on the age incidence of kernicterus. These subjects need more attention than they have been so far accorded.

Summary and conclusions. Bilirubin added to the ambient fluid in concentrations above 20 to 25 mg % depresses the respiration of chopped rat brain by approximately 25%. This inhibition can be counteracted by oxidation of the bilirubin and by the action of methylene blue on the bilirubin. Cytochrome C also reverses the inhibition. The mechanisms of these reversals appear to be different.

1. Schmorl, G., *Verhandl. d. deutsch. orthop. Gesellsch.*, 1903, v6, 109.

2. Allen, F. H., Jr., Diamond, L. K., and Vaughan, V. V., III, *Am. J. Dis. Child.*, 1950, v80, 779.

3. Crigler, J. F., and Najjar, V. A., *Pediatrics*, 1952, v10, 169.

4. Zuelzer, W. W., and Mudgett, R. T., *ibid.*, 1950, v6, 452.

5. Vogel, F. S., *J. Exp. Med.*, 1953, v98, 509.

6. Hsia, D. Y., Allen, F. H., Jr., Gellis, S. S., and Diamond, L. K., *New England J. Med.*, 1952, v247, 668.

7. Day, R., and Haines, M. S., to be published.

8. Najjar, V. A., Remarks in Proc. of M & R Conference on Erythroblastosis Fetalis, to be published.

9. King, L. S., Research Publications of the Assn. for Research in Nerv. Mental Dis., 1938, v18, Chap. 6.

10. Dereymaeker, A., *L'Ictère Nucléaire du Nouveau-né.* 1949, Editions Arsicia, Bruxelles.

11. Day, R., unpublished observations.

12. Potter, V. R., Schneider, B. S., and Liebl, G. L., *Cancer Research*, 1945, v5, 21.

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Urinary Estrogens and Related Compounds in Postmenopausal Women With Mammary Cancer: Effect of Cortisone Treatment. (20850)

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This paper presents data on the quantification of estrogens and related compounds in the urines of 15 postmenopausal women. Eight were normal and 7 had cancer of the breast. The effect of cortisone therapy upon urinary estrogens was investigated at various times in 4 of the 7 cancer patients. Observations were also made concerning the influence of advancing disease and of the surgical removal of X-rayed ovaries.

Methods. From the 8 normal women 72- to 96-hour specimens were analyzed while only 24- to 48-hour collections were available on the cancer patients. The accuracy of each collection was checked by creatinine determination and the volume corrected when necessary. One aliquot of each specimen was subjected to the usual HCl hydrolysis(1) and a second to Zn-HCl hydrolysis(2) prior to continuous benzene extraction(1) for bioassay. The total estrogenic activity released by these 2 procedures is designated T_o and T_{zn} respectively. The residual urines after benzene extraction of the HCl hydrolyzed aliquots were further studied by methods previously described(3) in order to determine how much of the difference between T_o and T_{zn} values might be accountable to more complete hydrolysis of conjugated estrogens. The results of these latter studies are not included in this report since in no instance could the difference observed between T_o and T_{zn} values be explained on this basis. It would appear to be safe to assume that the very high T_{zn} values found in many of the cancer urines represent unknown constituents, possibly estrogen oxidation products or precursors(3), related to the estrogens but exhibiting estrogenic activity only after Zn-HCl treatment. T_o values, on the other hand, represent estrogens excreted as such.

Bio-assay was performed by a standardized procedure(3) on spayed mature female rats. Unless more than 50 I.U. of T_o or more than

TABLE I. Estrogens in Urine of Women without Ovarian Function. Effect of mammary cancer and cortisone therapy.

Group	No. of cases	No. of urines	Urinary estrogens (I.U./24°) — HCl hyd. Range	HCl hyd. Avg*	Zn-HCl hyd. Range	Zn-HCl hyd. Avg*
Normal, postmenopausal	4	10	25- 50	37	50- 100	75
" , ovariectomized	4	10	12- 50	30	25- 100	75
Cancer, X-ray castrated						
Not receiving cortisone	5	6	<50-385	138	<100-1960	640
During cortisone therapy	4	6	<50- 80	13	<100- 575	158
Cancer, ovariectomized						
Not receiving cortisone	4	5	<50-125	25	<100-4700	1370
During cortisone therapy	2	3	<50	<50	<100	<100

* Because of the wide range of observed values in urines of cancer patients, the average figures presented in this table merely show the trends of excretion rates in these 4 groups. Individual value for each "cancer" urine is given in Table II. When the activity of a specimen was established at less than 50 I.U./24 hr volume, that value was considered as zero when calculating the avg. This, of course, lowers the avg figures, but does not detract from the significance of the trends demonstrated in this table, especially when one considers the range of values in each group and the very large differences observed between the groups being compared.

