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Electrophoretic profile of epididymal proteins in the rat under normal and experimental conditions

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Fisiologia. — *Electrophoretic profile of epididymal proteins in the rat under normal and experimental conditions* (*). Nota di RAKESH K. RASTOGI, FRANCA CIOFFI, CARLO BASILE e MARIA ARENA, presentata (**) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — Gli AA. hanno studiato il comportamento elettroforetico delle proteine solubili dell'epididimo di ratto in diverse condizioni sperimentali, quali la castrazione, la terapia androgenica e il trattamento antiandrogenico. È stata dimostrata la presenza di tre bande specifiche nella testa e quattro nella coda dell'epididimo, tutte androgeno-dipendenti.

INTRODUCTION

The mammalian epididymis is now ascertained to be something more than a simple tubular passage for sperm. These infact undergo a process named maturation. In the epididymis they are exposed to a complex chemical environment containing elements like carnitine, glycerylphosphorylcholine, glutamic acid, sialic acids, steroids, inorganic ions and a wide array of enzymes. We know a great deal of this sex accessory but our understanding of its physiology is limited (see Hamilton, 1975; Orgebin-Crist *et al.*, 1975; Rastogi, 1979). Recent reports also indicate that sperm coating by glycoproteins is an important step in epididymal sperm maturation (Johnson, 1975).

Protein synthesis and secretion in the epididymis are now known to be influenced by androgens in several species (Prasad *et al.*, 1974; Blaquier, 1975; Kanka and Kopecny, 1977; Rastogi *et al.*, 1979). Regarding the epididymal protein fractions several workers have shown that some proteins present in this duct fluid are not detectable in the rete testis fluid and blood serum and are thus considered to be of epididymal origin (Koskimies and Kormano, 1975; Amann *et al.*, 1973). At the present, however, it is not clear whether the protein fractions of epididymal origin are influenced by the androgen status of the animal. One approach to the problem is an electrophoretic analysis of the epididymal proteins under different experimental conditions. In the present work in fact an electrophoretic analysis of epididymal proteins was done in castrated, androgen-treated and antiandrogenized rats.

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(**) Nella seduta dell'8 novembre 1980.

Material and methods

Sexually mature (Charles River) rats were used. Each batch of 5 animals was treated as follows:

a) intact animals;

b) castration (for 7 days);

c) castration for 7 days + 50 μg DHT/day for 10 days;

d) intact animals treated daily for 10 days with 1 mg cyproterone acetate (CA).

At the end of each experiment animals were killed by ether and tissue samples were separated. Testes and caput and cauda segments of the epididymis were homogenized in cold deionized water. A small sample of each homogenate was used for the determination of total proteins (Lowry *et al.*, 1951). The rest of the homogenate was centrifuged at 15 000 g for 30 min. Blood serum was prepared by centrifuging (2 500 g for 15 min) clotted blood. For electrophoretic analysis we used a 7 % polyacrylamide gel with a tris-glycine buffer (pH 8.6) with bromphenol blue as the tracking dye. Generally, a fixed volume of supernatant and blood serum containing approximately 100 μg proteins was applied to a gel. Following separation soluble protein components were stained with amido Schwarz 10 R followed

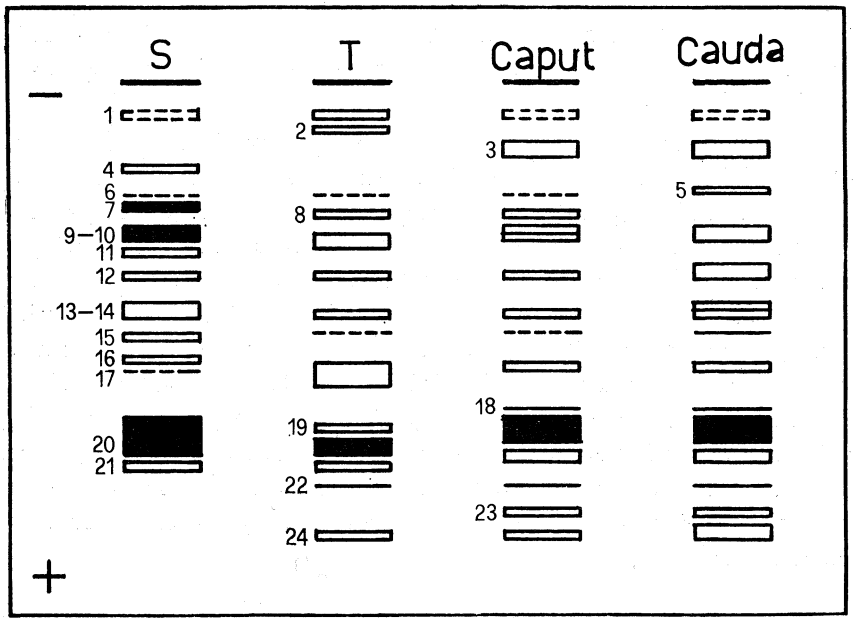

Fig. 1. – Polyacrylamide gel electrophoretograms showing amido Schwarz 10 R-stained protein bands in the serum (S), testis (T), caput epididymis and cauda epididymis of the normal rat.

