THE DISTRIBUTION AND FUNCTION OF ZINC IN NORMAL AND MALIGNANT TISSUES

PART I.

UPTAKE AND DISTRIBUTION OF RADIOACTIVE ZINC, ^{65}Zn

by

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Observations by one of us (HEATH[1]) have shown the extremely wide range of values which the natural zinc concentrations in human and other mammalian tissues may have. Occasionally in this work certain malignant tumours have been found to have much higher zinc contents than the tissues supporting them. This, coupled with the fact that the functions of zinc in cell metabolism are as yet only very slightly understood, made a study of the distribution and function of this element very desirable. Further observations by the same worker (HEATH[6]) had also shown that nuclear nucleoprotein (desoxyribose type) obtained from calf thymus and mouse tumour cells by MIRSKY's method (MIRSKY AND POLLISTER[2]) usually contained some natural zinc, whereas unfragmented nuclei obtained from the same tissues by DOUNCE's method[3] did not contain a detectable amount of this element. These observations on the naturally occurring concentrations of zinc, which for the most part have been made polarographically, will be published in detail later, together with the analytical methods. The present paper shows the results obtained in some studies of the distribution of radioactive zinc 65 in the tissues of tumour-bearing mice following its subcutaneous injection as zinc chloride. Further studies on the metabolism of zinc in normal and malignant tissues suggested by these results are now in progress.

The distribution of ^{65}Zn has been followed by a GEIGER-MÜLLER counting method after injection into mice carrying either a transplanted mammary carcinoma or a transplanted spindle-cell sarcoma of the leg. In the first place the distribution of the injected ^{65}Zn among the various body tissues has been assessed, and secondly an attempt has been made to follow the distribution of the ^{65}Zn in different fractions of the same tissue, namely, isolated nuclei, nuclear desoxyribose nucleo-protein and cytoplasmic residues.

EXPERIMENTAL METHODS

Production of Tumours

The two types of tumour used were:

1. A transplanted C3H mammary adeno-carcinoma originally spontaneous in pure line C3H

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mice and during these experiments in its 33rd–51st transplant in C3H hybrids (C3H crossed once with laboratory strain).

2. A transplanted spindle-cell sarcoma, originally induced by dibenzanthracene and during these experiments in its 265th–273rd transplant in the rear-leg of white mice (Clarke No. 0.1 strain).

Transplantations were always done by injecting a mince of freshly dissected tumour in physiological saline, subcutaneously below the ventral surface of the right flank for the mammary carcinoma, and intramuscularly into the right rear leg for the sarcoma. Each tumour was transplanted into a batch of mice of approximately uniform age and weight. The subsequent zinc uptake experiment was then done on a given number of mice from such a batch all bearing a tumour of identical age and origin. In general the sarcomas took 14–28 days to reach optimum size and the carcinomas 10–14 days.

Preparation and injection of Zinc Solution

The ^{65}Zn was supplied in solution as chloride with an excess of hydrochloric acid. In the first experiments noted in Tables I and II the ^{65}Zn was carrier-free. In later experiments the ^{65}Zn had a carrier of natural zinc. The presence of this carrier coupled with the relatively low specific activity (*i.e.*, activity/unit mass of natural zinc) meant that the toxic limit was nearly reached in attempting to give all tissues a radioactive content great enough for accurate assay. Toxicity tests with ordinary zinc as chloride showed that a 20 g mouse could usually just tolerate 1.2 mg zinc as chloride. With ^{65}Zn of specific activity 16 μc/mg Zn (the highest available) this allowed a maximum dose of about 20 μc/20 g mouse. Higher zinc doses were likely to cause convulsions, tremors, and death, and where this did not ensue severe tissue necrosis occurred at the injection site after about 3–4 days. The solution of ^{65}Zn as chloride was always neutralized with sodium hydroxide and then back titrated with the minimum quantity of hydrochloric acid to take up any precipitate of zinc hydroxide or oxychloride. The neutralized solution was made up to a known volume with double distilled water (from pyrex still) to form the stock solution.

