Polarographic Analysis of Certain Fractions Obtained from the Urine of Patients with Malignancy and from Normal Individuals (32913)

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Very many chemical procedures have been submitted for the diagnosis of malignancy, but none of the proposed methods have proved specific or useful for most types of cancer. An excellent review of suggested diagnostic cancer tests and criteria which should be applied to the latter have been published by Homberger (1). Since the polarograph offers the possibility for the quantitative and qualitative determination of minute amounts of various oxidizable and reducible organic substances, several fractions of the urine of cancer patients and of normal individuals were examined with this instrument. This approach provides a chance for the detection of various organic compounds, the nature and amount of which might be affected by the presence of neoplastic growths.

Methods. Overnight urine specimens were obtained from patients with early and somewhat advanced cancer (17 patients with carcinoma of the cervix, 9 with carcinoma of the breast, 30 with carcinoma of the gastrointestinal tract and 12 with leukemia of several types), and from 31 normal individuals (20 females and 11 males) of age comparable to the patients.

The urine samples were extracted on the day of collection, or refrigerated for not more than 1 or 2 days prior to extraction. The urine samples were extracted in a manner somewhat similar to that of Talbot *et al.* (2) and the fractions obtained contained steroids along with other substances. Briefly the specific gravity of the urine specimen was taken and then 50–125 ml of it were extracted 3 times with 25–50 ml of ether (peroxide-free ethyl ether was used in all the extractions) in a separatory funnel. This ether extract (ether fraction) was then washed three times with distilled water. The ether extracted urine was hydrolyzed with 15 volumes% HCl by boiling for 10 min. After cooling, the urine was extracted three times with 50- to 100-ml portions of ether in a separatory funnel. The combined ether solution was washed three times with distilled water and then extracted four times with 10 ml of 1 *N* NaOH to remove the phenolic fraction. The neutral fraction, which remained in the ether layer, was washed with distilled water until it was alkali free. The phenolic fraction was treated with 20 ml of 4.5 *N* H_2SO_4, and then it was extracted four times with 25- to 50-ml portions of ether. The latter was washed with distilled water in a separatory funnel until acid free. Then the washed ether solutions of the ether, neutral and phenolic fractions were dried by passing them through anhydrous Na_2SO_4 in a funnel into glass stoppered Erlenmeyer flasks. The Na_2SO_4 was rinsed with several small portions of ether, and the fractions were brought to dryness under nitrogen on a steam bath. The samples were stored at 0–4°C.

The materials in each fraction were dissolved in the Erlenmeyer flask by the addition of 1 ml of purified dioxane (3) and 1 ml of phosphate buffer, pH 6.95–7.0. Electrolysis was carried out in Heyrovsky microvessels with side arms for the introduction of nitrogen through the sample and for the maintenance of this gas over the solution during reduction at the dropping mercury electrode. Current voltage curves of each sample were made at 25°C directly against a saturated calomel electrode (SCE) after removing any oxygen with a dioxane-saturated stream of nitrogen. A Leeds and Northrup Electro-Chemograph, type E, was employed for the polarographic measurements. The pH of each sample was determined after electrolysis with a Beckman, model G, pH meter.

The half-wave potentials of the reducible compounds in each fraction were measured and the diffusion currents were determined from the polarograms following the extrapolation method of correcting for the residual

