Minor and Trace Elemental Contents of Cancerous Breast Tissue Measured by Instrumental and Radiochemical Neutron Activation Analysis

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ABSTRACT

Many minor and trace elements influence the permeability of cell membranes by competing for binding sites, and exert direct or indirect action on the carcinogenic process. Instrumental and radiochemical neutron activation analysis has been employed for the determination of more than 20 elements in normal and cancerous breast tissues of 6 patients. Most trace elements, viz., Zn, Cu, Mn, Co, Se, Br, As, Sb and Cd, are elevated in cancerous tissue, whereas lower levels are observed for Fe, Cs, I, and Sr. Similarly, concentrations of minor constituents, such as Na, K, P, Cl, and Mg, are enhanced compared to normal tissue. Several elements incorporate into the normal cell, change its enzymatic activity, and accelerate the growth of tumor.

Index Entries: Trace elements; neutron activation analysis; breast cancer; neoplastic tissue; malignancy; carcinogenic; solvent extraction; gamma spectrometry.

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INTRODUCTION

The essentiality of trace elements in a number of biological processes, through their action as activator or inhibitor of enzymatic reactions, is now well established. Furst (*1*) suggested that perhaps metals, aided by viruses, may penetrate living cells and either enhance or retard the kinetics of anabolic or catabolic enzymes. Versieck (*2*) has critically reviewed the trace elemental contents in human body fluids and various tissues, especially with regard to reference materials. During the last few years, several attempts have been made to correlate trace metal analysis data with the tumor tissues and serum from cancerous patients. Mega et al. (*3*) found a positive correlation between mortality from stomach cancer and zinc content of ground water and rice. Berg and Burbank (*4*) have compared trace metal concentration with the death rate from cancer, for major water basins in the US. Wright and Dormondy (*5*) have found elevated zinc levels in cancerous liver tissue. Some workers (*6–10*) have found elevated copper levels in serum of patients suffering from acute leukemia, lung cancer, and oral cavity.

There has been growing recognition that metal compounds are an important class of environmental and occupational carcinogens. Many compounds of Cd, Co, Cr, Ni, Zn, and Pb have been used to induce cancer in experimental animals, indicating that metal ions can interact with nucleic acids to influence base pairing and conformation (*11,12*). Such effects have been known to cause somatic mutations leading to cellular transformations. Cr, Ni, As, and Be have been shown as human carcinogens, and exhibit genetic toxicity in a number of test systems, suggesting that metagenesis is involved in the initiation of cancer by these metals.

The literature contains several studies of trace metal concentrations in paired samples of cancerous and normal tissues from the same human breast of an individual. Choice of paired samples avoids variations resulting from differences in age, diet, sex, hormonal status, medication, genetics, and other environmental factors. De Jorge et al. (*13*) were the first to employ colorimetric methods for determining Cu, Ca, Mg, and P in 13 sample pairs, from which Cu was found to be significantly higher in the tumors. Mulay et al. (*14*) reported the measurement of Al, Ba, Ca, Cr, Cu, Fe, Mg, Mn, Sn, Sr, Te, and Zn in 8 paired samples, by emission spectroscopy. In recent years, neutron activation analysis (NAA), an ideal technique for trace elemental analysis of biological samples, has been widely used by several workers (*15–18*). This technique has an advantage of having simultaneous multielemental capability, freedom from reagent and laboratory contamination, and an adequate sensitivity for most elements of interest (*19,20*). Schwartz et al. (*15*) first used NAA for the determination of 17 elements in 9 paired samples, and observed substantial differences for certain trace elements in malignant and normal breast tissues. Increase in the concentration of essential trace ele-

ments has been attributed to enhancement in the metabolism and enzymatic activity of cancerous tissue. Santoliquido et al. (*16*) have observed significantly higher concentrations of Mg and Zn in cancerous tissue. Most recently, Rizk and Sky-Peck (*21*) have reported the determination of 16 elements in 25 patients, using the EDXRF method, and found a characteristic pattern of elevation for Ca, V, Cu, Zn, Se, and Rb in neoplastic tissues. Also, Mangal and Kumar (*18*) have employed INAA for the determination of 10 elements in 17 paired samples, and observed a positive correlation of Fe, Zn, Cr, and so on, with the corresponding change in neucleocytoplasmic ratio.