For each experiment a portion of this stock solution was taken, diluted to give 20 μc in each 0.4 ml aliquot injected, and rendered isotonic by addition of sodium chloride. A radioactivity standard was prepared from each injection solution by taking 1 ml after a further known dilution. The mice were usually injected with 0.4 ml of the isotonic ^{65}Zn solution subcutaneously in the nape of the neck at 3.0 p.m. and killed by cervical dislocation or chloroform at 9 or 10 a.m. the following morning, approximately 18–19 hours afterwards.

Preparation of animal tissue

The gross weight of the freshly killed animals was quickly determined and the tumours and other required tissues removed. The tissues taken were control normal mammary gland from unaffected breast in the carcinoma cases, control leg muscle from opposite unaffected leg in sarcoma cases, liver, kidneys, spleen, pancreas, stomach and intestines. At all stages the tissue temperature was kept as near 0° C as possible. Like tissues or organs from all animals in a batch were combined, minced and weighed, and weighed samples put to dry to constant weight in 100 ml conical Pyrex flasks at 95–100° C in an electric oven. Where the combined tissues or organs of a given sort weighed less than a few grams they were weighed and dried in their entirety in a similar flask. Tissues usually dried to constant weight in 4–6 days and were then wet-ashed by a modification of BUELL's method[4]. 15 ml double distilled water and 15 ml conc. redistilled nitric acid (pyrex still, metal free) were added to 1 g dry tissue in each conical pyrex flask and the digestion allowed to proceed at 90° C (thermostat) for 3–4 hours. 2 ml of 72% perchloric acid (redistilled, metal free) was added and digestion continued at 90° C until the residues were just dry. Very rarely a little extra nitric and perchloric acid was required to complete digestion. Provided care was taken not to overdry the digestion residues these were usually quite water soluble when slightly warmed. Occasionally hydrochloric acid (redistilled in pyrex still, metal free) at a concentration of 20% or less was required to effect solution. It must be pointed out here that the white crystalline digestion residues obtained in this way are not devoid of organic compounds contrary to BUELL's belief. Residual organic compounds at first seriously interfered with the above mentioned polarographic analyses and methods devised to overcome this difficulty will be described when the polarographic work is published. BUELL defatted her specimen tissues before ashing and this may account for her being able to claim completely inorganic residues by this ashing method. For the present work, provided the digestion residue could be completely dissolved in a small volume of water, a slight soluble organic component was of no consequence. The dry residues were then dissolved in the minimum measured volume of double distilled water and 1 ml of this solution was taken for measurement of radioactivity.

The bulk of the tumour tissue was treated immediately after mincing by the method developed by MIRSKY[2] in which 0.14 *M* and 1 *M* sodium chloride solutions are used to extract nuclear desoxyribose nucleoprotein. The combined 0.14 *M* sodium chloride washings from this extraction containing the bulk of the cytoplasmic material were further divided into two fractions by heating nearly to the boiling point. At this temperature a large proportion of the suspended and dissolved solids was

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coagulated and after being cooled was centrifuged off. This portion is the "cytoplasm heat coagulated" entry of Table IV. The supernatant from this centrifuging, evaporated to dryness, is the "cytoplasm-supernatant" entry of Table IV. In all of the above fractions, *i.e.*, nuclear nucleoprotein, and the two cytoplasmic portions, due allowance was made in dried solid residues for any sodium chloride introduced in the extraction process where this had not been removed by washing. When tumour cell nuclei were required the citric acid method of DOUNCE[3] was used.

Comparative tests were conducted with liver tissue to see how far the report of SAHYUN AND FELDKAMP[5] that trichloracetic acid in aqueous solution would extract all of the zinc contained in an animal tissue was correct. Such a method, if giving complete extraction, would have considerable advantages over the ashing method. The minced liver tissue was blended at high speed in the Waring blendor with 4% aqueous trichloracetic acid solution in the proportion of 3 ml of solution to 1 g of fresh wet liver and the suspension left for 24 hours. After the suspension had been centrifuged the residue was again extracted with the same volume of trichloracetic acid solution as before. The radioactivity of the trichloracetic acid extracts was estimated directly without ashing, by measuring 1 ml into a standard tube and counting as for the ashed tissue samples.