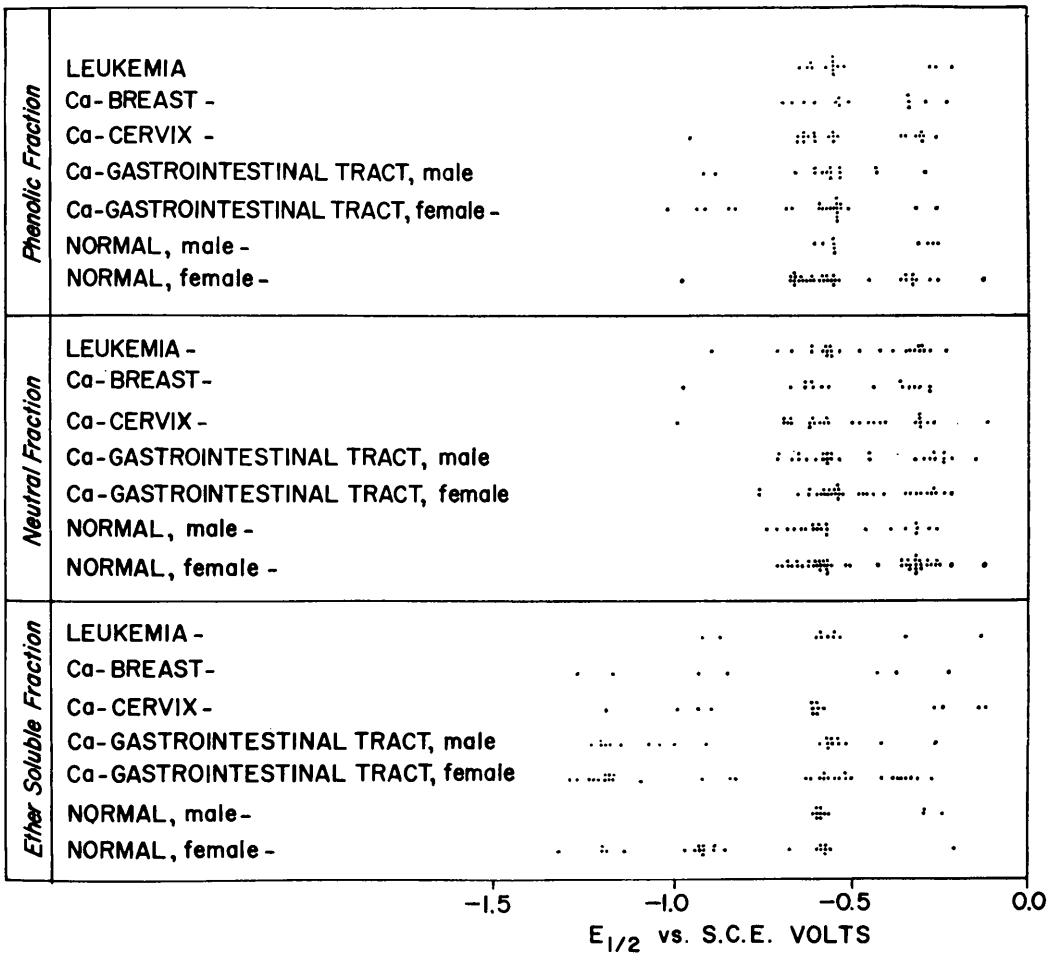

FIG. 1. Scattergram showing the half-wave potentials of the reducible substances extracted from the urine of patients with cancer and normal individuals. The $E_{1/2}$ of the number of reducible compounds present in the ether, neutral, and phenolic fractions of the urine from normal persons and from patients with cancer of the various organs are plotted against $E_{1/2}$ vs SCE in volts. (Ca = carcinoma).

current (4). The amount of each reducible substance in microamperes (diffusion current) was calculated for a liter of urine from the product of the diffusion current, specific gravity of the urine, and 1000/volume of urine extracted.

Results. A scattergram (Fig. 1) shows the half-wave potentials of the reducible substances present in the ether, neutral, and phenolic fractions of the urine of normal individuals and patients with neoplastic growths. The current–voltage curves were well defined. The scattergram indicates that there is a fairly good separation of the $E_{1/2}$ vs SCE of the reducible compounds in the three fractions into one group more negative than —0.5 V and another group more positive than 0.5 V. Also in the ether fraction there is a separation into a group of reducible compounds more negative than —0.76 V and another group more positive than —0.76 V. The number of reduction waves more negative and more positive than —0.5 vs SCE as well as the ratio of these values are given in Table I. In the phenolic fraction males and females with gastrointestinal cancer had much larger ratios of 4.3 and 7.0, respectively, than did this fraction from the

TABLE I. The Number of Polarographic Waves More Negative and Positive Than —0.5V vs SCE Found in the Various Urine Fractions.

Urine fraction	Specimen[a]	Sex	Number of reduction waves at potentials vs SCE more — (—0.5V)	more + (—0.5V)	Ratio —/+
Phenolic	Ca–GI	♂	13	3	4.3
	Normal	♂	6	4	1.5
	Ca–GI	♀	21	3	7.0
	Normal	♀	23	11	2.2
	Ca–cervix	♀	15	8	1.9
	Ca–breast	♀	7	5	1.4
			Mean[b] 1.8 ± 0.3		
Neutral	Ca–GI	♂	15	12	1.25
	Normal	♂	14	8	1.7
	Ca–GI	♀	19	14	1.35
	Normal	♀	25	22	1.14
	Ca–cervix	♀	14	14	1.0
	Ca–breast	♀	9	8	1.1
			Mean[c] 1.3 ± 0.2		
Ether	Ca–GI	♂	19	2	9.5
	Normal	♂	9	3	3.0
	Ca–GI	♀	25	9	2.8
	Normal	♀	25	9	2.8
	Ca–cervix	♀	11	4	2.7
	Ca–breast	♀	4	3	1.3
			Mean[d] 2.5 ± 0.7		

[a] Abbrev.: Ca = carcinoma; GI = gastrointestinal tract.

[b] Mean ± SD of 4 lowest ratios.

[c] Mean ± SD of 6 ratios.

