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Comparison between Concentrations of Trace Elements in Normal and Neoplastic Human Breast Tissue[1]

Sherif L. Rizk and Howard H. Sky-Peck[2]

Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612

ABSTRACT

Histologically normal and neoplastic human breast tissues obtained from 25 patients at the time of mastectomy were homogenized (200 mg/ml) in distilled water and 5-μl aliquots dried on Formvar films for trace element analysis by energy-dispersive X-ray fluorescence. The elements measured were calcium, vanadium, copper, zinc, iron, chromium, manganese, nickel, selenium, molybdenum, bromine, rubidium, strontium, mercury, arsenic, and lead. In general, significantly large increases ($p < 0.001$) in calcium, vanadium, copper, zinc, selenium, and rubidium were found in breast tumors, with a less significant increase ($p < 0.05$) for nickel. When a comparison was made between histologically normal and neoplastic tissues from the same individual, zinc and rubidium were found to be consistently higher in the tumor, whereas calcium, copper, and vanadium levels varied from normal to high. In no instance were the tissue changes in calcium, copper, zinc, or rubidium reflected in the blood levels, which were within normal limits. The distribution of calcium, copper, and zinc in urine varied among individuals with primary tumors; however, rubidium levels tended to be consistently elevated. An attempt is being made to correlate these various differences with the extent of the primary disease at the time of surgery, the postoperative tumor-free interval, and subsequent therapy.

INTRODUCTION

It has become well established that many trace elements play an essential role in a number of biological processes through their action as activators or inhibitors of enzymatic reactions by competing with other elements and proteins for binding sites, by influencing the permeability of cell membranes, or through other mechanisms. It is therefore reasonable to assume that these trace minerals would exert action, directly or indirectly, on the carcinogenic process (19, 23, 27).

During the past decade, there has been a growing recognition that metal compounds are an important class of environmental and occupational carcinogens. Several reviews on metal carcinogens have been published which demonstrate both epidemiologically in humans and experimentally in animals the possible carcinogenicity of such elements as arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel, zinc, and iron (8–10, 18, 19, 23, 24). Numberous epidemiological studies have implicated arsenic, cadmium, chromium, and nickel as human carcinogens, while compounds of beryllium, cadmium, cobalt, chromium, iron, nickel, lead, titanium, and zinc have been used to induce cancers in experimental animals. Many of these studies have indicated that metal ions can interact with nucleic acids to influence base-pairing and conformation. Such effects have been known to cause somatic mutations, a consequence of base-pairing errors or frame-shift mutations by deletion, leading to cellular transformation. For example, magnesium, manganese, and zinc are cofactors of many enzymes, especially RNA and DNA polymerases (32). In magnesium deficiency, errors during cell division occurred more frequently. The effects of various metal ions on the accuracy of *in vitro* DNA synthesis was measured using DNA polymerase from myeloblastosis virus and a synthetic deoxypolynucleotide template of defined composition. If magnesium was displaced by other cations such as chromium, nickel, cadmium, manganese, and cobalt, the frequency of incorporation of noncomplementary nucleotides during DNA replication was markedly increased (21). In another study, misincorporation of the deoxynucleotide (dTTP) into RNA was 6-fold greater in the presence of elevated manganese than with magnesium (24). Thus it would appear that manganese, by competing with magnesium, impaired the ability of RNA polymerase to discriminate between ribonucleotides and deoxynucleotides. Many metals complete for the same cellular sites of action. For example, manganese antagonism of nickel carcinogenesis may reflect competition between manganese and nickel ions for binding of specific sites on DNA and/or RNA polymerases (20).

Sufficient experimental data have been accumulated about chromium, nickel, arsenic, and beryllium to indicate that they are human carcinogens and exhibit genetic toxicity in a number of test systems which suggest that matagenesis is involved in the initiation of cancer by these metals. While arsenic has not been shown to induce tumors in experimental animals, it has been shown that arsenic depresses the level of DNA polymerase in human epidermal cells, inducing a reduction of the DNA repair mechanism, which in turn renders human cells vulnerable to DNA damage by secondary factors; *i.e.*, exposure to arsenic and cigarette smoking significantly increased the incidence of chromosomal aberrations in lymphocytes, suggesting that arsenic may have acted as a cocarcinogen (13).