In addition to separating nuclei from tumour cells one sample of liver was repeatedly washed with 0.14 *M* NaCl in order to remove as much cytoplasm as possible and thus leave a residue considerably enriched in nuclear material. Although an exact quantitative measure of the amount of cytoplasm thus removed was not attempted, a rough estimate showed that something greater than 50% had been removed, and stained smears of the residual tissue showed masses of nuclei and very little cytoplasmic material. MIRSKY[2] indicates that about 60–70% of mammalian liver substance, mainly cytoplasm, can be removed by repeated washing with 0.14 *M* NaCl at approximately pH 7. The nuclear-enriched material was ashed in the usual way and its activity determined.

One sample of liver and one sample of tumour (sarcoma) were subjected to an extraction process in which distilled water, acetone, acetone-ether, and glycerol were used successively in that order. The quantity of each solvent used was about 3 × volume of the original tissue. The various extraction liquors were kept after being cleared by centrifugation and 1 ml of each fraction was measured into a standard tube for radioactive assay. In addition, the final tissue residues, after all of the extractions, were ashed and their specific activities determined.

Finally two batches of sarcoma-bearing mice, which had had no treatment other than tumour inoculation, were killed and their tissues analysed for naturally occurring zinc by the above mentioned polarographic methods. The natural zinc content of these tissues given in Table VII is appended as a base line.

Measurement of Radioactivity

^{65}Zn emits both positrons and γ-rays and has a half-life of 250 days. For convenience and to eliminate self-absorption effects the γ-ray emission was used for the assay of the radioactivity.

Radioactivities were determined with a GEIGER-MÜLLER counter tube (G.E.C. type G.M. 2) followed by a pre-amplifier, pulse-shaper and scaler (scale of 4) made by one of us (J. L.–M.). The G.M. 2 tube had a copper cathode wall 0.75 mm thick and a 20 mg/cm^2 copper end window 2.4 cm diameter and was suitable for γ-ray counting. Most of the measurements with the above counter were duplicated with a CINTEL γ-ray counter (Type G.M. 4) and parallel results obtained.

1 ml of the tissue digest solution was measured carefully, without splashing, into the bottom of one of a series of similar glass tubes. These tubes were selected from a large batch so that 1 ml occupied a depth not less than 1.3 cm and not greater than 1.6 cm. This tolerance had been previously shown to be acceptable by experimental determinations. 1 ml of the solution of ^{65}Zn prepared by a known dilution of the isotonic solution as injected was measured into a similar tube and used as a standard, being measured for radioactivity along with the relevant batch of tissue digest solutions. Under these conditions the water and the glass wall of the specimen tube were sufficient practically to eliminate the β-rays, leaving the γ-rays as the radiation to be measured.

A uranium oxide standard (provided by the Radiotherapeutic Research Unit, Hammersmith Hospital, London) was used at the beginning of each set of experiments to check the efficiency of the counting tube.

The total number of counts collected for each specimen was at least 800, but more often 1000, the statistical accuracy achieved being then calculated to be of the order of $\pm$ 3.2–3.5%. All the counting data obtained formed a very satisfactory aggregate both in relation to the analytical and volumetric work connected with the biochemical aspect of the research, and to the actual counting results. For instance, measurements on standard samples prepared separately and on different days from the initial solution of ^{65}Zn, agreed to 2% or better after the decay had been taken into account. The combined errors, including the error on the background rate are given for one of the following tables (Table II).