In the present work, we have analyzed histologically normal and neoplastic human breast tissues obtained from 6 patients, by instrumental and radiochemical neutron activation analysis. The elements determined were Na, K, P, Cl, Zn, Cu, Mn, Co, Se, Br, As, Cd, Fe, Cs, Sr, Mg, I, and Sb. Ga, Rb, and Ag were also detected in some samples. An attempt has been made to understand the degree of disease relationship with the concentration of elements and their role in regulating tumor growth.

EXPERIMENTAL

Sample Collection

Paired samples of normal and malignant tissues were obtained from 6 patients who had undergone radical mastectomy for breast cancer. In each case, two specimens were removed from the same breast, using tungsten carbide scissors so as to avoid any metal contamination. A piece of cancerous breast tissue, weighing about 10–15 g, and an equal weight of normal breast tissue from the diagonally opposite site to the cancerous mass at a maximum distance were taken from the same breast. Microscopic examination of the malignant tissue showed infiltrating duct carcinoma, and care was taken to separate the two types of tissues. The excised surgical specimens were washed thoroughly to remove any blood, and kept in isotonic saline water (0.9% sodium chloride solution) in polythene containers.

Sample Preparation

After bringing the samples to the chemical laboratory on the same day, they were cut into pieces and thoroughly washed in boiling double-distilled water, to dissolve most of the low-melting fats. At least 10–15 washings were performed in each case, and all handling was done in Corning™ petridishes, employing a glass rod and a Teflon™-coated spatula. Afterwards, all water was removed by decantation, and then samples were dried under an infrared lamp at ~70°C, avoiding any charring. After complete drying, tissues were powdered in an agate mortar and irradiated in a cobalt-60 Gamma Chamber-900 to a dose of 2.5 Mrads.

Irradiation and INAA Counting Procedure

About 50 mg each of samples, synthetic standard (prepared by impregnating an appropriate mount of pure salt solution in nitrate form on Whatman filter paper), and comparators (NBS SRM 1577a Bovine Liver and IAEA H-4 Animal Muscle) were packed in pure aluminium foil (INDAL). For INAA, irradiations were carried out for 10 min, 1 h, and 10 h at a thermal-neutron flux of 10^{12}n cm $^{-2}$ s $^{-1}$ in the APSARA reactor at BARC, Bombay. For RNAA work, however, 80–100 mg each of samples and standards were encapsulated in quartz ampules and irradiated in the CIRUS reactor at a thermal-neutron flux of 10^{13}n cm $^{-2}$ s $^{-1}$ for 2 wk.

After a delay of 40 min samples were opened, decontaminated, and counted on a 45 cm^3 HPGe dector (EG & G ORTEC) and Canberra Series 35 Multiparameter MCA system in a fixed geometry at the radiochemistry laboratories of BARC, Bombay. Various nuclides were identified by their γ-ray energies. At least 4 countings were performed at successive intervals to follow the half-lives of short-lived nuclides (*22*). Phosphorus was determined by β^- counting ^{32}P after a delay of 3–4 wk, employing a gas-flow proportional counter (ECIL, Hyderabad) and Al filter (28 mg cm^{-2}), as already described (*23*).

RNAA Procedure

After a delay of 2 wk, samples were transported to our laboratories and decomposed in 5 mL of aquaregia and 2 mL of perchloric acid. Carrier solutions containing about 5–10 mg each of Fe, Zn, and Co from high-purity chemicals (AR, more than 99%) were added and heated to dryness. The contents were dissolved in 10 mL of 6 *M* HCl, and the solution was made to 50 mL. From a 10-mL aliquot of the stock solution, Fe was extracted with diethyl ether, followed by a 6% aqueous solution of cupferron and $CHCl_3$ at pH 4, from which rediochemically pure fractions were obtained. To the aqueous phase, 5% KCN was added to mask Co, and then Zn was extracted using 0.1 *M* 2-thenoyltrifluoroacetone (TTA) in isobutyl methylketone (IBMK) at pH 6. Finally, Co was extracted using α-nitroso β-naphthol in chloroform at pH 5 (*24*).