New analytical techniques, such as neutron activation (11) and EDXRF,[3] make possible the simultaneous determination of ultratrace quantities of elements in human tissues and body fluids. By using such techniques, it is possible to determine whether the simultaneous monitoring of the less abundant trace metals has diagnostic or prognostic significance. Such systematic studies which include a broad spectrum of trace elements with large patient populations have not been conducted to our knowledge.

The present study was initiated to determine the feasibility of using a micro-X-ray fluorescent system to measure simultaneously a number of trace elements in human tissues and to compare any significant differences which may exist between

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[2] To whom requests for reprints should be addressed, at Department of Biochemistry, Rush Medical College, 1753 W. Congress Parkway, Chicago, IL 60612.

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[3] The abbreviation used is: EDXRF, energy-dispersive X-ray fluorescence.

normal and neoplastic breast tissues obtained from the same individual at time of surgery.

MATERIALS AND METHODS

Normal and neoplastic human breast tissues were obtained from the same individual at the time of mastectomy. The excised surgical specimens were immediately sent to the pathology laboratory, where the pathologist removed samples of tumor and normal breast tissues for trace-element analysis. Unanalyzed tissues were stored in polypropylene vials at −20°. Tissues were homogenized in distilled water at a concentration of 200 mg/ml (wet weight) with a Teflon homogenizer. Using a micropipet with a disposable plastic capillary tip, 5-μl aliquots of sample were pipeted onto the surface of a Formvar film suspended on 35-mm plastic slide holder. The samples were allowed to dry in a laminar flow hood. The dried samples were placed into the vacuum chamber of the ultramicro-EDXRF which was then air evacuated prior to X-ray analysis.

X-ray energy fluorescence is a technique for rapid, simultaneous multielement analysis. When excited by an appropriate source, a sample will emit X-rays of energies that are characteristic for the elements composing the sample. By measuring the energies of X-rays that are emitted from an excited sample and counting the number of X-rays of each energy, EDXRF allows the identificaiton of the elements present in the sample, and also allows determination of the relative concentration of each of the elements present. A schematic representation of the X-ray fluorescence system used in this study has been published previously (22). The entire system, including excitation source, pulse processor, detector, and computer system was obtained from Kevex Corporation, Foster City, CA.

Biological samples subjected to X-irradiation exhibit a background continuum. This background is the result of primary charged particles and secondary electron interactions in the sample, and to a lesser extent in the silicon (lithium) detector, with the production of bremsstrahlung, Rayleigh, and Compton scatter emissions. Therefore, prior to the quantitative analysis of trace elements in the sample, this background interference must first be removed. This is accomplished with the aid of the computer.

In order to quantitate the energy emission spectra from each element under investigation, a series of internal element standards was added to the samples. The effects of background scatter were subtracted directly from the acquired spectra, and the area under each energy peak was integrated. These data of known concentrations of element standards were placed in a standard calibration file in computer memory for the quantitative determination of trace elements in unknown samples and reported directly as μg of element per g dry weight of sample. Details of the principles and methodology of EDXRF have been published previously by Sky-Peck and Joseph (22) and Valković (29).

RESULTS

Tissues were obtained from 25 patients with primary carcinoma of the breast and analyzed by EDXRF for calcium, vanadium, chromium, manganese, iron, nickel, copper, zinc, arsenic, selenium, bromine, rubidium, strontium, molybdenum, lead, and mercury. Chart 1 is a representation of a computer video display showing the differences in the distribution of these trace elements in normal and neoplastic breast tissues taken from the same individual. These differences appear to be most prominent with respect to the levels of potassium, calcium, copper, zinc, and rubidium. Quantitative results for these 16 trace elements were obtained from 22 patients and are presented in Table 1. The mean concentrations and S.D.s are shown and represent the results of 66 determinations for each element. Statistical analysis using Student's *t* test reveals significantly higher concentrations of calcium, vanadium, copper, zinc, selenium, and rubidium in malignant breast tissue. These results for calcium, copper, and zinc are in general agreement with data obtained by other investigators (6, 12, 15, 16).

