A SEX-ASSOCIATED PROTEIN IN LIVER TISSUES OF THE MALE RAT

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Antigenic differences between the tissues of male and female inbred rats and mice prevent skin homotransplants when the donor is male and the recipient female. However, the reverse procedure is typically successful. In both the rat and the mouse, the reaction has been attributed to histocompatibility genes on the Y chromosome (Billingham and Koprowski, 1959; Billingham and Silvers, 1958, 1959; Eichwald et al., 1957). That protein differences should exist between male and female rats and mice may be inferred from the skin transplantation studies. This report demonstrates the presence of a protein component present in the liver of the male rat and absent from the female.

EXPERIMENTAL

Male and female Sprague-Dawley rats ranging in ages from 1 1/2 to 5 months were used in this study. The rats were stunned and decapitated. The livers were perfused, homogenized and the 105,000 X g supernatants prepared according to the methods of Anderson (1956).

Before chromatography the supernatants were routinely dialyzed 4 hours in the cold against 0.01 M Tris chloride buffer at pH 8.0. For comparison, supernatants were also dialyzed on Sephadex G-50† columns. This was done to reduce the possibility that slowly dialyzable materials were contributing to the observed differences between the sexes.

*Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

† Pharmacia, Uppsala, Sweden

DEAE‡ in the hydroxide form was packed into 2 X 25 cm columns under 15 lb sq in. air pressure. The columns were equilibrated with 0.01 Tris chloride buffer at pH 8.0 before they were loaded with the dialyzed supernatant. Proteins were then eluted with the same Tris chloride buffer at a flow rate of 1.5 ml/minute.

The sex-associated protein component was collected and concentrated by either ultrafiltration or lyophilization before further study.

RESULTS

Fig. 1 depicts typical 280 mμ chromatograms obtained from liver supernatants of male and female rats. The characteristic male chromatogram was confirmed twenty-four

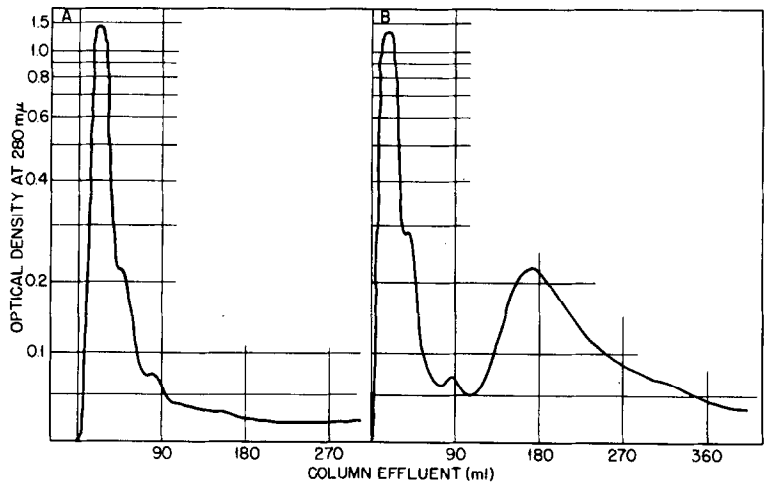

Figure 1. DEAE chromatograms of male and female rat liver soluble proteins eluted with 0.01 M Tris chloride buffer, pH 8.0. A—Female proteins eluted from a column loaded with 193 mg of protein; B—male proteins eluted from a column loaded with 247 mg of protein; note the presence of the additional peak missing from the female.

times with separate liver supernatants during the course of various investigations. The female chromatogram similarly was repeated four times for comparison. The entire female complex shown in Fig. 1 represents 16% of the total 280 mμ extinction of the material

‡ Eastman Kodak Co., Rochester, N. Y.

placed onto the columns. The entire male complex correspondingly represents 28% by 280 mμ extinction and 24% by nitrogen determinations. The peak missing in the female but present in the male (called the "male component" for brevity) represents 8% of the total material as judged by 280 mμ extinction and has a 280:260 ratio of 1.78 at the peak. The $s_{20,w}$ is 3.13 and the material appears homogeneous during ultracentrifugation. The mobility of the material at pH 7.5 is -1.6×10^{-5} cm^2/volt/sec. Electrophoretically, there is evidence of a minor slower moving contaminant in the material being studied. Further attempts to characterize the "male component" will be deferred until it has been properly purified.

DISCUSSION

A sex-associated protein component is present in the male but absent from the female rat liver. Some preliminary information concerning the physical properties of the material are reported, but no attempt has been made to assess the physiological importance of the "male component" or to look for it in other tissues at this time. It cannot be ignored, however, that histoincompatibility exists between male and female rats. The possibility that the "male component" shown here may be involved in the histoincompatibility is presently under investigation.

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