[d] Mean ± SD of 5 lowest ratios.

urine of normal persons. The latter and females with cancer of the cervix and breast had a value of 1.8 ± 0.3 (mean ± S.D.). There were no differences in the $E_{1/2}$ of the reducible compounds in the neutral urine fraction of normal persons or patients with cancer. The mean ± standard deviation of the ratio for this group was 1.3 ± 0.2. The ether fraction of the urine of males with gastrointestinal cancer had a ratio of 9.5, or more than 3 times that found in this same fraction of the urine of normal males (Table I). There was no significant difference in the ether fractions of the urine samples of the remaining normal and cancer patients. The mean ± standard deviation for these was 2.5 ± 0.7. In the ether fraction the ratio of the number of reduction waves more negative than —0.76 V vs SCE to that more positive than —0.76 V is given in Table II. A striking difference was found between the ether fraction of the urine of males with cancer (10/11) as compared with normal males (0/12). There also appeared to be a possible significant difference in this ratio between the urine of normal females and females with gastrointestinal cancer and carcinoma of the cervix.

TABLE II. The Number of Polarographic Waves More Negative and Positive Than —0.75 V vs SCE Found in the Ether Urine Fraction.

Specimen[a]	Sex	Number of reduction waves at potentials vs SCE more — (—0.75 V)	more + (—0.75 V)	Ratio —/+
Ca–GI	♂	10	11	0.9
Normal	♂	0	12	0
Ca–GI	♀	15	19	0.79
Normal	♀	15	9	1.66
Ca–cervix	♀	4	11	2.75
Ca–breast	♀	4	3	1.33

[a] Abbrev.: Ca = carcinoma; GI = gastrointestinal tract.

In the various urine fractions examined by polarography no compound specific for cancer was found in the urine of patients with several types of cancer.

The amounts of the reducible substances found in the various fractions in the urine of normal and diseased individuals did not show any significant differences. There was a wide variation in the amount of each reducible substance in both groups of individuals. No attempt was made to ascertain the nature of the polarographically reducible compounds since the prime purpose of this study was to determine whether qualitative or quantitative differences existed in the excretion of the ether, neutral, and phenolic substances in the urine of normal persons as compared to that in the urine of cancer patients. Further study would be required to determine which, if any, of the reducible compounds in the various urine fractions are steroids, since some of these compounds are directly reducible at the dropping mercury electrode (4,5).

Summary. The ether, neutral, and phenolic fractions of the urine of normal individuals and of patients with carcinoma of the breast, cervix, gastrointestinal tract, and with various leukemias were analyzed polarographically. Reducible compounds were found in all the fractions. In the urine phenolic fraction the ratio (of the number of polarographically reducible compounds with an $E_{1/2}$ more negative than —0.5 V vs SCE to that more positive than—0.5 V) was significantly different for male and female patients with gastrointestinal cancer as compared to normal persons of both sexes. There was no difference in this ratio between the neutral urine fraction of normal persons and cancer patients. The ether fraction of the urine of males with gastrointestinal cancer had a much higher ratio (number of compounds with an $E_{1/2}$ more negative than —0.5 V vs SCE/number of compounds with $E_{1/2}$ more positive than —0.5 V) than that of the same fraction of normal males. Finally a significant difference was found in the ratio (of the number of reducible compounds with an $E_{1/2}$ more negative than —0.76 V vs SCE to that more positive than —0.76 V) of the ether fraction of normal male urine as compared with that of men with gastrointestinal cancer. Patients with various types of cancer did not excrete reducible compounds characteristic of any form of this disease.

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An *in Vitro* Demonstration of Cellular Sensitivity in Experimental Autoimmune Nephrosis in Rats* (32914)

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The precise mechanism by which injections of Freund's adjuvant and kidney produce the nephrotic syndrome in rats is not completely clear. Recent studies in our laboratory (1) would indicate that antigens from the apical, periluminal portion of the proximal tubular cell, complexed to the antibody they initiate, deposit in the glomeruli of the sensitized animal, inducing glomerular damage. The role, if any, that might be played by a delayed hypersensitivity phenomenon has not been fully elucidated, though this mechanism has been suggested (2). Independent reports by Heymann (3) and by Hess (2) demonstrate that a nephrotic disease can be produced in normal, immune tolerant Sprague-Dawley rats by the transfer of splenic or lymph node cells from donors with autoimmune nephrosis. The fact that this transfer has not been repeatable in an isogeneic strain (4) does not negate or explain that something was induced in the recipients of sensitized cells that was not induced in recipients of normal or control cells. It seemed reasonable, therefore, to examine the cells involved from both allogeneic and isogeneic rats for evidence of a cellular sensitivity similar to that seen in delayed hypersensitivity, namely the specific inhibition of cell migration from capillary tubes in tissue culture by the sensitizing antigen.

Materials and Methods. Autoimmune nephrosis was produced in ten Sprague-Dawley and ten Lewis rats by intraperitoneal injections of a suspension of complete

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