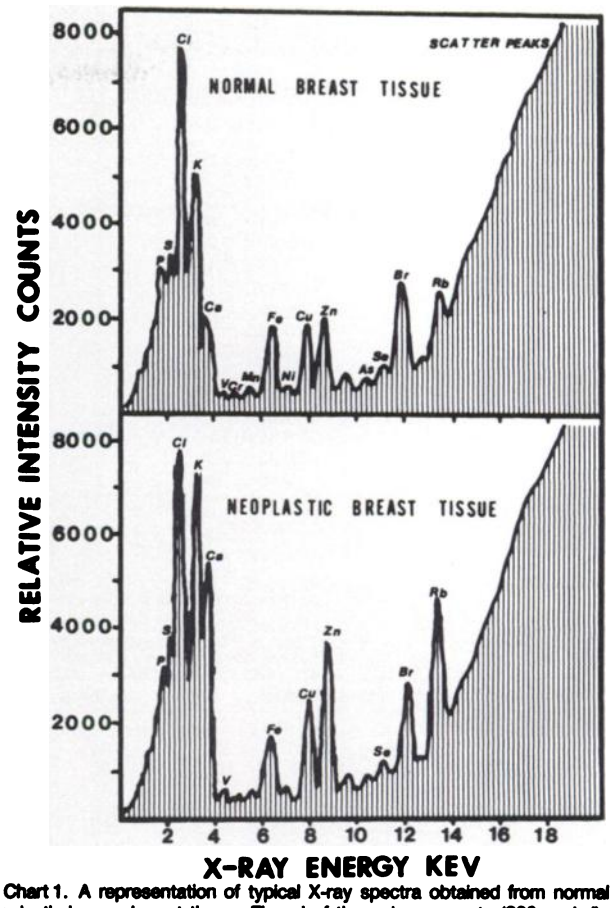

Chart 1. A representation of typical X-ray spectra obtained from normal and neoplastic human breast tissue. Five μl of tissue homogenate (200 mg/ml) were dried on Formvar films and exposed under vacuum (200 μm) to X-irradiaton at 18 ma and 35 kV for 500 sec.

Table 1

Distribution of trace elements in normal and neoplastic human breast tissue

Normal and neoplastic tissue was obtained by surgical incision from 22 individuals. Each tissue was analyzed in triplicate.

	Distribution (μg/g, dry wt)		
	Normal tissue	Tumor tissue	p^a
Calcium	357.5 ± 85.7[b]	871.7 ± 374.5	0.0001
Vanadium	0.78 ± 0.46	1.34 ± 0.76	0.004
Chromium	1.26 ± 0.65	1.48 ± 0.80	0.16
Manganese	1.37 ± 0.66	1.42 ± 0.73	0.41
Iron	218.4 ± 149.3	238.5 ± 113.0	0.30
Nickel	0.96 ± 0.47	1.27 ± 0.59	0.04
Copper	9.3 ± 2.3	21.0 ± 10.7	0.0001
Zinc	25.6 ± 12.1	68.1 ± 26.9	0.0001
Arsenic	0.19 ± 0.13	0.13 ± 0.09	0.04
Selenium	0.70 ± 0.30	1.02 ± 0.43	0.003
Bromine	24.4 ± 11.5	23.4 ± 10.7	0.39
Rubidium	9.2 ± 3.2	19.7 ± 6.1	0.0001
Strontium	1.29 ± 0.70	1.48 ± 1.13	0.25
Molybdenum	2.70 ± 0.95	2.45 ± 0.67	0.15
Lead	1.33 ± 0.66	1.55 ± 1.24	0.23
Mercury	0.87 ± 0.45	0.77 ± 0.57	0.25

[a] Calculated from Student's *t* distribution and χ^2 probability.
[b] Mean ± S.D. based on 66 determinations for each element.

