ENZYME	EC number	METHOD APPLIED
Δ^5–3 β–*hydroxysteroid dehydrogenase* . .	1.1.1.51.	BAILLIE *et al.* (1966)
11 β–*hydroxysteroid dehydrogenase* . . .	1.1.1.51.	BAILLIE *et al.* (1966)
Acid phosphatase	3.1.3.2.	BURSTONE (1962)
Alkaline phosphatase	3.1.3.1.	BURSTONE (1962)
β–*glucuronidase*	3.2.1.31.	FISHMAN and BAKER (1956)
Non–specific esterases	3.1.1.–.	PEARSE (1972)
Lactate dehydrogenase	1.1.1.27.	PEARSE (1972)
Glutamate dehydrogenase	1.4.1.2.	PEARSE (1972)
Glucose–6–phosphate dehydrogenase . . .	1.1.1.49.	PEARSE (1972)
Glucose–6–phosphatase	3.1.3.9.	WACHSTEIN and MEISEL (from PEARSE, 1972)
NADH–*diaphorase*	1.6.99.2.	BURSTONE (1962)
NADPH–*diaphorase*	1.6.99.1.	BURSTONE (1962)

RESULTS AND DISCUSSION

Histological examination of the different segments of the epididymis of intact and castrated mice showed that the extirpation of the testes caused the disappearance of spermatozoa from the ductule lumen of all the three segments. The lumen diameter and the height of the epithelium lining the ductules greatly decreased due to castration. These regressive changes were successfully reversed by androgen therapy.

The results of histochemical analysis are summarized in Table II. It can be noted that the intensity of enzyme reaction varied greatly in different regions of the epididymis and for the sake of clarity we have used the conventional terms of *corpus*, *caput* and *cauda* epididymides.

Bearing in mind the questions dealing with the basic cellular biochemistry of the epididymis in relation to its physiology the utilization of enzyme cytochemistry of mouse epididymis was considered one approach to solving,

TABLE II.

Enzyme histochemistry of mouse epididymis.

Enzymes studied	Experimental groups	Epididymal segments		
		Caput	Corpus	Cauda
Acid phosphatase	Normal	+++++	+++	+++++
	Castrate	++	++	++
	» + 5 α-DHT	+++++	+++	+++++
Non-specific esterases	Normal	+++	++	+++
	Castrate	+	+	+
	» + 5 α-DHT	+++	+++	+++
Alkaline phosphatase	Normal	+++++	++++	++++
	Castrate	++	++	++
	» + 5 α-DHT	++++	+++	++++
Lactate dehydrogenase	Normal	trace	+	++
	Castrate	—	+	+
	» + 5 α-DHT	++++	+++	++++
Glutamate dehydrogenase	Normal	++	+	trace
	Castrate	++	+++	+++++
	» + 5 α-DHT	++	++	+++
Glucose-6-phosphatase	Normal	+++	+	+++
	Castrate	—	+	—
	» + 5 α-DHT	trace	+	+
Δ^5-3 β-*hydroxysteroid dehydrogenase*	Normal	++	++	trace
	Castrate	+++	+++	++
	» + 5 α-DHT	+	+	trace
11 β-*hydroxysteroid dehydrogenase*	Normal	++	++	trace
	Castrate	++	+++	++
	» + 5 α-DHT	trace	trace	trace
NADH-*diaphorase*	Normal	+++	+++	+++
	Castrate	+++++	+++	+++++
	» + 5 α-DHT	+++	+	++
NADPH-*diaphorase*	Normal	+	+++	+
	Castrate	++++	++++	+++++
	» + 5 α-DHT	++	++	++
Glucose-6-phosphate dehydrogenase	Normal	++++	++++	+++
	Castrate	++	++++	+++
	» + 5 α-DHT	++++	++++	+
β-*glucuronidase*	Normal	++	++	+++
	Castrate	++	++	+
	» + 5 α-DHT	++	++	+++

Key: +++++ = very intense activity; ++++ = intense activity; ++ — +++ = moderate activity; + = weak activity; — = no activity.

even if partially, the existing interrogatives. It was for this reason that several representative enzymes having a role in tissue catabolism and anabolism were studied. These enzymes could be classified in the following functional categories: *acid phosphatase* and β-*glucuronidase* (predominantly of lysosomal localization) form a part of the intracellular digestive system; *alkaline phosphatase* is considered responsible for the breakdown of phosphate esters and is correlated with amino acid pool movements and transport of phosphate via the Golgi apparatus; *glucose-6-phosphatase* localized predominantly in the microsomes, is a specific enzyme capable of dephosphorylating glucose–6-phospate and thus is determinant of glucose supply; *glutamate dehydrogenase* is a key enzyme in the mitochondrial amino acid metabolism; *glucose-6-phosphate dehydrogenase* is a soluble NADP-linked enzyme and belongs to the glucose-6-phosphate oxidation system (pentose cycle); *lactate dehydrogenase* is a part of the glycolytic system; NADH and NADPH-diaphorase are flavoprotein enzymes; *non-specific esterases* are hydrolytic enzymes; β-*hydroxysteroid dehydrogenases* (mainly Δ^5-3 β-system) are concerned with steroid biosynthesis.

Correlations between results obtained by histochemical and biochemical methods may be very indicative. In fact concerning the activity of glucose-6-phosphate dehydrogenase in the various zones of mouse epididymis the present histochemical data confirm those reported earlier (biochemical analysis) by Rastogi (1979).

Although all twelve enzymes were demonstrated throughout the length of the epididymal duct the intensity of the enzyme reaction was found to differ in different regions of the epididymis.

Similarly the effects of castration and androgen-replacement therapy were also found to differ in different regions (cf. Brooks, 1976 b; Rastogi, 1979). The regional differences in the response of different enzyme systems of these treatments may thus be indicative in assuming that different epididymal segments may either have differing androgen thresholds or that various processes within a given segment of the epididymis can be influenced to differing degrees depending on the nature of the androgen (see also Vreeburg, 1975; Brooks, 1976 b; Ganjam and Amann, 1976; Jones, 1977; Rastogi, 1979). Furthermore the absence of spermatozoa (due to castration) may also be responsible for a particular response of an enzyme in a given epididymal segment.

Since castration and subsequent replacement therapy are able to alter the intensity of histochemical reaction of all enzymes studied it seems realistic to infer that the various processes within the epididymis are influenced by androgens.

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