In another radiochemical procedure for Sb and Se, respective carriers were added before dissolution, and a stock solution was prepared in 0.5 *M* HCl. To a 10-mL aliquot, 2 mL of 2% citric acid solution was added for masking of Sb. Selenium was complexed with o-phenylenediamine, using a 0.1% solution in absolute alcohol. Two extractions were carried out, each with 10 mL of benzene. The aqueous phase was reduced to 2 mL and added 6 mL of 6 *M* H_2SO_4, 1 mL of 1 *M* KI, and 1 mL of 0.1% aqueous solution of 4-(2-pyridylazo)-resorcinol (PAR). Finally, Sb was extracted with 10 mL of benzene (*25*). In each of these cases an aliquot of the organic phase was counted on a well type 2 × 1.75 in (5.1 cm × 4.4 cm) (Tl) detector, coupled to a Nuclear Data ND 62 1K MCA and a Centronics 154 printer. Corrected areas for the respective photopeaks

were used for the calculation of elemental concentrations. Pure elemental standards were used as primary standards, and NBS SRM 1577a and IAEA H-4 were used as comparators for quality assurance.

RESULTS

In general, the precision of our INAA analysis is within the range of ±5% at ppm (μg/g) levels and ±5–10% at ppb (ng/g) levels, based on replicate analyses. It is observed that our INAA data for various elements in biological standards NBS SRM 1577a (Bovine Liver) and IAEA H-4 (Animal Muscle) are in good agreement with the certified or literature reported values (Table 1). Typical γ-ray spectra for cancerous and normal tissues, along with that of Bovine Liver, are shown in Fig. 1. Ranges of elemental abundances and mean values, along with the standard deviation of the mean concentration obtained by INAA and RNAA procedures, are given in Table 2. Enhancement or depression is expressed as the percent change in the mean value. Some other nuclides, such as those of Tb, Eu, Cr and so on, were also identified in some cases, but could not be quantified because of nonavailability of suitable standards. All values are expressed on a dry wt basis. In most cases, these concentrations are within the normal range for human tissues (*26*). Histograms of the mean concentrations of the trace elements in paired samples obtained from 6 patients are shown in Fig. 2.

DISCUSSION

It is observed from Table 2 that concentrations of all the minor and most trace elements are enhanced for all paired samples (not given separately) of cancerous and normal breast tissue. The increase in the mean concentration of Se is maximum (by almost twofold) in cancerous compared to that in normal tissue. It is followed by Co, As, P, K, Ga, Cd, Mg, and Cu, which all show an enhancement of up to 40%. However, concentration for quite a few trace elements, viz., Fe, Cs, Sr, and I, are depressed or only marginally changed. It is apparent that the change in concentration of these elements in cancerous tissue is real and establishes a definite pattern in the carcinoma of the breast.

The essentiality of Se has been recognized in recent years, but in excess it may be deleterious (*27, 28*). Several workers have observed its enhancement in cancer of the breast (*15,18,21*) and other tissues (*12,29*). Ehmann and coworkers (*30–32*) have observed significant differences in Se level for Alzheimer's disease patients, and its role in aging. On the other hand, Vernie et al. (*33*) have not found any significant change in Se levels of whole blood and plasma from breast cancer patients. Further, Se also seems to be correlated with other trace metals such as Fe, Co, and Zn. Selenium and Zn have been found to play an important role in

Table 1
Comparison of Elemental Concentrations in Standards

Element	Bovine liver SRM 1577a		Animal muscle H-4	
	This work	Lit. value	This work	Lit. value
Na (%)	0.239 ± 0.003	0.243	0.208 ± 0.020	0.206
P (%)	1.097 ± 0.028	1.110	0.693 ± 0.013	0.683
K (%)	0.994 ± 0.018	0.996	1.56 ± 0.04	1.58
Fe (μg/g)	191 ± 2.0	194	51.1 ± 0.9	49.1
Mn (μg/g)	10.04 ± 0.15	9.9	0.49 ± 0.04	0.52
Cu (μg/g)	163 ± 4	158	3.62 ± 0.23	3.65
Zn (μg/g)	127 ± 7.8	123	80.6 ± 1.6	86.0
Cd (μg/g)	0.45 ± 0.02	0.44	4.5 ± 0.3 (ppb)	4.9 (ppb)
Cs (μg/g)	0.26	NA	–	0.12
Sr (μg/g)	–	0.138	0.325	NA
Se (μg/g)	0.76 ± 0.12	0.71	0.24 ± 0.02	0.28
Br (μg/g)	9.46 ± 0.59	9.0	4.12 ± 0.05	4.10
Ga (μg/g)	1.66 ± 0.06	–	1.80 ± 0.16	–
I (ng/g)	3.30 ± 1.5		–	14.3
Sb (ng/g)	2.4	3.0	1.33	–
As (ng/g)	43.3 ± 2.0	47.0	7.77 ± 0.33	7.0