In order to ascertain the contribution of the methodology to the variability of the results obtained in Table 1, a sample of

Table 2
Reproducibility of analyses
The analyses were based on the variations of 10 aliquots from the same tumor sample, each counted separately.

	μg/g dry wt of tissue	Coefficient of variance (%)
Calcium	1262.60 ± 5.30[a]	0.4
Vanadium	0.13 ± 0.02	15.3
Chromium	0.25 ± 0.04	16.0
Manganese	1.51 ± 0.09	5.3
Iron	198.40 ± 0.76	0.3
Nickel	2.70 ± 0.10	3.7
Copper	16.10 ± 0.14	0.9
Zinc	49.50 ± 0.9	0.2
Arsenic	0.13 ± 0.02	15.3
Selenium	0.93 ± 0.02	2.1
Bromine	23.70 ± 0.15	0.6
Rubidium	25.80 ± 0.18	0.7
Strontium	1.50 ± 0.05	3.3
Molybdenum	2.31 ± 0.12	5.2
Lead	0.83 ± 0.05	6.0
Mercury	0.55 ± 0.04	7.2

[a] Mean ± S.D.

breast tumor was homogenized, and ten 5-μl aliquots were each analyzed under identical conditions. The results are summarized in Table 2 where the mean, S.D., and coefficient of variance are presented. As would be expected, the lower the element concentration the higher the variance. In addition, detection efficiency of X-ray fluorescence is a function of atomic number. Elements of higher atomic number, *e.g.*, molybdenum, strontium, or selenium, have a greater sensitivity than do vanadium, chromium, or manganese. In spite of these limitations, a comparison of the data presented in Tables 1 and 2 indicates that the biological variation of tissue samples far exceeds any contribution from the methodology.

One problem in the study of trace metals in relation to disease is defining normal baseline concentrations. A number of investigators (25, 27, 33) have shown that trace element concentrations in healthy tissue vary considerably from one individual to another. Many of these variations can be attributed to age, sex, hormonal status, diet, medication, or environment. An attempt was made

Table 3
Comparison between the distributions of trace metals in paired samples of normal and neoplastic breast tissues
Histologically normal and neoplastic tissues obtained from the same individual at time of surgery were compared, each patient acting as her own control.

