A STUDY OF SOME SELECTED TRACE ELEMENTS IN NORMAL AND CANCEROUS TISSUE BY NEUTRON ACTIVATION ANALYSIS

A. Danielsen* and E. Steinnes

Rikshopitalet, Oslo, Norway and Institutt for Atomenergi, Isotope Laboratories, Kjeller, Norway

Neutron activation analysis is known to be a sensitive analytical technique for the determination of most chemical elements, showing high accuracy even at very low concentration levels. This technique has therefore appeared to be advantageous for the study of minor and trace elements in small biological samples (*1,2*).

The possible role of trace elements in cancer formation has not been extensively studied. In the work of Wester *et al* (*3*) a number of elements were determined in a few samples obtained at autopsy.

In the present work another approach to this type of research has been attempted. Small biopsies were removed by surgical operation. If possible, cancerous tissue as well as normal tissue from the same organ was taken. In most cases three parallels or more were collected. In this manner the possibility is good of obtaining a representative sample, unaffected by surface contamination.

In most previous investigations of trace elements in soft tissue, the concentrations of the elements have been related to the wet weight of the samples. In the authors' experience it is difficult to determine the correct wet weight of samples as small as those studied in the present work because of the continuous loss of weight due to evaporation during the weighing process. It was therefore decided to relate the results to dry weight obtained as described in the experimental section.

SELECTION OF ELEMENTS TO BE STUDIED

The present study was concentrated mainly on copper and zinc, because these elements are known to have certain biological functions in man and also because they are readily determined by neutron activation analysis.

Copper is located predominantly in the nuclei of the cells but is also found in the mitochondria (*4*). The element participates in the cell metabolism, being present in various enzymes such as tyroxinase, uricase and cytochrome oxidase—mainly those concerned with oxidation. Excessive tissue deposition of copper is observed in Wilson's disease (hepatilenticular degeneration), characterized by deficiency of ceruloplasmin, the copper-binding globulin of normal plasma (*5*). The highest concentrations of copper are found in the liver, particularly in newborn children. Increased concentrations of liver copper have been observed in connection with cirrhosis, hemochromatosis and tuberculosis (*6*).

Zinc is a constituent of several enzymes participating in the chemical processes within the cells, such as carbonic anhydrase, glutamic, lactic and alcoholic dehydrogenases and alkaline phosphatase. The element is also important in activating certain enzymes (*7,8*). It is located almost entirely in the cytoplasm of the cell (*4*). High concentrations of zinc in human tissue have been measured in the prostate gland (*8*), in the pancreas (*8*) and in the skin (*9*). It has been shown that zinc deficiency during pregnancy results in congenital malformations in rats (*8*). Zinc injections in rats protect against malignant tumor development induced by cadmium. On the other hand, zinc has been shown under certain conditions to have cancerogenic activity when injected in testis of rats (*10*).

Because most of the known biochemical functions of copper and zinc are concentrated within the cells, it seemed useful to relate the results to some property coherent with the intracellular space.

Potassium is mainly localized intracellularly, where its concentration is high and relatively constant compared to the small amounts of trace elements. The determination of potassium was therefore included in the analysis.

Received July 7, 1969; revision accepted Jan. 26, 1970.

For reprints contact: E. Steinnes, Institutt for Atomenergi, Isotope Laboratories, Kjeller, Norway.

* Present address: Flekkefjord Sykehus, Flekkefjord, Norway.

Rubidium. Little is known about the function of the alkali metal rubidium in human medicine. Rubidium is easily determined simultaneous with potassium by the analytical method used in this work. Hence, the values for this element were also recorded.

METHODS

Collection of samples. The specimens of cancerous and corresponding normal tissue were obtained at surgical operations. A few normal liver samples used for testing the method were obtained at autopsy of persons who had died by accidents. The samples were dissected with a thoroughly rinsed quartz knife and placed on plastic plates for drying at a temperature of 80°C, maintained long enough to obtain constant weight, usually 1–2 hr. Weighing was performed with a Kahn balance, the samples being kept on the plastic plates. Specimens of the plastic plates were investigated by neutron activation analysis to make sure that the plastic did not contain the actual elements in concentrations sufficiently high to contaminate the biopsy samples significantly.