malignant breast tissue (*18,21*). Selenium deficiency has been asserted to be the cause of muscular dystrophy, pancreatic fibrosis, hepatosis, dietetosis, dietetica, cancer, and certain disorders attributed to vitamin E deficiency. Selenium and vitamin E protect membranes from oxidative degradation, and prevent exudative diathesis. Recently, Se deficiency in soils and diets in the Keshan province of China has been stated to cause heart disease (*33*). It is well known that Se is incorporated into at least 6 sulfur-containing amino acids, and Se can replace S, making them ineffective (*34*). Therefore, it is quite likely that replacement of S by Se may accelerate the growth of tumors.

Experimentally, Zn deficiency and its supplementation have each shown both inhibitory and stimulatory responses on tumor growth (*35*). It has been shown that Se and Zn are antagonistic to each other in a number of metabolic systems (*36*).

Several workers have reported the elevation of Zn and Cu levels in breast carcinoma (*16,18,21*). The present study also confirms these results with a consistently elevated level of both the elements in all 6 paired breast cancer tissues. The exact role of Zn in carcinogenesis is unknown (*37*). However, it is known to be essential for more than a hundred different metabolic functions. Copper is absorbed as an amino acid complex in the intestinal walls, and also is deposited in the brain of the patients with obvious pathological consequences (*38*). A better understanding of the interrelationships of these trace metals is needed in order to better understand their roles in regulating tumor growth.