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EXPERIMENTAL RESULTS

The results in Tables I and II were obtained in preliminary experiments undertaken to get an idea of the range of tissue specific activities likely to be encountered. The first ^{65}Zn samples obtained* and used for these experiments were carrier-free, so that the values given are not strictly comparable with subsequent values. The individual mouse tissues tested as shown in Table I were used separately and not combined; average values are therefore not given and the table shows the sort of variation to be expected from one animal to another. This table also shows that the mammary tumours have taken up much more ^{65}Zn/gram of wet tissue than the normal mammary tissue during the 18 hours or so of the experimental period. In Table II the like mouse tissues were combined so that the results are average ones; for simplicity in this preliminary survey the tissues were not dried to constant weight, so for this reason also tissue specific activities here may not be compared directly with later ones. However, the dry weight

TABLE I

DISTRIBUTION OF ^{65}Zn IN INDIVIDUAL C3H HYBRID MICE WITH MAMMARY ADENOCARCINOMA, 18–19 HOURS AFTER SUBCUTANEOUS INJECTION

Dose 36.2 μC/mouse. ^{65}Zn as $ZnCl_2$ carrier-free. Age of tumour – 13 days.

Mouse		Organ Specific Activity in counts/minute/gram wet weight			
No.	Weight g	Right Mammary Gland	Left Mammary Gland	Liver	Kidneys (both)
1	26.2	1160 Tumour	418 No Tumour	3650	1950
2	24.9	1455 Tumour	177 No Tumour	4190	1825
3	22.7	1385 Tumour	306 No Tumour	3520	— not taken
13	24.8	396 No Tumour	511 No Tumour	3230	1575
14	29.3	346 No Tumour	0 No Tumour	2770	— not taken
		Right Mammary Gland (Tumour)	Left & Right Mammary Glands (No Tumour)		
Mean	25.58	1333	307.7	3472	
Standard deviation	2.428	154.2	172.9	524.6	
Coefficient of variation	9.50	11.56	56.2	15.1	
Standard error of mean	± 1.09	± 89.1	± 65.5	± 235	

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—wet weight ratios given later may be used for an approximate comparison if we bear in mind that the ^{65}Zn in Table II was carrier-free, whereas the ^{65}Zn in subsequent experiments had a considerable amount of carrier.

TABLE II

AVERAGE DISTRIBUTION OF ^{65}Zn IN A GROUP OF WHITE MICE (CLARKE 0.1 STRAIN) WITH LEG SARCOMAS, 18–19 HOURS AFTER SUBCUTANEOUS INJECTION. AGE OF TUMOUR = 15 DAYS.

Dose 22.6 μC/mouse. No. of mice in experiment = 10. Average weight of mouse = 36.9 g. ^{65}Zn as $ZnCl_2$ carrier-free. 1 μC equivalent to 1106 counts/min. on the given counting arrangement.

Tissue	Weight of Tissue Sample (wet) grams	Number of Counts/minute /sample	Specific Activity of Tissue in counts/min/gram wet weight
Sarcoma (right leg)	2.483	764 ± 31	307
Leg muscle normal (left leg)	1.793	282 ± 10	157
Sarcoma Cytoplasm	15 approx.	3450 ± 84	230
Sarcoma nuclear nucleoprotein	1 approx.	356 ± 42	356
Liver	2.807	4580 ± 63	1630
Kidney	1.584	1570 ± 42	990
Spleen	2.009	2280 ± 52	1135
Pancreas	1.850	3040 ± 52	1650
Intestines & Stomach	2.870	3160 ± 42	1100
Carcase	2.850	940 ± 42	330
Standard A (Volumetrically identical)		795 ± 10	
Standard B (Volumetrically identical)		790 ± 15	
Standard C (Volumetrically identical)		792 ± 10	

Tables III and IV give a complete analysis of the results obtained in five separate experiments with batches of sarcoma-bearing mice and one experiment with a batch of carcinoma-bearing mice. These Tables show that the specific activities of sarcoma tissue range from 1.79 × to 5.3 × the specific activities of the muscle tissue from the opposite unaffected leg. In the carcinoma group the tumour specific activity is 3.6 × that of the unaffected mammary glands from the opposite flank of the mouse and 1.6 × that of whole foetal tissue from one of the mice in the batch which was pregnant. Specific activities of liver tissues were always very high and remarkably constant, being usually between 5 × and 10 × as high as the specific activity of the tumour tissue from the same batch of animals. For the carcinoma group the liver specific activity is only 3.6 × that of the tumour tissue.

