J. Hyg. Camb., 1958, v56, 466.
17. Warren, J., Ziherl, F. A., Kish, A. W., Ziherl, L. A., J. Am. Med. Assn., 1955, v157, 633.
Received July 16, 1964. P.S.E.B.M., 1965, v118.

Sex Dependent Protein Component of Rat Liver.* (29754)

DAVID BARZILAI† AND GREGORY PINCUS
Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Bond(1) has reported the presence of a sex-associated protein component in extracts from livers of male rats and absent in the extracts from livers of females. The response of this liver-protein fraction to castration and treatment of animals with estradiol or testosterone has also been described(2). Initial analytical data indicate that the sex-associated protein fraction contains a major single component and 2 minor components(2). This report confirms the initial observations of Bond with respect to the difference observed in male and female liver extracts and the influence of estradiol or testosterone treatment. The results agree well though different levels and times of hormone treatment were used. We have also studied the effect of progesterone treatment on the level of this sex-associated protein fraction in liver extracts.

Methods. Male and female Sprague-Dawley rats were received at the age of 14 days (average weight, 20 g). Immature animals used were 14 days old. Gonadectomy was done on the same day. Hormones were administered by intramuscular injection into the respective groups of animals twice a week for 13 weeks. Amount of hormone per injection was 2.5 mg testosterone propionate, 0.25 mg 17-Beta estradiol and 5 mg progesterone. All animals were sacrificed by decapitation 108 days after the initial injection. Livers were perfused with Locke's solution, homogenized, and the 105,000 $\times$ *g* supernatants prepared, according to the methods of Anderson (3). Chromatography of the supernatants was performed as described by Bond with DEAE cellulose (Carl Schleicher & Schuell Co., Type 20), equilibrated with 0.01 M Tris-chloride buffer, pH 8. Proteins were eluted with the buffer in 3 ml fractions at 1.5 ml/minute. Optical densities of the fractions were read at 280 mμ in a Beckman DU spectrophotometer.

Results. The typical chromatogram for a liver supernatant from a normal adult male is shown in Fig. 1. The sex-associated peak appears between 140-230 ml of effluent. As shown in Fig. 1, the supernatant from livers of immature males does not show this peak. The chromatograms obtained with liver supernatant from adult males 93 days after castration show a demonstrable but greatly reduced amount of protein in this area (Fig. 1). Bond has also shown that after castration the concentration of the protein was reduced to one-half of normal values(2).

Bond has shown that results from Kjeldahl nitrogen determinations compare favorably with the spectrophotometric measurements of the amount of material in the area of the sex-associated protein and that these areas can be used to assess the amount of protein present(2). Our data confirm that the concentration of the protein in the 105,000 $\times$ *g* supernatant from adult male liver is approximately 3%

Fig. 2 contrasts the chromatograms obtained from livers of normal adult males and females, and shows the absence of any protein peak in the corresponding area in the pattern obtained from livers from female rats.

When intact or castrated females are injected with testosterone propionate, the protein peak appears in the chromatograms (Fig. 3). After a single injection of 10 mg testosterone propionate, Bond reported a

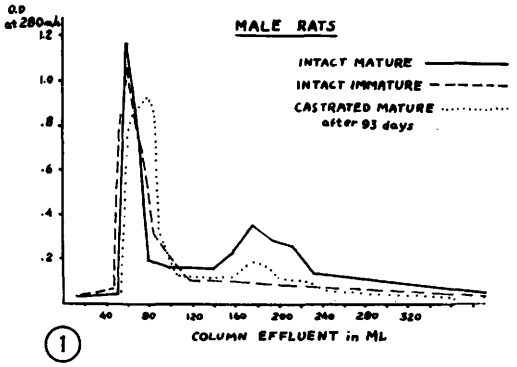
* Supported by a grant made to the Training Program in the Physiology of Reproduction by the Population Council and by G. D. Searle & Co.