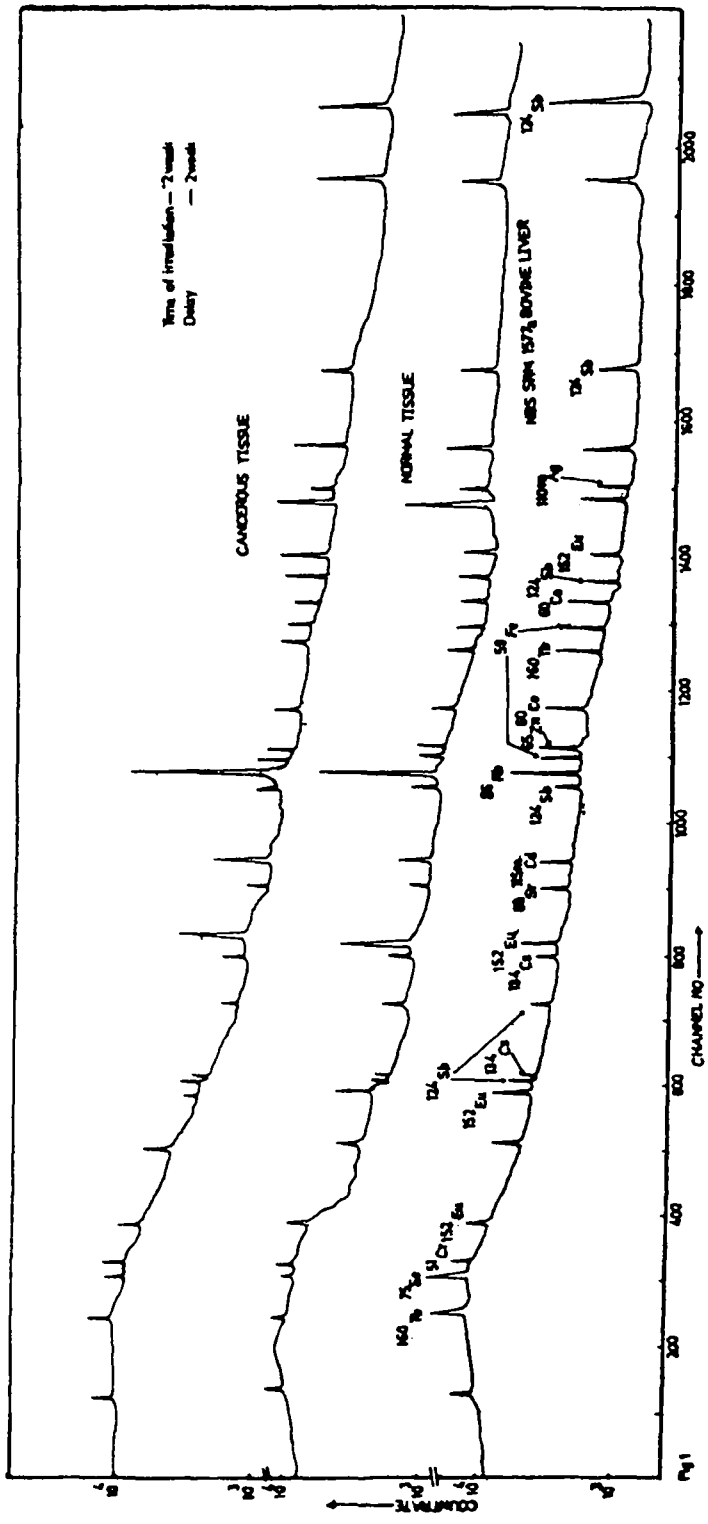

Fig. 1. Typical γ-ray spectra of cancerous and normal breast tissue, along with NBS SRM 1577a.

Table 2
Elemental Concentrations in Normal and Cancerous Human Breast Tissue

Element	Normal		Cancerous		Change, %†
	Range	Mean ± SD	Range	Mean ± SD	
Na (%)	0.09–0.49	0.22 ± 0.15	0.16–0.61	0.31 ± 0.14	+40.9
K (%)	0.06–0.31	0.18 ± 0.10	0.16–0.44	0.29 ± 0.11	+61.0
Mg (%)	0.04–0.17	0.11 ± 0.07	0.10–0.18	0.14 ± 0.04	+38.1
Cl (%)	0.09–0.31	0.18 ± 0.09	0.13–0.35	0.23 ± 0.08	+30.0
P (%)	0.11–0.37	0.25 ± 0.13	0.30–0.50	0.41 ± 0.08	+65.2
Fe (μg/g)	19.0–43.1	32.0 ± 8.3	20.0–45.0	25.8 ± 7.8	−19.3
Zn (μg/g)	22.0–55.0	36.6 ± 10.2	30.0–62.6	43.8 ± 10.5	+20.0
Cu (μg/g)	0.76–2.33	1.32 ± 0.55	1.11–3.62	1.80 ± 0.92	+36.4
Se (μg/g)	0.64–0.93	0.81 ± 0.08	1.20–1.84	1.58 ± 0.10	+95.0
Cd (μg/g)	0.15–0.50	0.36 ± 0.13	0.32–0.80	0.52 ± 0.18	+44.2
Mn (μg/g)	0.23–0.60	0.43 ± 0.12	0.38–0.62	0.49 ± 0.08	+13.9
Br (μg/g)	2.0–54.0	19.0 ± 21.0	6.0–70.0	25.0 ± 21.0	+31.6
Ga (μg/g)	1.4–2.3	1.9 ± 0.4	1.7–2.5	2.8 ± 0.3	+47.4
Cs (μg/g)	1.4–5.0	3.7 ± 0.6	2.0–5.5	3.2 ± 1.4	−13.5
Rb* (μg/g)	–	4.3	–	6.1	+42.8
Sr (ng/g)	10.0–105	62.3 ± 27.6	30.0–107	73.8 ± 32.7	−18.4
As (ng/g)	5.6–14.0	9.0 ± 3.0	5.0–32.0	16.6 ± 10.1	+84.4
Co (ng/g)	1.0–5.0	3.1 ± 1.1	1.3–7.0	4.9 ± 1.9	+55.4
Sb (ng/g)	0.25–0.43	0.33 ± 0.13	0.18–0.64	0.35 ± 0.19	+6.1
I (ng/g)	3.3–19.0	8.8 ± 5.6	2.5–12.0	5.2 ± 3.7	−41.1
Ag (ng/g)	25–54	43.8 ± 11.6	43–58	53 ± 5.8	+21.0

*These values are for a single case only.