100 I.U. of T_{zn} are present per 24-hour volume of urine, the end-point criteria of this method cannot be met in analyses of less than 72-hour collections. Negative values at these levels can be established, however, and are so expressed in Tables I and II.

Results. In the urines of normal women whose ovaries have been removed or who have passed the menopause we have never found more than 50 I.U. of estrogenic activity per 24-hour volume after HCl hydrolysis (T_o) or more than 100 I.U. after Zn-HCl hydrolysis (T_{zn}). This was true of the 20 specimens collected from the 8 control women of this study. The findings in these and in 20 specimens from the 7 women with mammary cancer are summarized in Table I. When not receiving cortisone, the average output of estrogens excreted as such (T_o) by cancer patients with X-rayed ovaries was about 4 times as high as the average output by normal postmenopausal women. This was not true in the cancer patients whose ovaries had been surgically removed. Only one of these had an abnormally high level of T_o. The pretreatment output of the unknown compounds (T_{zn}) that may represent estrogen oxidation products or precursors, however, averaged 9 times the normal in the cancer patients with X-rayed ovaries and 18 times the normal in the cancer patients who had been oophorectomized. In both types of cancer patients these very high levels of excretion, both of the estrogens excreted as such and of related compounds, were markedly lowered by cortisone therapy. They were brought to within the normal range or below in all but 2 of 9 experiments (Table II).

In Table II the findings on the 7 cancer patients are presented in sufficient detail to demonstrate in the individual cases not only the effect of cortisone therapy but also the influence of advancing disease and of the surgical removal of X-rayed ovaries. Higher excretion rates with advancing disease were apparent in studies on all three of the patients who were followed over long periods of time. Mrs. E., whose output of T_o and T_{zn} were well above normal 2 years after X-radiation, was found to have an even higher level of excretion in both fractions 17 months later when her disease had become more advanced. In Mrs. C. and Mrs. B. the effect of surgical removal of X-rayed ovaries as well as of advancing disease was observed. After oophorectomy the high levels of estrogens excreted as such (T_o) disappeared from the urines of both of these women, while the T_{zn} values on both rose with advancing disease despite the absence of any ovarian tissue. This was especially striking in the case of Mrs. C. By April, 1953, 7 months after oophorectomy, she had very widespread cancer with pain. Her urinary output

TABLE II. Estrogens in Urine of Postmenopausal Women with Mammary Cancer.

Patient	Condition of ovaries	Not receiving cortisone: Date of urine	Urinary estrogen (I.U./24°) T_o	T_{zn}	During or just after cortisone treatment: Date of urine	Cortisone given: Daily dose, mg	Duration of dose	Urinary estrogen (I.U./24°) T_o	T_{zn}
#1 Mrs. E., aged 43-46	X-ray cast. in 1947	3/20/49	100	425	8/18/51	25	7 days	<50	<100
		8/10/51	165	665	10/18/51	25	2 mo	<50	<100
						200 to			
#2 Mrs. C., aged 42	X-ray cast. in 1948	7/ 2/52	125	665	7/10/52	50	7 days	<50	375
					9/11/52	25	2 mo	<50	<100
#3 Mrs. B., aged 37-38	X-ray cast., Oct. 1951	12/ 9/51	60	125	10/ 6/52	25	10 "	<50	<100
#4 Mrs. V., aged 50	X-ray cast., 1950	9/29/53	385	1960	11/16/53	75	5 wk	80	575
#5 Mrs. L., aged 48	X-ray cast., May 1952	10/30/52	<50	<100					
						150 to			
#2 Mrs. C. (cont.)	Oophorect., Sept. 1952*	10/18/52	<50	750	10/ 5/52	25	3 mo	<50	<100
		4/24/53	<50	4700	5/14/53	75	3 wk	<50	<100
#3 Mrs. B. (cont.)	Oophorect., April 1953*	6/22/53	<50	250	7/ 5/53	75	2 "	<50	<100
#6 Mrs. M., aged 52	Oophorect., Feb. 1953*	4/28/53	125	1150					
#7 Mrs. S., aged 33	Oophorect., Feb. 1953	5/ 7/53	<50	<100					

* Sections of these ovaries were examined by Dr. George V. Smith and found to exhibit typical stromal hyperplasia as first described by him(10) in the ovaries of postmenopausal women with cancer of the endometrium.

of estrogens excreted as such was not elevated as it had been on July 2nd, 1952, before oophorectomy. Her urinary T_{zn}, on the other hand, was 7 times as high as it had been then and 60 times as high as the average level in normal surgical castrates. Cortisone therapy was particularly effective in relieving the pain in this patient. After 3 weeks on cortisone her T_{zn} value was within or below the normal range. In only one patient of this study did cortisone therapy for 2 weeks or more fail to eliminate excessive excretion of estrogens and related compounds. Mrs. V., an X-ray castrate, had received the same dosage of cortisone as Mrs. C. and for an even longer period of time by 11/16/53. Her urine still contained excessive amounts of estrogens excreted as such and of urinary products rendered estrogenic by Zn-HCl treatment, although the levels of both were considerably lower than they had been before treatment. It is perhaps worthy of note that this patient was the only one of the 4 treated cases who derived no definite relief of pain from cortisone, as well as the only one whose urinary output of T_o and T_{zn} was not lowered to within or below normal limits by this form of therapy.