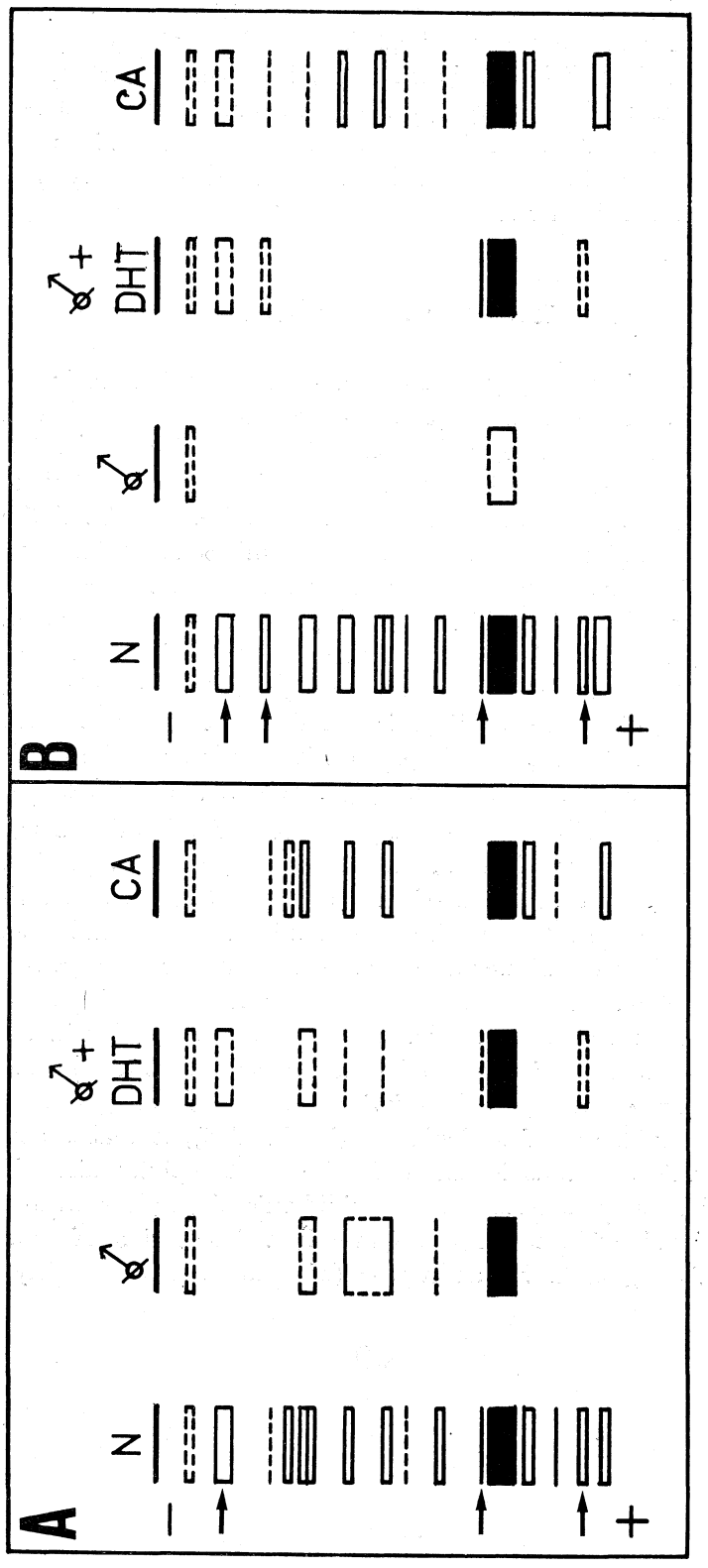

Fig. 2. – Electrophoretograms showing changes in protein pattern of the caput (2 A) and cauda (2 B) epididymes following castration, castration + DHT therapy and cyproterone acetate (CA) treatment. Arrows indicate epididymis specific, androgen-dependent protein bands.

by destaining with 7 % acetic acid in GD4 Pharmacy destainer. The location of each protein band was determined in 2–3 duplicate gels per sample. There was no obvious difference between duplicate gels of each and between gels of all animals of the same group. The data were therefore pooled. Estimates of the relative mobility and relative concentrations of amido Schwarz-stained proteins were based on densitometric analysis using a Cellomatic CGA densitometer.

Results and discussion

A diagrammatic summary of the protein patterns is seen in Figures 1 and 2. Since we used total homogenates for all tissues, blood contamination was obvious. The specific albumin band (band 20) found in all the four tissues examined had a similar relative mobility and a similar colour density. In the testis, however, there was an additional band (band 19) with the same relative mobility. One prealbumin band (band 21) and a few postalbumin bands present in the blood serum were also found in the testis and caput and cauda epididymes. Bands 8, 22 and 24, present in the testis and absent in the blood serum, were found also in the two segments of the epididymis. On the contrary, band 2, typical of the testis, was absent from the epididymis. The epididymis, on the other hand, showed bands (bands 3, 18 and 23) characteristic of both its segments. The cauda epididymis had one additional band (band 5), absent in the caput segment.

Castrated males showed the disappearance of several bands, except for those most probably of serum origin. Androgen therapy of castrated animals induced the appearance of bands 3, 5, 18 and 23, showing that these proteins are secreted in the epididymis and are under androgen control. In fact they are affected also in animals treated with an antiandrogen, cyproterone acetate. The results thus appear to be consistent with the earlier data on the rat and ram that there are some proteins in the epididymis evidently secreted in the testis and transported into the caput epididymis (Amann *et al.*, 1973; Koskimies and Kormano, 1975). Part of these proteins, evidently of testicular origin (band 8), is reabsorbed in the caput epididymis and thus is absent in the cauda epididymis. Similarly new protein bands are secreted in the epididymis, dependent on androgens. This is additional evidence for the synthesis and secretion of proteins by the epididymis regulated by androgens (cf. Kaňka and Kopečný, 1977).

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