STUDIES ON ESTROGEN-SENSITIVE TRANSHYDROGENASE

The Effect of Estradiol-17β on α-Ketoglutarate Production in Noncancerous and Cancerous Human Breast Tissue

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PREVIOUS studies from this laboratory[3-5] have indicated that many samples of noncancerous and cancerous human mammary tissue contain a steroid-sensitive transhydrogenase that is sensitive to low concentrations of estradiol-17β. It was demonstrated that many homogenates of these tissues formed more reduced diphosphopyridine nucleotide (DPNH) from diphosphopyridine nucleotide (DPN) in the presence of estradiol when isocitrate was present as a substrate. The equation for the enzymatic reaction potentiated by estradiol is:

$$d\text{-isocitrate} + \text{DPN} \longrightarrow \text{DPNH} + \alpha\text{-ketoglutarate}$$

The present report demonstrates the increased rate of α-ketoglutaric acid formation when certain noncancerous or cancerous breast tissue homogenates are incubated in the presence of a small quantity of estradiol and isocitrate.

MATERIALS AND METHODS

Preparation of Tissue Homogenates. Surgical specimens were placed in cracked ice in the operating room and brought promptly to the laboratory, where representative areas of tissue were selected both for enzymatic assay and for microscopic examination. The tissues for enzymatic assay were then rapidly minced, using a Mickle slicer adjusted for the formation of 0.05-mm. cubes. A 20% homogenate was made from this mince in 0.25*M* sucrose at 0° C. Homogenization was effected with Potter type homogenizers with Teflon pestles that were operated for 20 seconds. The homogenates were then strained through gauze and centrifuged for 45 minutes at 50,000×*g* in a Spinco Model L ultracentrifuge. The supernatant fraction was removed and assayed immediately.

TABLE 1

REPRODUCIBILITY OF THE ENZYMATIC ASSAY*

μM KG in absence of estradiol-17β	μM KG in presence of estradiol-17β
0.0275	0.0359
0.0286	0.0361
0.0301	0.0367
0.0308	0.0378
0.0312	0.0398
0.0315	0.0398
0.0323	0.0411
0.0323	0.0411
0.0337	0.0418
0.0337	0.0422
0.0343	0.0433
0.0315	0.0396
±0.0021 S.D.	±0.0026

*The data are corrected for the apparent α-ketoglutarate present before incubation. The difference in α-ketoglutarate production is due to $4\times10^{-6}M$ estradiol-17β. $P<0.01$ by Fishers' t test.

Assay Procedure. The supernatant fraction (0.30 ml.) was incubated for 3 hours at 30° C. with 0.60 ml. of substrate solutions in the presence and absence of estradiol. The preparation of the substrate solutions and the method for α-ketoglutarate analysis has been described.[4]

RESULTS

The precision possible with the present assay procedure is illustrated in Table 1. These data represent a study of in vitro stimulation of the transhydrogenase activity of a homogenate of apparently normal breast tissue by estradiol. This particular sample of breast tissue was selected for illustration because it represents quite modest activity. All of the tumor tissue homogenates described in this report as exhibiting significant sensitivity to estradiol had enzymatic activities greater than this sample. This slightly active homogenate exhibited reproducible and significant stimulation by estrogen in vitro. Table 2 summarizes our studies of breast cancer tissue done

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TABLE 2

IN VITRO ESTRADIOL-SENSITIVE AND -INSENSITIVE CANCEROUS HUMAN BREAST TISSUE

Pt.*	Age	Microscopic diagnosis	KG production†			P	N	% incr.
			In absence of estradiol	In presence of estradiol	Due to estradiol ±S.E.M.			
A.M.D.	38	Inf. duct ca.	0.0130±0.002	0.0229±0.003	0.0099±0.0019	<0.01	4	76.2
E.M.J.	48	Inf. duct ca.	0.0456±0.010	0.0707±0.003	0.0251±0.006	<0.02	3	55.0
M.S.B.	71	Inf. duct ca.	0.034	0.107	0.073		1	214.7
D.B.O.	42	Inf. duct ca.	0.0405±0.003	0.0570±0.004	0.0165±0.0037	<0.05	2	39.5
J.C.R.	50	Inf. duct ca.	0.0317±0.0045	0.0447±0.0022	0.120 ±0.0028	<0.02	3	36.7
H.B.S.	44	Inf. duct ca.	0.0589±0.001	0.0607±0.001	0.0018±0.0014	N.S.	3	...
A.M.S.	69	Inf. duct ca.	0.0563±0.0024	0.0539±0.002	−0.0024±0.0022	N.S.	3	...
J.Y.A.	64	Inf. duct ca.	0.0151±0.003	0.0146±0.0037	−0.0004±0.005	N.S.	2	...
P.R.C.	36	Undiff. ca.	0.0447±0.014	0.0217±0.003	−0.0230±0.010	N.S.	3	...
M.M.D.	65	Inf. duct ca.	0.0385±0.0049	0.0253±0.0014	−0.0132±0.0036	N.S.	2	...
P.S.L.	59	Inf. duct ca.	0.0709±0.0022	0.0229±0.0038	−0.0480±0.003	<0.01	3	−67.7
A.D.	43	Inf. duct ca.	0.0493±0.0032	0.0626±0.0046	0.0133±0.003	<0.01	4	27.0
M.B.	72	Inf. duct ca.	0.0301±0.0026	0.0359±0.0027	0.0058±0.009	N.S.	3	...
R.E.	80	Inf. duct ca.	0.0824±0.004	0.0801±0.012	0.0023±0.035	N.S.	3	...