The dry weight of the samples varied from 3 to 35 mg, most of them weighing 10–20 mg.

Irradiation. The dry specimens were sealed in clean separate silica ampoules and irradiated together with an ampoule containing 0.100 ml of a standard solution with copper, zinc, potassium and rubidium in known concentrations. Irradiations were performed for 5 days at a neutron flux of about 1.5×10^{13} n/cm^2/sec in the JEEP II reactor (Kjeller, Norway).

Radiochemical separation procedure. After a delay of one day to allow the decay of the 2.6-hr ^{31}Si activity in the ampoules, the separation of the desired activities was started. The content of the standard ampoule was quantitatively transferred to a 100 ml volumetric flask and diluted to the mark with dilute nitric acid. From this solution, three aliquots of 1.00 ml each were withdrawn and treated separately in the same manner as the biopsy samples. The approximate content of each element in a standard aliquot was as follows: copper, 1 μg; zinc, 5 μg; rubidium, 5 μg; and potassium 100 μg.

Samples and standard aliquots were transferred to 100-ml Ehrlenmeyer flasks containing 5 ml concentrated HNO_3, 1 ml concentrated H_2SO_4 and 1 ml of the standard mixture acting as carrier. These were treated in the following manner:

1. The solution was heated on a hot plate until white fumes of SO_3 occurred. If the solution turned dark, more HNO_3 was added.
2. Five milliliters of H_2O and 1 ml 30% hydrogen peroxide were added, and the solution was again heated to incipient fumes of SO_3. This step was performed to release possible ^{82}Br activity.
3. After cooling, 10 ml 6 *M* HCl was added, and the solution was transferred to the top of an anion exchange column (Dowex 1–X8, 100–200 mesh), pre-equilibrated with 6 *M* HCl. The column was washed with 5 x 2 ml 6 *M* HCl. Eluate and washings were combined and set aside for the determination of potassium and rubidium.
4. Copper was eluted with 3 x 5 ml 0.5 *M* HCl. The column was washed with additional 5 ml to remove possible traces of ^{59}Fe.
5. Zinc was eluted with 3 x 5 ml 2% NH_4OH.
6. The eluate from Step 2 was evaporated down to a few milliliters and made alkaline with 1 *M* NaOH. Potassium and rubidium was precipitated from hot solution with 100 mg sodium-tetraphenylborate in 10 ml 1 *M* NaOH. After 2 hr the precipitate was filtered onto a membrane filter, washed with 1 *M* NaOH and transferred to a counting vial.

Activity measurements. The gamma activity of the separated fractions was recorded by a well 3 x 3-in. NaI(Tl) detector connected to a 400-channel pulse-height analyzer. The following radionuclides were made a basis for the analyses: 12.8-hr ^{64}Cu, 245-day ^{65}Zn, 12.5-hr ^{42}K and 18.6 day ^{86}Rb. The measurements of copper and potassium were performed a short time after the end of the separation while zinc and rubidium were measured after 1 week. The use of 13.8-hr ^{69m}Zn was rejected because of interference in some samples from 2.7-day ^{198}Au which emits gamma rays of similar energy. The peak-area measurements were performed according to the method of Covell (*11*).

Chemical yield. The chemical yields of the separation procedure were tested by experiments with radioactive tracers and found to be higher than 95% for all four elements involved. Because of the equal chemical treatment, the yield is not significantly different for samples and standards. A separate step for chemical yield determination was therefore found unnecessary.