†Expressed as ratio of change in mean value and mean concentration in normal × 100; +ve sign indicates enhancement and −ve sign indicates depression.

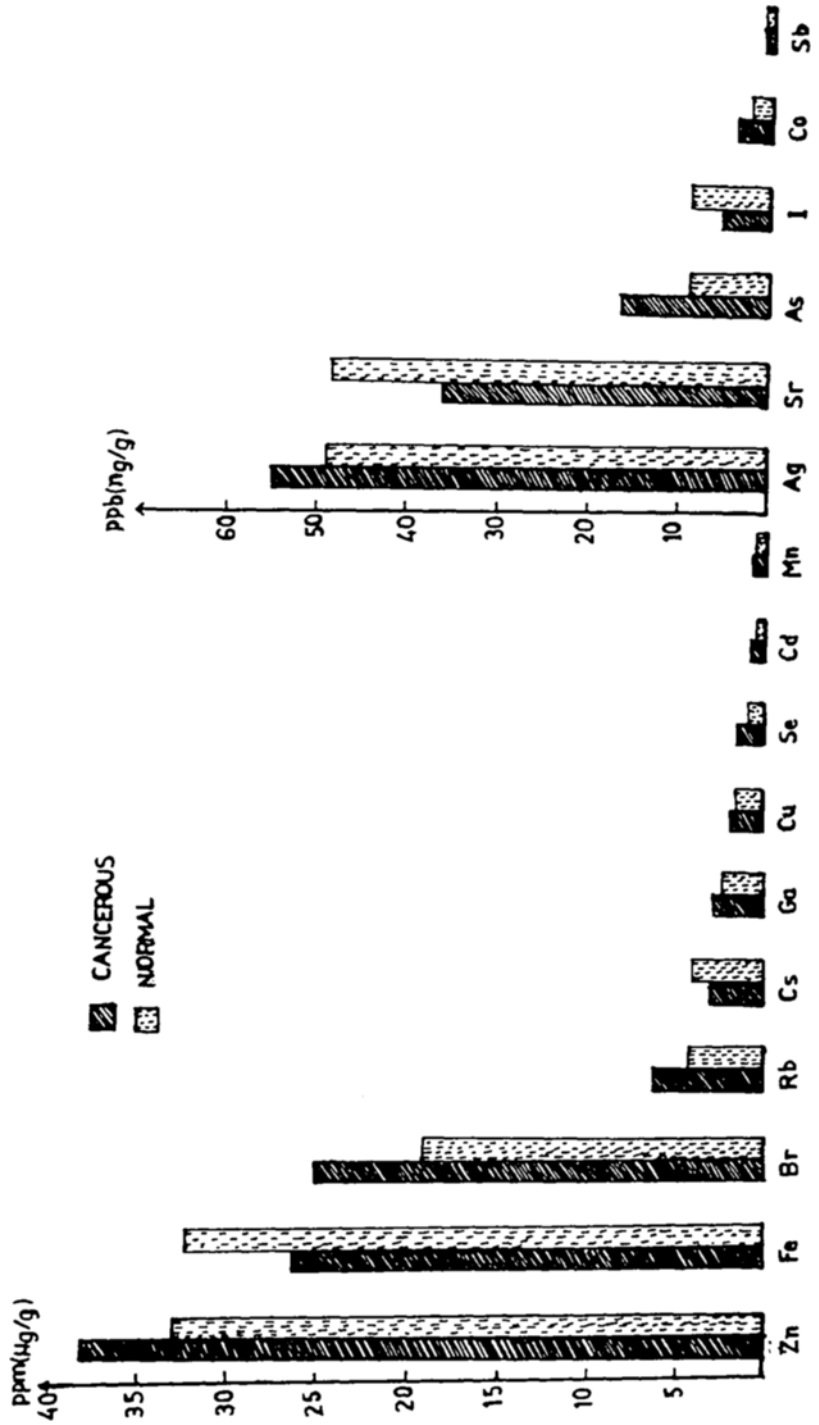

Fig. 2. Comparison of mean trace elemental contents in normal and cancerous breast tissues from 6 patients.