	μg/g dry wt of tissue, av. of 3 determinations					
	Calcium	Vanadium	Copper	Zinc	Selenium	Rubidium
Patient 1						
Normal	361	0.64	10.8	30.1	0.84	10.5
Tumor	821	1.80	15.2	83.9	1.80	28.5
Patient 2						
Normal	264	0.95	5.7	14.2	0.28	3.9
Tumor	1812	1.30	18.5	52.3	0.45	8.1
Patient 3						
Normal	302	1.66	8.6	31.5	0.82	5.9
Tumor	1696	2.12	21.9	75.2	1.05	34.3
Patient 4						
Normal	287	0.87	7.6	11.0	0.32	3.5
Tumor	1013	1.26	25.6	54.2	0.95	8.1
Patient 5						
Normal	317	0.91	10.9	42.2	1.10	17.6
Tumor	650	3.00	20.2	89.7	1.80	26.9
Patient 6						
Normal	421	0.30	8.6	42.5	0.87	10.4
Tumor	511	0.33	9.3	70.8	1.20	36.1
Patient 7						
Normal	347	1.86	8.9	27.7	1.00	9.8
Tumor	712	1.66	12.4	37.7	0.67	18.5
Patient 8						
Normal	308	0.57	7.5	14.7	0.42	7.9
Tumor	667	2.18	8.7	48.4	0.97	16.8
Patient 9						
Normal	411	0.42	9.5	32.8	0.92	10.7
Tumor	482	3.10	18.2	43.7	0.66	17.1
Patient 10						
Normal	407	0.57	7.4	23.6	0.49	8.5
Tumor	716	0.81	12.4	61.1	0.68	18.1
Patient 11						
Normal	325	0.11	8.4	29.5	0.50	10.2
Tumor	867	0.38	16.7	35.8	0.55	21.7
Patient 12						
Normal	355	0.95	12.6	40.0	1.05	11.1
Tumor	1252	1.66	19.0	51.2	0.63	25.7
Patient 13						
Normal	303	0.40	13.7	21.9	0.55	4.2
Tumor	382	0.94	8.4	40.4	0.74	9.4
Patient 14						
Normal	672	1.18	20.3	52.4	0.63	16.0
Tumor	683	1.02	12.8	53.2	0.91	21.3
Patient 15						
Normal	307	0.87	9.1	40.9	0.56	6.7
Tumor	361	0.56	10.9	74.9	0.93	15.5
Patient 16						
Normal	658	1.20	9.9	32.0	1.15	10.1
Tumor	887	1.60	16.5	80.1	1.35	12.3
Patient 17						
Normal	375	0.34	8.6	20.1	0.85	6.4
Tumor	941	1.12	10.5	35.8	0.46	21.0
Patient 18						
Normal	211	0.95	6.3	10.6	0.29	3.3
Tumor	1129	0.98	12.4	32.1	1.04	9.8
Patient 19						
Normal	425	1.24	7.4	13.5	0.61	3.5
Tumor	356	1.07	7.9	60.4	1.30	22.7
Patient 20						
Normal	617	1.20	8.4	38.6	0.68	12.6
Tumor	581	0.70	8.2	45.9	1.25	18.1
Patient 21						
Normal	663	0.70	7.8	35.9	0.70	9.7
Tumor	661	0.89	9.2	77.7	0.92	30.5
Patient 22						
Normal	412	0.39	6.3	24.0	0.67	7.4
Tumor	1098	0.56	34.9	103.1	0.83	20.9
Patient 23						
Normal	397	0.35	9.4	34.1	1.17	15.4
Tumor	634	0.84	9.8	49.6	1.20	38.1
Patient 24						
Normal	423	0.47	9.8	41.9	0.80	10.6
Tumor	499	0.37	9.8	71.6	0.66	21.0
Patient 25						
Normal	289	1.10	9.6	36.2	0.82	6.5
Tumor	1787	1.70	46.9	110.6	1.06	34.2
p[a]	<0.0001	0.0036	0.0015	<0.0001	0.0017	<0.0001

[a] Calculated from paired Student's *t* distribution.

to avoid these complications in the present study by using paired samples of cancerous and noncancerous tissue obtained from the same individual. Results of a paired analysis of normal and neoplastic breast tissues from 25 patients are shown in Table 3. Only those metals, namely, calcium, vanadium, copper, zinc, selenium, and rubidium, which showed significant differences (Table 1) were analyzed by the paired Student t test using the mean values from each individual. p was calculated from the t distribution. It is apparent that the increased concentrations of these metals in tumor tissue is real and establish a pattern in carcinoma of the breast.

Contrary to results reported by others (19), the levels of calcium, copper, zinc, and selenium in the serum and urine obtained from this group of patients were within normal limits and showed no characteristic pattern of distribution.

DISCUSSION

A comparison of trace element levels in normal and neoplastic human breast tissues has shown a relatively consistent and characteristic pattern of elevation for calcium, vanadium, copper, zinc, selenium, and rubidium. The explanation for these differences cannot be made at this time. However, the results obtained for calcium, copper, and zinc in the present study are similar to those obtained by others (6, 12, 15, 16) who also compared these trace metals in paired samples of normal and neoplastic breast tissues. In one study (18), the investigators assumed that elevated tissue calcium levels were in conflict with previous reports (2, 7) and attributed these differences to methodology. These investigators failed to recognize that their data on breast cancer was being compared with gastric carcinomas. In a yet unpublished study by the present authors, 8 patients with primary gastric adenocarcinoma had levels of 638 ± 243 (S.D.) μg/g of normal tissue and 381 ± 178 μg/mg of cancer tissue. These results indicate that the previous data were not in conflict and that it is presumptuous to assume that all cancers regardless of tissue origin have a similar distribution of trace elements.