Testing the method. The present method was tested on six series of liver biopsies, each consisting of five samples, from different persons. The results of this investigation are summarized in Table 1. The relative standard deviation of a single value is presented for each element as a measure of the precision of the method. Besides factors normally affecting the analytical precision such as neutron-flux gradients, statistical counting errors, weighing errors and variation

TABLE 1. PRECISION OF METHOD IN ACTUAL CONCENTRATION REGION*

Sample	Average weight (mg)	Cu (%)	Zn (%)	K (%)	Rb (%)
A	3.35	5.7	9.1	5.4	6.3
B	4.27	3.8	9.7	3.9	3.6
C	9.7	4.5	10.6	3.4	5.8
D	11.2	4.4	4.8	8.1	7.5
E	18.1	3.8	7.0	5.5	8.2
F	30.2	4.0	8.3	4.0	4.7
Average		4.4	8.2	5.0	6.0

* Expressed as relative standard deviation of single value.

in chemical yield, these figures also include possible variation due to inhomogeneous distribution of the actual elements in the tissue. The precision is seen to be essentially independent of the sample weight, indicating that the observed spread is purely analytical. Consequently, the samples of normal liver studied in this work are probably homogeneous with respect to the elements studied.

RESULTS

Table 2 shows a summary of results on the samples of cancerous tissue analyzed in this work. The patients from whom the biopsies have been taken were all operated upon for malignant tumors. As can be

TABLE 2. SUMMARY OF RESULTS ON MALIGNANT TUMOR PATIENTS

Sample no.		Cu (ppm)	Zn (ppm)	K (%)	Rb (ppm)	K/Cu	K/Zn	K/Rb	Sex	Age	Diagnosis
1	N	20.8 ± 0.5	161 ± 8	1.03 ± 0.07	32.4 ± 2.0	490	64	320	M	1	Hepatoma (carcinoma hepatis)
	T	28.2 ± 4.0	78 ± 9	1.72 ± 0.20	43.2 ± 6.5	620	219	400			
2	N	28.2 ± 2.7	266 ± 3	1.24 ± 0.01	21.4 ± 0.7	440	46	580	M	72	Hepatoma (carcinoma hepatis)
	T	15.4 ± 5.5	85 ± 2	1.80 ± 0.01	25.0 ± 1.4	1,220	212	720			
3	N	27.5 ± 0.4	171 ± 13	1.38 ± 0.01	55.3 ± 2.5	500	81	250	M	47	Carcinoma coli
	T	15.5 ± 3.9	213 ± 1	2.11 ± 0.13	73.5 ± 2.1	1,400	99	290			
4	N	11.1 ± 3.6	122 ± 21	0.53	—	490	44	—	F	84	Carcinoma coli
	T	16.3 ± 2.1	84 ± 17	0.34 ± 0.02	—	210	42	—			
5	N	13.2 ± 4.5	82 ± 16	1.10 ± 0.12	58.0 ± 9.0	810	136	190	M	50	Carcinoma ventriculi
	T	11.4 ± 0.7	33 ± 8	0.89 ± 0.09	41.9 ± 4.6	780	279	210			
6	N	4.4 ± 0.7	76 ± 37	0.98 ± 0.26	16.5 ± 3.8	2,200	133	590	F	68	Carcinoma ventriculi
	T	4.7 ± 0.4	88 ± 3	1.93 ± 0.04	31.1 ± 1.0	4,110	220	620			
7	N	11.2 ± 0.4	94 ± 12	0.90 ± 0.08	26.2 ± 1.8	800	96	350	M	61	Carcinoma renis
	T	8.2 ± 0.7	38 ± 12	0.62 ± 0.17	13.9 ± 5.0	770	153	460			
8	N	11.2 ± 0.7	59 ± 12	0.78 ± 0.01	18.1 ± 2.1	700	134	440	M	60	Carcinoma pulmonis
	T	8.6 ± 1.6	63 ± 2	1.31 ± 0.05	30.0 ± 0.4	1,550	209	440			
9	T	9.7 ± 0.9	40 ± 1	0.56 ± 0.11	15.0 ± 2.4	580	140	370	M	63	Carcinoma pancreatis
10	T	9.6 ± 1.3	39 ± 12	0.76 ± 0.03	22.6 ± 4.8	800	200	340	F	53	Neurofibroma maligna pulmonis
11	T	8.8 ± 1.0	95 ± 25	0.67 ± 0.09	18.7 ± 1.7	770	73	360	F	70	Hepatoma (carcinoma hepatis)
12	T	10.2 ± 1.0	78 ± 17	2.02 ± 0.26	37.8 ± 4.2	2,060	257	530	M	71	Haemangiopericytoma maligna mesenterii

N = normal; T = tumor.