Rubidium has been added to the list of essential trace elements only recently, but at present it has no known physiological function. We obtained Rb data in only one case of significant enhancement, in agreement with the observaiton of Rizk and Sky-Peck (*21*). The physical properties of Rb closely resemble those of K, and may only reflect the higher concentration of K consistently found in various cancer tissues (*39*). It may simply be a reflection of the largely intracellular location of K in normal tissues, and the high cellular nature of malignancy. Our observaiton of enhancement in concentrations of minor constituents, such as K, Mg, and P, is in agreement with earlier reports (*13–16*). Seltzer at al. (*40*) have reported a twofold increase in Mg concentration in human breast cancer. Interestingly, Cs, another alkali metal, and Sr, an alkaline earth, both show depletion in cancerous tissue.

The decrease in Fe concentration of the cancerous tissue, though small, is in agreement with the observation of other workers (*12,14,15*), even though Santoliquido et al. (*16*) have found increased Fe in lymphatic leukemia. It is well known that ferritin serves as a major iron storage constituent of the tissue, but it has also been found to be associated with the blood circulation in patients with disease (*41,42*). This could possibly be the cause for depletion of Fe in cancerous breast tissue. Among the halogens, Br is enhanced, but I is depressed in cancerous tissue.

These studies only suggest that some elements are incorporated into normal cell, change its enzymatic activity, and help in reduplication of neoplastic growth. Generally, enzymatic activities change in cancerous cell, resulting in variations in trace elemental concentrations, which may act as cofactors of these enzymes.

Although present data are not conclusive, they do give guidelines for future study into the possible role and interaction of essential as well as nonessential trace elements in the carcinogenic process. The increased awareness of the role of trace elements, and their interactions in metabolism and cancer growth, suggested the need for a multielemental microanalytical method that could provide rapid analysis in the same biological specimen. Clearly NAA employing short irradiation and radiochemical procedures is most suitable for such studies. At this stage, it can only be said that further work is required to understand fully the role of trace elements in neoplastic growth.

SUMMARY

A sequential irradiation neutron activation analysis with radiochemical procedures has been developed, and permits the determination of more than 20 elements in cancerous and normal breast tissue. Concentrations of several trace elements are enhanced, whereas those of others are depressed, indicating their role in the growth of tumor.

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REFERENCES

1. A. Furst, *Metal Binding in Medicine,* M. J. Seven and L. A. Johnson, eds. J. B. Lippincott, Philadelphia and Montreal, 1960, pp. 336–344.
2. J. Versieck, *CRC Crit. Rev. Clin. Lab. Sci.* **22,** 97 (1985).
3. T. Mega, S. Tonici, and H. Aruchi, *Nara Igaku Zasshi* **23,** 201 (1972).
4. J. W. Berg and F. Burbank, *Ann. NY Acad. Sci.* **199,** 249 (1972).
5. E. B. Wright and T. L. Dormondy, *Nature* **237,** 166 (1972).
6. M. Hrgovic, C. F. Tessmer, and B. W. Brown *Progr. Clin. Cancer* **5,** 121 (1973).
7. M. Hrgovic, C. F. Tessmer, and F. B. Thomas *Cancer,* **31,** 1337 (1973).
8. H. T. Delves, F. W. Alexander, and H. Lay *Br. J. Haematol.,* **24,** 525 (1973).
9. S. Inutsuka, S. Araki, and I. Kusaba, *Rinsho Byori* **21,** 632 (1973).
10. I. T. Tsilyunyk, M. M. Ischenko, and S. U. Rusenko, *Klin. Med.* **43,** 18 (1965).
11. M. A. Sirover and L. A. Loebb, *Science* **194,** 1434 (1976).
12. P. C. Mangal and K. B. Verma, *Indian J. Med. Res.* **69,** 290 (1979).
13. F. B. DeJorge, J. G. Sampalo, J. L. Guedes, and A. B. DeUlhoa, *Clin. Chim. Acta* **12,** 403 (1965).
14. I. L. Mulay, R. Ray, B. E. Knox, N. H. Suhr, and W. E. Dalaney, *J. Natl. Cancer Inst.* **47,** 1 (1971).
15. A. E. Schwartz, G. W. Leddicotte, R. W. Fink, and E. W. Friedman, *Surgery* **76,** 325 (1974).
16. P. M. Santoliquido, H. W. Southwick, and J. H. Oliwin, *Surg. Gynecol. Obstet.* **142,** 65 (1976).
17. I. Othman and N. M. Spyrou, *Trace Elements in Medicine and Biology,* P. Bratter and P. Schramel, eds., Walter de Gruyter Co., New York, (1980), p. 199.
18. P. C. Mangal and S. Kumar, *Indian. J. Phys.* **58A,** 355 (1984).
19. J. C. K. Lai, A. W. K. Chan, M. J. Minski, T. K. C. Leung, L. Lim, and A. N. Davison, *Metal Ions in Neurology and Psychiatry,* S. Gabay, J. Haris, and B. T. Ho, eds., Alan R. Liss, New York, 1985, p. 323.
20. W. D. Ehmann, W. R. Marksbery, E. J. Kasarskis, D. E. Vance, S. S. Khare, J. D. Hord, and C. M. Thompson, *Biol. Trace Element Research,* **13,** 19 (1987).
21. S. L. Rizk and H. H. Sky-Peck, *Cancer Res.* **44,** 5390 (1984).
22. H. K. Wankhade, D. L. Samudralwar, and A. N. Garg, *J. Radioanal, Nucl. Chem. Letters* **105,** 95 (1986).
23. R. G. Weginwar, D. L. Samudralwar, and A. N. Garg, *J. Radioanal. Nucl. Chem.* **133,** 317 (1989).
24. R. G. Weginwar and A. N. Garg, Radiochemistry and Radiation Chemistry Symposium, IGCAR, Kalpakkam, Jan 4–7, 1989, RA-01.
25. R. G. Weginwar and A. N. Garg, unpublished results.