Nuclear nucleoprotein (desoxyribose type) from tumour tissue (Table IV), always showed a fairly high specific activity of the order of one-third of that of the tumour tissue itself, whereas the cytoplasmic material (heat coagulated fraction) had practically

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TABLE III

AVERAGE DISTRIBUTION OF ^{65}Zn IN 5 GROUPS OF WHITE MICE (CLARKE O.1 STRAIN) WITH LEG SARCOMA AND IN 1 GROUP OF C3H HYBRID MICE WITH MAMMARY ADENOCARCINOMA 18–19 HOURS AFTER SUBCUTANEOUS INJECTION

^{65}Zn as $ZnCl_2$ with carrier Zn. Specific activity 16.1 μC/mg Zn when first received.

Group No.	No. of mice in Group	Age of Tumour in days	Average weight of mice in group grams	Dose in μc per mouse	Tumour		Normal tissue		Ratio tumour specific activity: Normal tissue specific activity	Liver		Foetus	
					Ratio dry weight: wet weight	Specific activity counts/ min/gram dry weight	Ratio dry weight: wet weight	Specific activity counts/ min/gram dry weight		Ratio dry weight: wet weight	Specific activity counts/ min/gram dry weight	Ratio dry weight: wet weight	Specific activity counts/ min/gram dry weight
					Right leg sarcoma		Left leg muscle						
1	10	26	33.02	19.7	0.175	238	0.257	133	1.79	0.241	2420		
2	10	13	32.82	19.3	0.188	459	0.268	87	5.28	0.244	2270		
3	8	19	27.80	17.7	0.178	392	0.266	117	3.35	0.248	2550		
4	10	25	31.63	20.0	0.177	380	0.260	113	3.36	0.246	2470		
5	11	26	29.80	19.3	0.161	311	0.261	95	3.27	0.236	1950		
Mean		21.80	31.01	19.2		356		109			2332		
Standard deviation		5.718	2.206	0.889		84.31		18.28			236.7		
Coefficient of variation		26.2	7.12	4.63		23.7		16.8			10.2		
Standard error of mean		±2.56	±0.99	±0.40		±37.8		±8.18			±106.2		
					Mammary carcinoma right breast		Normal mammary gland left breast						
6	11	13	26.1	18.8	0.162	605	0.431	166	3.65	0.240	2180	0.138	373

TABLE IV

^{65}Zn DISTRIBUTION IN VARIOUS FRACTIONS OF TUMOUR AND LIVER TISSUES FROM THE SAME GROUPS OF MICE AS IN TABLE III

Group No.	Specific activities counts/minute/gram dry weight								
			Tumour fractions					Liver fractions	
	Tumour (Whole tissue)	Liver (Whole tissue)	Nuclear Nucleoprotein	Cytoplasm heat coagulated	Cytoplasm supernatant	Nuclei (citric acid method)	Residue after repeated extractions of whole tissue with water, acetone, ether, glycerol	Nuclear-enriched liver residue after repeated washing with 0.14 *M* NaCl of whole tissue	Liver residue after repeated extractions of whole tissue with water, acetone, ether, glycerol
	Leg sarcoma								
1	238	2420	79	253	229				
2	459	2270	150	520	151				
3	392	2550				12			
4	380	2470				9		1025	
5	311	1950					190		995
	Mammary carcinoma								
6	605	2180	278	609	266				

TABLE V

SAME GROUPS AS TABLES III AND IV

Ratios of observed specific activities of tissues to expected average specific activity of whole body tissue, assuming no losses by excretion etc. during the period of the experiment and uniform distribution of the injected dose. 1 μc equivalent to 1106 counts/minute.