TABLE 3. CONTENT OF Cu, Zn, K AND Rb IN SAMPLES OF NORMAL LIVER

Sample no.	Cu (ppm)	Zn (ppm)	K (%)	Rb (ppm)	Sex	Age (yr)
13	30.6 ± 2.0	268 ± 30	1.05 ± 0.06	51.0 ± 3.9	M	43
14	20.7 ± 1.5	180 ± 18	0.97 ± 0.04	26.9 ± 1.2	M	70
15	28.2 ± 1.2	184 ± 12	0.87 ± 0.05	26.0 ± 2.2	M	10
16	65.0 ± 2.1	92 ± 9	1.02 ± 0.04	52.1 ± 2.6	F	45
17	22.4 ± 1.2	182 ± 9	0.71 ± 0.05	30.6 ± 2.0	M	15
18	39.4 ± 3.7	192 ± 21	0.79 ± 0.05	17.2 ± 0.6	F	10
19	27.1 ± 3.0	137 ± 12	0.89 ± 0.04	13.6 ± 1.7	M	
20	27.1 ± 1.2	228 ± 4	1.00 ± 0.04	34.0 ± 1.4	F	56
Mean value, dry weight	33.0 ± 14	183 ± 52	0.91 ± 0.12	31.0 ± 14		
Mean value, wet weight	6.6 ± 2.8	37 ± 10	0.18 ± 0.02	6.2 ± 2.8		
Pan and Taylor (12), wet weight	6.9 ± 1.7	67 ± 20	—	—		
Underwood (5), wet weight	—	54.9	—	—		
Tipton and Cook (13), dry weight	19	130	0.74	—		
Yamagata (14), dry weight	—	—	—	30		

seen from the table, the types and sites of the tumors were different. In eight of 12 cases, normal as well as malignant tissue has been analyzed. Ten of the tumors were carcinomas while the two remaining were sarcomas. All diagnoses have been verified by histological examination.

Besides data for copper, zinc, potassium and rubidium, Table 2 also shows the ratios K/Cu, K/Zn and K/Rb. The figures are mean values of 2–5 single samples. The limits of error quoted for each value is standard deviation calculated from the observed range. The limits of error have been included in the table to emphasize the fact that in some cases, especially in some of the tumors, the observed scattering of single values is considerably higher than the corresponding spread obtained in the normal material tests. This indicates an inhomogeneous distribution of the actual element in the tissue from which the samples have been taken.

In Table 3, abundance data for the four elements on normal liver from eight individuals are presented. These samples were obtained either at autopsy or at operations on patients assumed to have normal liver function. The mean values of this table have been transformed to wet weight basis assuming that the dry weight constitutes about 20% of the wet weight (*3*) to facilitate a comparison with previous literature data on human liver related to wet weight (*5,12*) as well as dry weight (*13,14*). The present data appear to be in reasonable agreement with the literature values in most cases.

DISCUSSION

The basis for comparison of normal and cancerous tissue is not the same in all kinds of tumors. For some of the tumors, such as hepatomas and pancreatic cancer, the cells and structure of tumor tissue and normal tissue are so similar that they can be directly compared. The specimens of normal tissue from the wall of colon and ventricle, however, do not contain only epithelial or glandular cells from which the tumor cells are developed. The wall also contains different amounts of other cells, such as muscle cells and fibrous tissue. Furthermore, the inner surface of colon and ventricle may have been contaminated by food rests. These assumptions are supported by the spread of parallels observed in analysis of the normal samples of colon and ventricle. For the other types of organs, the results for tumor samples generally show higher scattering of single values than do the corresponding results for normal tissue.