The diagnostic value of knowing that tissue calcium content is elevated in different neoplastic breast tissues seems limited at this time due to the wide variability found among individuals and because serum levels have been shown not to be altered or to reflect tissue levels.

The elevation of zinc and copper levels in breast carcinoma have been reported by numerous investigators. The present study confirms these results with a consistently elevated level of zinc in all 25 paired tissues obtained from patients with breast cancer. While copper was statistically elevated ($p < 0.0001$), the levels were not as consistent as with zinc when compared by paired analysis. Furthermore, unlike other malignant disease such as lymphoma and bronchogenic carcinoma (31), serum and urine levels did not reflect the state of the disease. Hypozincemia associated with marked urinary zinc excretion was not seen in these patients with primary breast cancer.

The exact role of zinc in carcinogenesis is unknown. However, zinc is known to be essential for more than a hundred different metabolic functions. Important among these are included RNA and DNA polymerases, phosphodiesterases, adenylkinase, membrane-bound adenylcyclase and lipid peroxidase, blastogenic transformation of lymphocytes, and immune function. All suggest a role in carcinogenesis. Experimentally (30), zinc deficiency and zinc supplementation have each shown both inhibitory and stimulatory responses on tumor growth, adding confusion to the role of zinc in human cancer.

The levels of vanadium and selenium were significantly elevated in breast cancers compared to normal tissue. Vanadium is a ubiquitous essential trace metal experimentally linked to the membrane bound Na-K ATPase pump system (1, 3). High levels of vanadium reversibly inhibit the pump system and adenylkinase while stimulating adenylcyclase, glucose oxidation, and transfer.

While selenium in large concentrations is toxic, it is also an essential trace metal at low concentrations (4, 26). Deficiencies of selenium have been related to a cause of muscular dystrophy, pancreatic fibrosis, hepatosis dietetica, cancer, and certain disorders attributed to prostaglandin and vitamin E deficiency. Selenium and vitamin E protect membranes from oxidative degradation and prevent exudative diathesis. Selenium functions as a metalloenzyme glutathione peroxidase to reduce peroxides before they can attack the cell membrane. There have been numerous reports that geographical areas low in selenium have higher incidences of various cancers, heart disease, and muscular dystrophy (26).

In all 25 patients studied, the level of rubidium was 2- to fourfold higher in breast cancer than in normal breast tissue. Rubidium has no known physiological function; however, results obtained from various normal tissues indicate that, like other essential trace elements, rubidium has a comparatively low coefficient of variation which suggests some as yet unknown biological function. The physical properties of rubidium resemble those of potassium and may only reflect the higher concentration of potassium consistently found in various cancer tissues (5, 14).

Although the various findings presented here generate more questions than answers, they do give guidelines for future study into the possible roles and interactions of essential trace elements in the carcinogenic process. For example, vanadium, selenium, and zinc each appear to play an important role in various membrane functions. Selenium and zinc have also been shown to be antagonistic to each other in a number of metabolic systems (19, 28). A better understanding of the interrelationships of trace metals is obviously needed to better understand their role in regulating tumor growth.

This increased awareness of the role of trace elements, their interactions in metabolism and disease suggested the need for a multielement micromethod of analysis which could provide data quickly and efficiently for several elements in the same biological sample. The present study has shown the applicability of a photon excitation energy-dispersive X-ray spectrometer for the simultaneous determination of trace elements in microsamples of human tissues. This makes practical the gathering of needed base-line data on large populations.

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