Group No.	Calculated average specific activity over whole body. Counts/min/gram wet weight	Tumour		Normal tissue		Liver	
		Observed tissue specific activity counts/min/gram dry weight	Ratio* Observed tissue specific activity: whole body specific activity	Observed tissue specific activity counts/min/gram dry weight	Ratio* Observed tissue specific activity: whole body specific activity	Observed tissue specific activity counts/min/gram dry weight	Ratio* Observed tissue specific activity: whole body specific activity
		Right Leg Sarcoma		Left Leg Muscle			
1	659	238	0.063	133	0.052	2420	0.88
2	650	459	0.133	87	0.036	2270	0.85
3	704	392	0.099	117	0.044	2550	0.90
4	699	380	0.096	113	0.042	2470	0.87
5	716	311	0.070	95	0.035	1950	0.64
		Right Breast Mammary Carcinoma		Left Breast Normal Mammary Gland			
6	799	605	0.123	166	0.090	2180	0.66

* Before taking this ratio the observed tissue specific activity is brought to a wet weight basis using the dry: wet weight ratios of Table III.

TABLE VII

CONCENTRATIONS OF NATURALLY OCCURRING ZINC IN THE TISSUES OF WHITE MICE (CLARKE O.I STRAIN) BEARING THE LEG SARCOMA DETERMINED POLAROGRAPHICALLY

Group No.	Age of Tumour (days)	No. of mice in Group	Average weight of mice grams	Natural Zinc Content grams/gram of dry tissue							
				Right Leg Sarcoma	Sarcoma nuclear nucleoprotein	Sarcoma tissue after nucleoprotein extraction	Left Leg muscle	Liver	Kidney	Spleen	Pancreas
7	26	12	31.14	0.788×10^{-4}			0.424×10^{-4}	1.23×10^{-4}	0.64×10^{-4}	0.955×10^{-4}	0.82×10^{-4}
8	26	7	27.90	1.15×10^{-4}	0.864×10^{-4}	0.342×10^{-4}	0.671×10^{-4}	1.33×10^{-4}	0.708×10^{-4}	0.958×10^{-4}	1.06×10^{-4}

the same specific activity as the tumour itself. The remaining cytoplasmic fraction (supernatant left after heat-coagulation) had a specific activity less than that of the whole tumour tissue. It should be pointed out here that the nucleoprotein extracted was a well-washed sample, whereas the cytoplasmic material contained all of the tissue fluids as well as the solid matter from tumour tissue. Whether the nucleoprotein zinc is present in a combined form, or as a contaminant, or as a component of some closely associated enzyme system is now being investigated.

Nuclei extracted from sarcoma tissue by the citric acid method of DOUNCE showed very little specific activity. The work of SAHYUN AND FELDKAMP on the trichloracetic acid extraction of zinc from tissues and the results obtained during the present experiments with this method of extraction and shown in Table VI, lead one to expect that probably citric acid will also extract zinc from tissues, and thus may easily remove a large proportion of any zinc contained in the nuclei. This is in spite of the clean integral appearance of the freed nuclei. Since mammalian tissue nuclei are not readily obtained without the use of some chemical agent it is difficult to ascertain how much zinc is contained in, or taken up by, the nuclei under given conditions. The problem was approached however in another fashion as indicated earlier, by removal of a large part of the cytoplasm from some liver tissue by repeated washing with 0.14 *M* NaCl. The nuclear-enriched residue thus produced gave a specific activity rather less than one-half of that for the untreated liver tissue (see columns 3 and 10 of Table IV). Since this residual tissue consisted very largely of nuclei with only a few shreds of cytoplasm and intercellular substance, it seems certain that the nuclei do contain an appreciable quantity of zinc, although the present experiments do not give a quantitative answer.

TABLE VI

SAME GROUPS AS TABLES III, IV AND V

EXTRACTION OF ^{65}Zn FROM FRESH WET LIVER TISSUE WITH AQUEOUS TRICHLORACETIC ACID SOLUTION

In each extraction 3 volumes of 4% aqueous trichloracetic acid solution were used to 1 volume of the fresh wet tissue, the mixture blended in the Waring blendor and allowed to stand for 24 hours at 4° C.