*In 9 of the 14 patients the tissue examined was from the primary cancer; in the other 5 (A.M.D., E.M.J., M.S.B., P.R.C., and R.E.) the tissue examined was from a metastasis.

†Expressed as micromoles KG produced in 3 hr. ±standard deviation. In column 6, the amount is expressed as micromoles KG produced in 3 hr. due to estradiol 17-β ±the standard error of the mean.

by the ketoglutarate technique up to the time of writing. In studies previously reported from this laboratory[3] breast cancer was divided into two types. One type was sensitive in vitro to estradiol; the other was not. The previous studies were done by demonstrating an increase in the formation of DPNH. The present studies of α-ketoglutarate formation from isocitrate are in complete accord with the DPNH data. Three out of 5 specimens of metastatic breast carcinoma exhibited significant in vitro sensitivity by the α-ketoglutarate technique. The presence of the enzymatic system in samples of metastatic cancer eliminates the possibility that the in vitro hormonal sensitivity of carcinoma tissue homogenates is due to admixture of the tumor with normal mammary tissue elements. All but one of the carcinomas examined in the present study were of the infiltrating duct variety. They varied considerably with respect to the amount of connective tissue. It has not been found possible to make any generalization between the microscopic appearance of the tumor and its behavior, qualitative or quantitative, enzymatically. Table 3 summarizes the assay results for all breast carcinoma specimens studied to date by either the spectrophotometric or the α-ketoglutarate procedure. The correlation of estradiol sensitivity with age is shown in Fig. 1. Apparently there is a tendency for the estradiol-sensitive tumors to show increased sensitivity in older patients. No significant difference is apparent in the distribution of positive results (breast cancer tissue showing estradiol sensitivity) and the assay procedure. Approximately the same percentage of positive results have been obtained by both procedures. For technical reasons it has not been found feasible to compare a number of carcinomas simultaneously by both assay procedures.

Mammary Tissue. Normal mammary tissue could not be obtained for control studies. Instead, histologically normal tissue from mastectomy or biopsy specimens was used. Table 4 summarizes the present data on this tissue. In 9 patients treated by radical mastectomy, 3 carcinoma specimens were found to be sensitive in vitro to estradiol, and the associated breast tissue was also sensitive. In 6 carcinoma specimens that gave negative results when assayed, only 1 sample of associated breast tissue showed sensitivity. An in vitro–sensitive carcinoma has not been found associated with noncancerous mammary tissue that lacks this enzymatic system.

Clinical Behavior. Previously 2 cases of breast cancer in premenopausal women were described in which the tumor was not sensitive in vitro to estradiol, and oophorectomy failed to achieve clinical benefit.[3] In the present study, the in vivo and in vitro behavior of the tumor could be correlated in only 2 cases.

Case 1. A.M.D., a 38-year-old woman, had a radical mastectomy in 1953 for scirrhous carcinoma. A local recurrence in 1956 gradually progressed until May, 1957, at which time multiple skin nodules and a large malignant ulcer were present on the chest wall. Bilateral

TABLE 3

ESTRADIOL-SENSITIVE TRANSHYDROGENASE IN CANCEROUS HUMAN BREAST TISSUE

Assay method	Total no. specimens	Estradiol sensitive	
		No.	%
Spectrophotometric	22	9	40.9
α-Ketoglutarate	14	6	42.9
Both methods	36	15	41.7

TABLE 4

ESTRADIOL-SENSITIVE TRANSHYDROGENASE IN NONCANCEROUS HUMAN BREAST TISSUE

Assay method	Total no. specimens	Estradiol sensitive	
		No.	%
Spectrophotometric	25	12	48.0
α-Ketoglutarate	10	4	40.0
Both methods	35	16	45.7

oophorectomy at this time was followed by subjective improvement, marked objective regression of the ulcer, and disappearance of the skin nodules. This striking remission had lasted 7 months when a supraclavicular nodule appeared and the patient began to complain of backache so severe that she was confined to bed. Roentgen-ray examination of the previously negative spine demonstrated osteolytic metastases. Enzymatic assay of the metastatic nodule demonstrated unequivocal presence of in vitro estradiol sensitivity (Table 2). Bilateral adrenalectomy in January, 1958, was followed by prompt disappearance of bone pain and complete ambulation, but sufficient time has not elapsed to permit an objective evaluation of the results of this therapy. However, there is no question that the tumor has shown regression after removing the source of ovarian estrogen in 1957.