Discussion. That postmenopausal women with cancer frequently excrete more estrogen than normal postmenopausal women was reported by Pincus and Graubard(4) and has recently been confirmed by Dao(5) in patients with widespread mammary cancer. In 12 ovariectomized women who had been adrenalectomized for the treatment of breast cancer, however, Dao found no urinary estrogen. Since these patients were being maintained on cortisone, this treatment rather than adrenalectomy might have accounted for the absence of demonstrable estrogen in their postoperative urines. The adrenals, however, are logically assumed to be the source of urinary estrogen in males and in women without ovarian function. High titres have been observed in ovariectomized women following major surgery(6) and in the urines of males after severe burns(7), the inference being

that any condition of stress, with resultant hyperactivity of the adrenal cortex, may be accompanied by increased secretion of adrenal estrogen. The finding herein reported that cortisone suppresses estrogen excretion further implicates the adrenals since cortisone inhibits secretory activity in the human adrenal cortex (8). The findings herein presented also suggest, however, that X-rayed ovaries as well as the adrenals may be a source of estrogen in women with mammary cancer. Here again a condition of stress may be involved since pituitary release of gonadotropic as well as adrenotropic hormones has been demonstrated in animals in response to noxious stimuli(9). The stromal hyperplasia observed by Dr. George V. Smith in the ovaries of these patients (Table II) is of particular interest in this connection, especially since it is known to occur very much more frequently in the postmenopausal ovaries of women with cancer than in those of normal postmenopausal women(10,11). It has yet to be demonstrated, however, that stromal hyperplasia represents estrogen secretion. Another possibility is that it may influence the metabolism of adrenal estrogen or related compounds so that more is excreted in estrogenically active forms. It is tempting to postulate that high T_{zn} titres in these patients reflect adrenal secretion of estrogen precursors, but in our present state of knowledge any interpretation of these data must be purely speculative. The rising titres with advancing disease are in keeping with the hypothesis, however, that, whatever the source of the urinary estrogens and related compounds, metastatic cancer is in some way responsible for the excessive amounts observed.

Summary. In 7 postmenopausal women with mammary cancer the urinary output of estrogens and related compounds has been studied under various conditions and compared with that of 8 normal postmenopausal women. Five of the 7 cancer patients were found to excrete excessive amounts. In these 5, when not receiving cortisone, the output of estrogens excreted as such averaged nearly 5 times the mean normal, and that of compounds estrogenic after Zn-HCl hydrolysis averaged 16 times the mean normal. Cortisone therapy markedly lowered these titres and reduced them to normal levels in 3 of 4 patients so treated. Except when under cortisone treatment the titres of estrogen and related compounds rose with advancing disease. The surgical removal of X-rayed ovaries lowered the titres of estrogens excreted as such but not of the unknown compounds rendered estrogenic by Zn-HCl treatment.

1. Smith, G. V., and Smith, O. W., *Am. J. Physiol.*, 1935, v112, 340.

2. Smith, O. W., and Smith, G. V., *Endocrinol.*, 1941, v28, 740.

3. ———, *Recent Progress in Hormone Research*, Academic Press, Inc., New York, 1952, v7, 209.

4. Pincus, G., and Graubard, M., *Endocrinol.*, 1940, v26, 427.

5. Dao, T. L-Y., *Science*, 1953, v118, 21.

6. Smith, O. W., and Smith, G. V., Conference on Metabolic Aspects of Convalescence. Sponsored and privately printed by the Josiah Macy, Jr., Foundation, Chicago, Ill., 1944, 7th Meeting, pp. 69-71.

7. Heller, C. G., *ibid.*, pp. 60-66.

8. O'Donnell, W. M., Fajoms, F. S., and Weinbaum, J. G., *Arch. Int. Med.*, 1951, v88, 28.

9. Smith, O. W., *Am. J. Obst. and Gynec.*, 1947, v54, 201.

10. Smith, G. V., *New Eng. J. Med.*, 1941, v112, 340.

11. Sommers, S. C., and Teloh, H., *Arch. Path.*, 1952, v53, 160.

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