Inverse Changes of Serum Glucuronidase and Esterase of Breast Cancer Patients on Estrogen Therapy.* (18472)

SAUL L. COHEN AND ROBERT A. HUSEBY.

From the Department of Physiological Chemistry, the M. H. Hoffman Endocrinological Research Laboratory, and the Division of Cancer Biology of the Department of Physiology, University of Minnesota, Medical School.

It was pointed out(1) that during estrogen therapy of patients with advanced breast cancer the levels of serum β-glucuronidase were markedly elevated. Harris and Cohen (2) in studies on the effects of ovarian hormones on tissue enzyme activities in mice observed that in most instances there appeared to be an inverse relationship in the tissue glucuronidase and esterase activity responses. Thus, for example, while the uterine glucuronidase level was reduced to about one-half by ovariectomy, the esterase activity was almost doubled; both changes were reversed by the administration of estrogen. It was thought possible that a similar relationship of glucuronidase and esterase changes in the blood of patients with breast cancer and on sex hormone therapy might occur. The glucuronidase and esterase activities were therefore simultaneously determined for the sera of patients during various stages of sex hormone therapy. The data obtained definitely indicate an inverse relationship between the glucuronidase and esterase changes in these patients.

Methods. The selection and treatment of the patients followed the same criteria as that previously reported(1). Serum glucuronidase was determined by the method of Fishman *et al.*(3), using the modified order of reagent additions(4). Serum esterase was determined titrimetrically using a procedure modified from the histochemical procedure of Glick(5). The sera were first diluted 3:10 with distilled water and all reagents were used in amounts about 150 times as great as those proposed by Glick in order that the test be applicable on a macro scale. One enzyme unit is defined as that amount of enzyme which liberates 1 γ of phenolthalein or butyric acid per hour incubation from their corresponding substrates by the glucuronidase and esterase respectively.

Results. A total of 37 healthy control subjects,† 11 patients with advanced breast cancer and not on hormone therapy, and 12 patients with advanced breast cancer on estrogen therapy‡ (3 patients were being treated with ethinyl estradiol and 9 with diethyl stilbestrol) were studied in this series of experiments.

Fig. 1 shows values obtained for serum glucuronidase and the corresponding esterase activity levels obtained for (a) a group of healthy male subjects (ages 48-61, average 53), (b) a group of healthy female subjects (ages 47-69, average 53), (c) a group of patients (ages 45-71, average 62) with advanced breast cancer who were not on any hormone therapy, (d) patients (ages 52-71, average 62) on estrogen therapy whose glucuronidase levels were less than 1500 units %, and for (e) patients (ages 42-73, average 62) on estrogen therapy who showed glucuronidase levels greater than 1500 units %

* This work was aided by grants from the American Cancer Society, through an Institutional grant made to the University of Minnesota, and the University of Minnesota Graduate School.

1. Cohen, S. L., and Huseby, R. A., *Cancer Research,* 1950, v11, 52.

2. Harris, R. S., and Cohen, S. L., *Endocrinology,* in press.

3. Fishman, W. H., Springer, B., and Brunetti, R., *J. Biol. Chem.,* 1948, v173, 449.

4. Fishman, W. H., Kasdon, S. C., and Homburger, F., *J. Am. Med. Assn.,* 1950, v143, 350.

5. Glick, D., *Z. Physiol. Chem.,* 1934, v223, 252.

† Sera was obtained from these control subjects through the Cancer Detection Center of the University of Minnesota Hospitals.

‡ The hormones used in these studies were supplied through the Committee on Research of the Council on Pharmacy and Chemistry of the American Medical Association by the Abbott Research Laboratories, Schering Corporation, and Winthrop-Stearns.

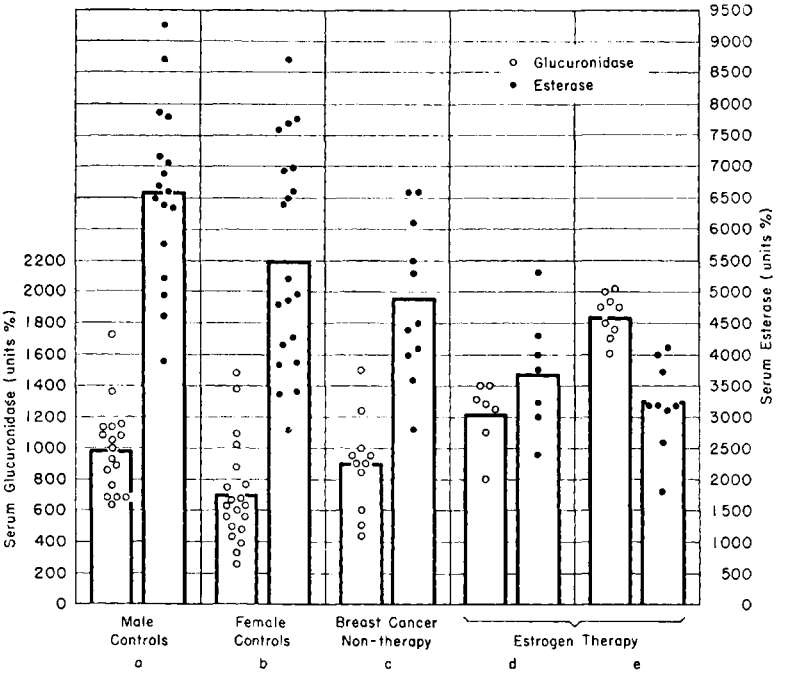

FIG. 1.
Glucuronidase and esterase values in normal and breast cancer patients. For description see text.