26. G. V. Iyengar, W. E. Kollmer, and H. J. M. Bowen, eds. *Trace Elemental Composition of Human Tissues and Body Fluids*, Verlag Chemie, Heidelberg, 1978 p. 151.
27. C. D. Thomsen and M. F. Robinson, *Am. J. Clin. Nutr.* **33,** 303 (1980).
28. J. E. Spallholz, J. L. Martin, and H. E. Ganther, eds., *Selenium in Biology and Medicine,* AVI Publishing Co. Inc., Westport, USA, 1981, p. 98.
29. P. C. Mangal and J. S. Sohal, *Indian J. Exp. Biol.* **16,** 685 (1978).
30. W. D. Ehmann, W. R. Markesbery, M. Alauddin. T. I. M. Hossain, and E. H. Brubaker, *Neurotoxicology* **7,** 197 (1986).
31. D. E. Vance, W. D. Ehmann, and W. R. Markesbery, *Neurotoxicology* **9,** 197 (1988).
32. W. R. Markesbery, W. D. Ehmann, M. Alaudin, and T. I. M. Hossain, *Neurobiol. Aging* **5,** 19 (1984).
33. L. N. Vernie, M. De Vries, C. Benekhuijsen, J. J. M. Goeij, and C. Zegers, *Cancer Lett.* **18,** 283 (1983).
34. Keshan Disease Research Group of the Chinese Academy of Medical Sciences, *Chin. Med. J.* **92,** 471 (1979).
35. L. M. Cummins and J. L. Martin, *Biochemistry* **6,** 3162 (1967).
36. E. J. Underwood, *Trace Elements in Human and Animal Nutrition, 4th Ed.,* Academic Press Inc., New York, 1977.
37. A. S. Prasad, ed. *Trace Elements in Human Health and Disease, Vol. 1 Zinc and Copper,* Academic Press, New York 1976.
38. A. Lipcsey, M. Ordogh, J. Fekete, and E. Szabo, *J. Radioanal. Nucl. Chem.* **88,** 57 (1985).
39. A. Danielson and E. A. Steinnes, *J. Nucl. Med.* **11,** 250 (1970).
40. M. H. Seltzer, F. E. Rosato, and M. J. Fletcher, *J. Surg. Res.* **10,** 159 (1970).
41. P. A. E. Jones, F. Miller, M. Warwood, and A. Jacobs, *Br. J. Cancer,* **27,** 212 (1973).
42. A. Jacob and M. Warwood, *Br. J. Cancer* **34,** 162 (1976).