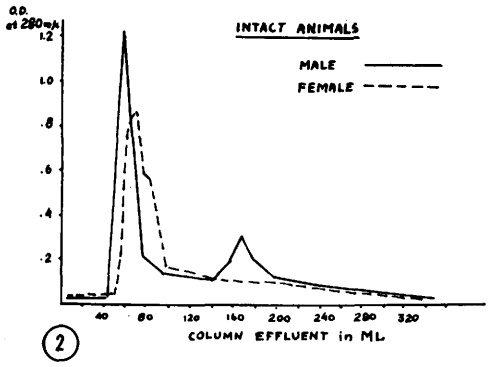
† Present address: Rambam Government Hospital, Haifa, Israel.

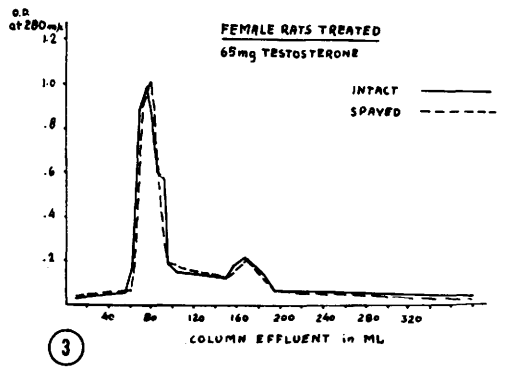
gradual increase in the protein component up to 33 days; it did not reach the level found in adult male extracts(2). In our studies, the amount of protein in the area is not as great as that found with male liver supernatants even after a total of 65 mg testosterone over a period of 13 weeks.

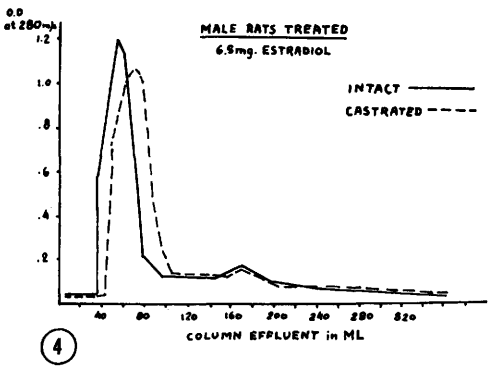
The chromatograms shown in Fig. 4 show that the amount of the sex-associated fraction is greatly decreased after treatment of intact or castrated males with 0.25 mg estradiol twice weekly for 13 weeks. At 7 to 11 days after a single injection of 2 mg estradiol propionate, a comparable decrease to approximately one-third of original values was observed by Bond(2).

Treatment of intact females with progesterone also results in appearance of a peak in the chromatograms. When castrated males are injected with progesterone, the amount of the sex-associated fraction is increased over that found for untreated castrate males.









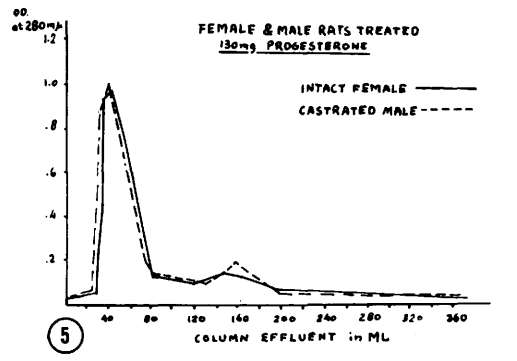


FIG. 1. Chromatography of rat liver supernatants on DEAE with 0.01 M Tris buffer, pH 8.0.

FIG. 2. Chromatography of adult male and female rat liver supernatants on DEAE with 0.01 M Tris buffer, pH 8.0.

FIG. 3. Chromatography of liver supernatants from female rats treated with testosterone (65 mg total, 2.5 mg per injection).

FIG. 4. Chromatography of liver supernatants from male rats treated with estradiol (6.5 mg total, 0.25 mg per injection).