(several patients contributed values used in more than one category of (c), (d) and (e)—*e.g.* patient A.M., Fig. 2b). Where several assays were available for a single patient in any one of these categories, these were averaged and each point in the various categories thus represents a different subject.

A comparison of the post-age 50 healthy male and female subjects shows: 1. A significantly higher average serum glucuronidase in males (990 ± 40)§ than in females (700 ± 65). This observation is in agreement with the findings reported earlier(1).

2. The serum esterase levels in the males (6600 ± 300) is likewise significantly higher than in the females (5500 ± 400) of this group.

The group of non-treated breast cancer patients showed a just significant increased serum glucuronidase activity level (900 ± 95) as compared to that of the healthy women. The non-treated cancerous patients showed on the other hand a lower (4800 ± 600) serum esterase than the healthy controls. The patients on estrogen therapy showed correspondingly lower esterase values (3700 ± 400) corresponding to glucuronidase values below 1500 units % (average 1220 ± 160) and still lower (3260 ± 250) for those glucuronidase values above 1500 (average 1840 ± 75).

The inverse relationship between serum glucuronidase and esterase levels is even more clearly demonstrated by following the changing enzyme activity concentrations in individual patients during and following endocrine therapy. In Fig. 2 are shown the enzyme activity concentrations for (a) a patient (E. F.) on diethyl stilbestrol therapy (15 mg/day); (b) a patient (A.M.) on ethyinl estradiol therapy (3 mg/day); (c) a patient (E.B.) from whom stilbestrol was withdrawn after 550 days of treatment; and for (d) a patient (E.H.) from whom estradiol was withdrawn after 275 days of treatment. It will be seen that in all cases there tends to be an inverse relationship between changing

§ The $\pm$ refers to the standard error.

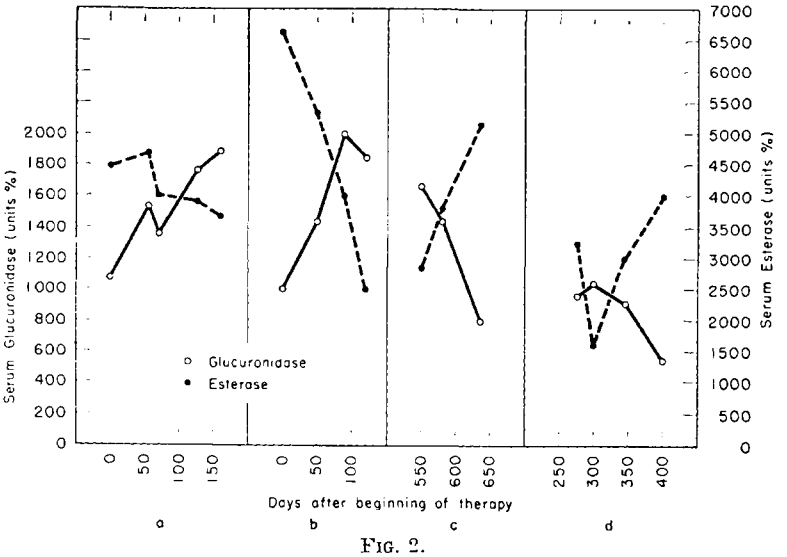

FIG. 2.

Glucuronidase and esterase changes during and following estrogen therapy to breast cancer patients. For description see text.

serum glucuronidase and serum esterase. Similar observations have been made on all the other patients studied.

Discussion. The data presented in this paper clearly illustrate that with a rising serum β-glucuronidase there is a reduced serum esterase and that with a falling glucuronidase there is an increased serum esterase. That these phenomena are not limited to cancer patients being treated with estrogens is attested to by the following observations: 1. Similar changes were frequently observed for various tissues in mice subjected to changes in the amount of circulating ovarian hormones(2). 2. Preliminary observations on mammary tumors in mice(6) indicate reduced esterase levels accompanying the increased glucuronidase activities levels in the tumorous tissue. 3. In a few scattered observations(7) where definite changes in serum glucuronidase levels have been found in subjects for whom several enzyme determinations have been made on different occasions, inverse changes in serum esterase levels have been observed.

