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Responsible Investigator: Clara Szego Roberts

University Department: Zoology

Financial Support 1954-55: UC $ 7,300.00.

Title of Project: The Metabolism of Steroid Hormones in Normal and Cancer-Bearing Animals.

The role of a liver enzyme system in promoting the binding of estrone to the proteins of the homologous serum incubation medium and to the hepatic proteins was first described from our laboratories (Szego, C. M. Endocrinology 52:669, 1953). A portion of these studies has now been confirmed and extended by independent observations at the University of Wisconsin (Riegel, I. and Mueller, G. C. J. Biol. Chem. 210:249, 1954). The significance of the overall process, not only as a normal metabolic pathway for the estrogenic hormones but as a possible intermediate in their interaction with physiological target tissues, has been emphasized in previous reports and publications from our laboratories. These findings gain emphasis from our more recent observations on the progressive loss of the enzyme with the development of liver neoplasms (1,2). The nature of the protein-binding defect in the pre-cancerous and tumor tissues appears, on the basis of preliminary evidence, to be failure of glucuronide conjugation which we had earlier demonstrated to be an intermediate step to

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association of the steroid hormone with protein. Moreover, the tumor tissues in vitro exhibited diminished capacity to metabolize the added hormone by alternate pathways as well. Two complete manuscripts are being prepared for publication. These observations appear to constitute an advance toward the biochemical characterization of neoplastic liver, and aid in the elucidation of normal metabolic pathways for the estrogenic hormones.

Related problems which have been studied for the past several years have now been completed (3,4,5). A portion of the above findings will be discussed in relation to the broad field of steroid biochemistry in an invited review (6).

Publications:

(1) Szego, C. M., and Wolcott, D. C. The Failure of Hepatoma to Promote Estrogen-Protein Binding in vitro. Fed. Proc., 13(1):149, 1954.

(2) Szego, C. M., and Wolcott, D. C. Aberrations of Estrone Metabolism in Rat Hepatoma. J. Clin. Endocrinol. & Metab. 14:796, 1954.

*(3) Beyler, A. L., and Szego, C. M. Correlation of Ovarian Cholesterol Levels with Changes in Beta-glucuronidase Activity of the Reproductive Tract of the Rat During the Estrous Cycle and Pregnancy. Endocrinology 54:323, 1954.

*(4) Beyler, A. L., and Szego, C. M. Steroid Hormone

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Interactions as Evidenced by Modification of the Beta-glucuronidase Activity of the Preputial Glands of the Rat. Endocrinology 54:334, 1954.

(5) Levey, H. A., and Szego, C. M. The Effects of Castration and Androgen Administration on Growth and Secretory Activity of the Male Sex Accessories. Endocrinology (in press).

(6) Roberts, S. and Szego, C. M. Biochemistry of the Steroid Hormones. Annual Reviews of Biochemistry 24, 1955 (in press).

*Cited "in press" in last year's report. Full reference now given.

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Responsible Investigator: Clara Szego Roberts

University Department: Zoology

Financial Support 1954-55: USPHS $15,000.00.

Title of Project: Studies on the Formation of Estrogen-Protein Complexes and Their Physiological Significance.

Considerable progress has been made in the course of the past year in establishing the nature of the protein-bound estrogen formed *in vitro* in the presence of liver preparations. Previous work from our laboratories which strongly suggested a glucuronide conjugate preliminary to association with the serum proteins has been substantiated by two independent enzymic approaches (1,2,3). Available data reveal that the linkage to the liver proteins which proceeds simultaneously *in vitro* occurs via another mechanism. Kinetic studies have demonstrated highly active systems. Substrate specificity is demonstrated by considerably greater reactivity of estrone compared to estradiol.

That the association of the hormonal moiety with the serum proteins in the presence of surviving liver tissue *in vitro* is not fortuitous or the result of artifact formation through chemical manipulations, has been successfully demonstrated by the application of paper electrophoretic analysis to incubation mixtures in which

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radioactive estrone was used as substrate (4). This represents a new departure since this analytical tool has not heretofore been utilized for the steroid hormones. Its potential usefulness in microanalytical separations appears to be great. These results will be reported at the April meetings of the Federated Societies for Experimental Biology (1,4) and two papers are in preparation. The data will be cited in a forthcoming review (5).

Publications:

Szego, C. M. The Nature of Protein-Bound Estrogen Formed *in vitro*. Fed. Proc. 14:(1), 1955 (in press).

Segal, H. L., and Szego, C. M. Studies on the Phenol Conjugating System of Rat Liver. Fed. Proc. 13:(1)132, 1954.

Segal, H. L. The Phenol Conjugating System of Rat Liver. J. Biol. Chem. (in press).

Szego, C. M., and Roberts, S. Paper Electrophoretic Studies of Protein-Bound Estrogen Formed *in vitro*. Fed. Proc. 14:(1) 1955, (in press).

Roberts, S. and Szego, C. M. The Biochemistry of the Steroid Hormones. Ann. Rev. Biochem. 24: 1955, (in press).

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