FIG. 5. Chromatography of liver supernatants from rats treated with progesterone (130 mg total. 5 mg per injection).

These chromatograms are shown in Fig. 5.

Discussion. The results described confirm the report of Bond(1,2) on the presence of a sex-associated protein component in liver extracts. The presence and absence of the protein fraction in response to varied hormone treatment of rats would indicate that it is a sex-hormone-dependent protein component.

The physiological significance of this liver component is not known. Reports indicate quantitative sex differences in liver-enzyme activities such as arginase, phenylalanine hydroxylase, and glucose-6-phosphatase(4,5,6, 7). Quantitative and qualitative differences due to sex have also been described for liver enzymes involved in metabolism of steroid hormones(8,9,10,11). The sex-associated protein was not found in extracts of male rat kidney, brain, or testis(2). It would be of interest if sex-hormone-dependent components exist in circulation in the blood. Attempts to isolate sex-associated proteins and to regulate the concentration of the protein components may be of aid in understanding the known problem of histoincompatibility between male and female rats(12,13,14,15).

Summary. The presence of a sex-associated protein component in extracts of livers from male rats has been confirmed. The protein fraction is not present in extracts of livers from females but appears on treatment of the female with testosterone or progesterone. The protein component is not present in livers of immature males and is decreased after treatment of males with estradiol.

We wish to express sincere thanks to Dr. Ailene Herranen for helpful guidance and discussion in this work.

1. Bond, H. E., Biochem. Biophys. Research Comm., 1960, v3, 53.
2. ———, Nature, 1962, v196, 242.
3. Anderson, N. G., Exp. Cell Research, 1956, v11, 186.
4. Campbell, R. M., Kosterlitz, H. W., J. Endocrinol., 1949, v6, 171.
5. Glock, G. E., McLean, P. S., Biochem. J., 1954, v56, 171.
6. Knox, W. E., Averbach, V. H., Lin. E. C. C., Physiol. Rev., 1956, v36, 164.
7. Freedland, R. A., Krakowski, M. C., Waisman, H. A., Am. J. Physiol., 1962, v202, 145.
8. Hubener, H. J., Amelung, G., Z. Physiol. Chem., 1953, v293, 137.
9. Forchielli, E., Brom-Grant, K., Dorfman, R. I., Proc. Soc. Exp. Biol. and Med., 1958, v99, 594.
10. Yates, F. E., Herbst, A. L., Urquhart, J., Endocrinology, 1958, v63, 887.
11. Rubin, B. L., Strecker, H. J., ibid., 1961, v69, 257.
12. Billingham, R. E., Koprowski, H., Nature, 1959, v184, BA6.
13. Billingham, R. E., Silvers, W. K., Science, 1958, v128, 780.
14. ———, J. Immunol., 1959, v83, 667.
15. Eichwald, E. J., Silmer, C. R., Wheeler, N., Ann. N. Y. Acad. Sci., 1957, v64, 737.

Received September 1, 1964. P.S.E.B.M., 1965, v118.

Effect of a Diet Deficient in Lipotropic Factors on the Lipoproteins of Rat Serum.* (29755)

J. C. FORBES, O. M. PETTERSON AND R. A. RUDOLPH
Department of Biochemistry, Medical College of Virginia, Richmond

Anionic polymeric polysaccharides have been extensively used as reagents for the precipitation of low density lipoproteins(1). Sakagami and Zilversmit(2) showed that under appropriate conditions dextran sulfate and calcium chloride precipitated the lipoproteins of densities less than 1.063 from hypercholesterolemic rabbit serum. Since these low-density lipoproteins are generally considered to be the chief vehicle for transporting triglycerides from the liver, it was decided to investigate the changes in lipid content of this fraction when animals were put on a choline-deficient diet. This report deals primarily with the changes in serum cholesterol and triglyceride content of this low-density

* Supported in part by NIH grant AM-07696-01.