More frequent determinations will have to be made in order to determine whether the changes in glucuronidase and esterase levels occur simultaneously or whether one change precedes the other. The data so far obtained, while far from conclusive, also tend to indicate that the relative degree of esterase and glucuronidase changes varies for different patients, and even in the same patient does not appear to be constant from time to time.

In view of the almost complete lack of evidence or hypotheses with regard to the function of esterase in the body and the suggested, though far from proven, hypotheses for glucuronidase function(2,8,9), it is felt that no interpretation can be made for the data presented in this paper. Two possibilities which have suggested themselves is that the esterase serves as a precursor for the more specific enzyme glucuronidase, or that some tissue or tissues involved in the production of esterase may under certain circumstances preferentially produce the more specific enzymes. However, until some evidence is available with regard to these suggestions they must be considered as interesting though highly speculative hypotheses.

Summary. Simultaneous sera glucuronidase and esterase determinations have been carried

6. Cohen, S. L., and Bittner, J., unpublished.
7. Cohen, S. L., unpublished.
8. Fishman, W. H., *J. Biol. Chem.*, 1947, v169, 7.
9. Kerr, L. M. H., Levvy, G. A., and Campbell, J. G., *Nature*, 1947, v160, 572.

out on the blood samples of 37 post-age 50 healthy control subjects (17 males and 20 females), 11 patients with advanced breast cancer and not on hormone therapy, and on 12 patients with advanced breast cancer receiving estrogen therapy. It was found that the control male subjects had both a higher serum glucuronidase and serum esterase than did the control female subjects. An inverse relationship was found for the changes in sera activity levels of glucuronidase and esterase in the patients with breast cancer subjected to variations in estrogen therapy.

We wish to thank Ruth Slaton Harris and Shirley Chapman Tennyson for their technical assistance in carrying out the assays reported in this paper.

Received January 3, 1951. P.S.E.B.M., 1951, v76.

Synnematin, an Antibiotic Produced by *Tilachlidium*. (18473)

R. Y. GOTTSHALL, J. M. ROBERTS, LUCILE M. PORTWOOD AND J. C. JENNINGS
(Introduced by C. W. Muehlberger)

From the Division of Laboratories, Michigan Department of Health, Lansing, Mich.

In a search for microorganisms capable of producing antibiotic substances, a mold belonging to the genus *Tilachlidium* Preuss which inhibits bacterial growth was isolated. In liquid medium the mold forms a substance which is more active against certain species of *Salmonella* than against *Escherichia coli* and which has low toxicity for mice. On the basis of the characteristic hyphal fascicles (Synnemata) of *Tilachlidium*, the name "synnematin" is proposed for the antibiotic.

Materials and methods. (a) *Method of assay.* One synnematin unit is defined as the minimum quantity/ml which completely inhibits the growth of *S. typhimurium* for 24 hours. Activity is assayed by the broth dilution test with *S. typhimurium* (Dr. P. R. Edwards, strain 9) as the test organism. Samples for assay are sterilized by Seitz filtration. F. D. A. broth* at pH 7.0 is inoculated with 1% of an 18 to 24 hour culture and 2-fold dilutions of the antibiotic are made with 2.0 ml volumes of the seeded broth. (b) *Production.* The synnematin used in these studies was produced by growing *Tilachlidium* sp. strain MDH 3590A, on a medium composed of Bacto Casamino Acids, 10.0 g; glucose, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; and $FeSO_4 \cdot 7H_2O$, 0.1 g/liter of tap water. The medium was adjusted to pH 6.7-6.9, dispensed in 2-quart milk bottles in 300 ml volumes and autoclaved at 121°C for 30 minutes. Each bottle was seeded with 6 ml of a 4-day-old culture in Czapek-Dox medium and incubated on the flat side at 24°C. Peak activity, 8 to 32 units, was obtained in 6 to 9 days. At this time the pH was 7.9 to 8.3. (c) *Purification.* The culture liquid was adjusted to pH 6 with H_3PO_4, mixed with 0.25% Nuchar C-190-N and filtered with the aid of 0.5% Standard Super-Cel. The antibiotic was then adsorbed from the filtrate on 1% Darco G-60. Synnematin was eluted from the Darco with 0.1 volume of 75% acetone, concentrated in vacuum at a temperature under 30°C and the residue was clarified with 2% Nuchar. The resulting filtrate was shell-frozen and dried. Crystalline synnematin has not been isolated; the most pure material thus far obtained contains 32 units/mg.

Characteristics. (a) *Stability.* Synnematin

TABLE I. Effect of pH on Activity of Synnematin.

	Units/ml for inhibition	
Broth, pH	*S. typhimurium*	*M. pyogenes*
5.8	1	2
7.0	1	4
7.9	2	8

* Unless otherwise stated, "broth" or F. D. A. broth refers to the nutrient broth portion of penicillin assay agar(1).

1. Federal Register, 1945, 10F.R., 11478.