By examining the data presented in Table 2 and Table 3, some characteristic features become apparent for each of the elements studied:

Zinc. It appears that the concentration of zinc is in general lower in cancerous tissue than in corresponding normal tissue. This difference becomes still more significant if the ratio K/Zn is looked at. In the normal liver samples (for example), with only one exception, this ratio is in the range 40–75, while the figures for cancerous samples are of the order of 200. In all samples except those of colon, this trend is evident.

Copper. No significant difference is observed by examining the ppm values of normal and cancerous tissue. If the results are related to potassium, however, the same trend is found as for zinc, although less pronounced.

Potassium. The potassium content is significantly different in cancerous and noncancerous tissue in all cases where comparison can be made. The most probable reason for this is perhaps a different content of fibrous tissue in the two types of samples. The possibility of a real difference in electrolyte concentration within the cells, however, cannot be excluded.

Rubidium. The ratio K/Rb does not show appreciable difference in tumor and corresponding tissue, thus making the possible role of rubidium in cancer formation unlikely. It should be noted, however, that the individual variation of this ratio in the same organ is appreciable; for example, the range for normal liver is 220–660.

As far as zinc is concerned, it has been previously shown experimentally that this element is associated with cell reproduction and wound healing (*15*). Furthermore, the leukocyte zinc concentration is markedly depressed in leukemias (*16*).

It must be emphasized that the present investigation is of a preliminary nature and that the experimental material is not sufficiently extensive to permit a statistical evaluation of the data. Nevertheless, certain trends in the results indicate that further research in this area might be worth while.

REFERENCES

1. BERGSTRÖM, J.: Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest.* **68**:14, 1962.

2. BRUNE, D. AND SJÖBERG, H. E.: Determination of magnesium in needlebiopsy samples of muscle tissue by means of neutron activation analysis. *Anal. Chim. Acta* **33**:570, 1965.

3. SAMSAHL, K., BRUNE, D. AND WESTER, P. O.: Simultaneous determination of 30 trace elements in cancerous and non-cancerous human tissue samples by neutron activation analysis. *Intern. J. Appl. Radiation Isotopes* **16**:273, 1965.

4. HARRISON, M. F.: Composition of the liver cell. *Biochem. J.* **55**:203, 1953.

5. UNDERWOOD, E. J.: *Trace Elements in Human and*

Animal Nutrition, Academic Press, New York, 1962.

6. COMAR, C. L. AND BRONNES, F.: *Mineral Metabolism,* vol. 2, part B, Academic Press, N.Y., 1964, p. 447.

7. COMAR, C. L. AND BRONNER, F., eds.: *Mineral Metabolism,* vol. 1, part A, Academic Press, N.Y., 1964, p. 158.

8. HUXLEY, L. S. AND SWENERTON, H.: Zinc deficiency and congenital malformations in the rat. *Nutr. Rev.* **25**:157, 1967.

9. HENZEL, J. H., DEWEESE, M. S. AND PORIES, W. J.: Significance of magnesium and zinc metabolism in the surgical patient. *Arch. Surg.* **95**:991, 1967.

10. GUNN, S. A., GOULD, T. C. AND ANDERSON, W. A. D.: Effect of zinc on cancerogenesis by cadmium. *Proc. Soc. Exp. Biol. Med.* **115**:653, 1964.

11. COVELL, D. F.: Determination of gamma-ray abundance directly from the total absorption peck. *Anal. Chem.* **31**:1785, 1959.

12. PARR, R. M. AND TAYLOR, D. M.: The concentration of cobalt, copper, iron and zinc in some normal human tissues as determined by neutron activation analysis. *Biochem. J.* **91**:424, 1964.

13. TIPTON, I. H. AND COOK, M. J.: Trace elements in human tissue. *Health Phys.* **9**:103, 1963.

14. YAMAGATA, N.: The concentration of common cesium and rubidium in the human body. *J. Radiation Res.* **3**:4, 1962.

15. PORIES, W. J. *et al:* Acceleration of wound healing in man with zinc sulfate given by mouth. *Lancet* **1**:121, 1967.

16. VALLEE, B. L.: Clinical significance of trace elements. Zinc. *Mod. Med.* **31**:118, 1963.