Case 2. E.M.J., a 48-year-old woman, had an inoperable breast carcinoma with lenticular metastases. Biopsy in January, 1958, revealed an infiltrating duct carcinoma, and the tumor showed definite in vitro sensitivity to estradiol (Table 2). Bilateral oophorectomy in January, 1958, was followed within a month by definite objective regression of the tumor as well as striking subjective improvement.

DISCUSSION

It is apparent that the similarity in the in vitro and in vivo responses of the tissues in the 4 patients studied thus far may be purely coincidental. Moreover, it is evident that more than 1 biopsy specimen ought to be taken from patients studied up to now. A great many more patients will have to be studied before a firm relationship between enzymatic and clinical behavior will emerge.

At present we have chosen to separate the tumors studied biochemically into sensitive and insensitive types. However, it is obvious from our data that there are large quantitative differences between tumors that exhibit sensitivity. The significance of these differences is not at all clear, but it is altogether possible that the gradations in in vivo re-

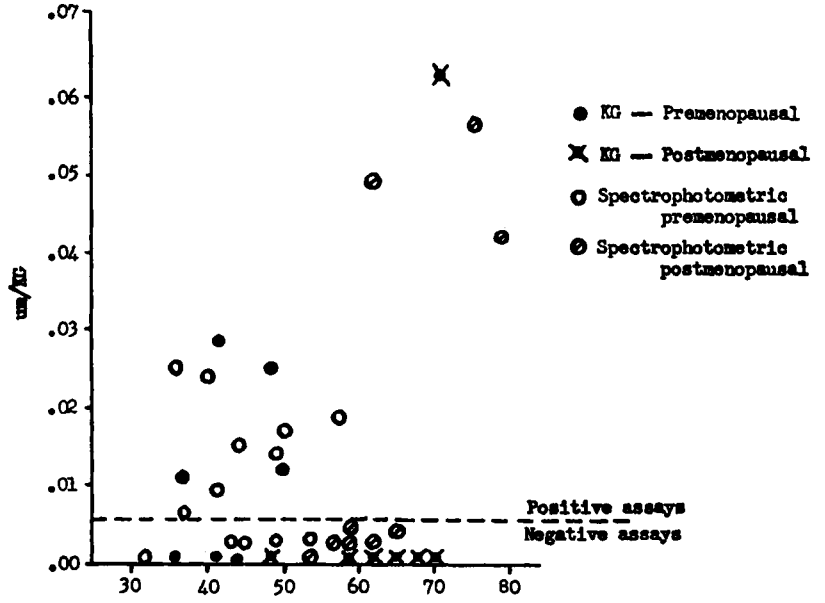

FIG. 1. Age distribution of patients with estradiol-sensitive breast cancer tissue.

sponses observed clinically may be mirrored in gradations of biochemical response.

The demonstration of in vitro sensitivity to estradiol-17β by means of the α-ketoglutarate production method has the advantage of higher precision and makes possible the study of a large number of replicates. On the other hand, the spectrophotometric method is more useful for kinetic studies since the extent of reaction at any time may be plotted. The spectrophotometric method cannot be applied to turbid homogenates or to homogenates with significant reduced diphosphopyridine nucleotide (DPNH) oxidase activity. The presence of DPNH oxidase does not interfere with the α-ketoglutarate procedure. In this laboratory both procedures are used, depending upon the nature of the particular study.

The studies on estradiol-sensitive mammary tissues have been carried out with techniques established for the estimation of the steroid-sensitive transhydrogenase described by Hagerman and Villee,[1, 2] Villee,[6] and Villee and Gordon[7] in placenta. We have no proof of the identity of the mammary and placental enzymes although our studies show that they have similar *p*H activity curves and similar thermal instability. A detailed comparison of the 2 enzymes must await a suitable method for purification of the breast tissue enzyme similar to that described for the placental tissue enzyme.

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GENETICS AND NEOPLASTIC GROWTH

The Thirteenth Annual Symposium on Fundamental Cancer Research is scheduled to be held at the University of Texas M. D. Anderson Hospital, Houston, Texas, Feb. 26, 27, and 28, 1959. The topic for discussion is Genetics and Neoplastic Growth. The programs deal with (1) Fundamental Aspects of Genetics in Carcinogenesis; (2) Gene Interaction in Neoplastic Growth; (3) Genetic Basis of Cell Resistance, and (4) Heredity and Human Cancer. Further information on the symposium may be obtained from the Editorial Office, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.