Group No.	Activity in counts/min/gram of dry untreated tissue extracted at 1st Extraction	Activity in counts/min/gram of dry untreated tissue extracted at 2nd Extraction	Activity in counts/min/gram of dry untreated tissue left in residual tissue after two extractions	Total activity in counts/min/gram of dry untreated tissue (sum of 2nd, 3rd & 4th columns)	Specific Activity in counts/min/gram of dry untreated tissue as determined by ashing
1	1900	458	72	2430	2420
2	1860	372	69	2301	2270
3, 4 & 5	No extraction made				
6	1890	345	56	2291	2180

The remaining extraction method in which water, acetone, acetone-ether, ether and glycerol are used in that order showed that in the case of the tumour (sarcoma) approximately 55% of the total tissue activity was removed in the aqueous portion of the

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extraction, a further 41% approximately in the glycerol extract, and the residual tissue after the whole sequence of extractions had a specific activity about 60% of that the unextracted tissue. For the liver tissue, approximately 82% of the total activity was removed in the aqueous extract and a further 16% approximately in the glycerol extract, the residual tissue after the whole sequence of extractions in this case having a specific activity of about 50% of that of the unextracted tissue. By contrast, the fat solvents, acetone and ether, extracted activities too small to be measured. In each case there was very little bulk of tissue left after this series of extractions so that although the specific activity of the residue was higher than might have been expected, yet the fraction of the total tissue activity remaining was very small.

CONCLUSIONS

1. Tumour tissue of either of the types used takes up considerably more injected ^{65}Zn/unit weight of tissue in the experimental period of 18 hours than do the control tissues.

2. In the sarcoma series an inspection of Table III suggests that the age of tumour may influence the uptake of ^{65}Zn/unit weight of tumour tissue.

3. Nuclear desoxyribose nucleoproteins from both types of tumour contain appreciable quantities of zinc.

4. Unfragmented microscopically intact nuclei obtained by the citric acid method contain very little zinc and the evidence points to this having been leached out by the acid. It is quite possible that nuclear enzyme systems depending on trace metals may be upset accordingly by the citric acid method.

5. One extraction of minced fresh tissue with 3 volumes of 4% aqueous trichloracetic acid will only extract 80% of the contained zinc. A second similar extraction will bring the total extracted up to 97%.

6. Table V shows that only a relatively small amount of the total injected ^{65}Zn is to be found in the tissues other than at the site of injection. It is concluded that most of the ^{65}Zn remains locked up at the injection site possibly as precipitated carbonate or phosphate, since a complete balance sheet of ^{65}Zn content of all organs and residual carcases in one experiment revealed a loss during the 18 hours of only 20% of the ^{65}Zn injected.

7. It may be stated in passing that it is unlikely that ^{65}Zn could be used in this fashion for tumour therapy by irradiation from the absorbed isotope. The liver, pancreas, spleen, intestine and kidney would all receive much higher radiation doses than the tumour for a given amount of ^{65}Zn, unless these organs excrete their stocks of ^{65}Zn much more rapidly than does the tumour.

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SUMMARY

The uptake and distribution of ^{65}Zn in normal and malignant mouse tissues and certain fractions derived from there have been studied following the injection of this element as chloride into tumour bearing mice. In both the sarcoma and carcinoma studied the malignant tissue took up more of the ^{65}Zn/unit weight of tissue in the given time of 18 hours than the normal tissue supporting tumour growth. Nuclear desoxyribose nucleoprotein from the tumour cells showed an appreciable content of ^{65}Zn and provided a suitable method of preparation was used so also did liver cell nuclei.

RÉSUMÉ

Nous avons étudié l'absorption et la distribution du ^{65}Zn dans des tissus normaux et malins et dans certaines fractions obtenues à partir de ces tissus, après injection de cet élément sous forme de chlorure dans des souris affectées de tumeurs. Aussi bien dans le cas du sarcôme que du carcinôme étudiés le tissu malin absorbait davantage de ^{65}Zn par unité de poids de tissu dans le temps donné de 18 heures que le tissu normal soutenant la croissance de la tumeur. La désoxyribose-nucléoprotéine préparée à partir de noyaux de cellules de tumeurs montraient une teneur appréciable en ^{65}Zn, et il en était de même des noyaux de cellules de foie, pourvu qu'une méthode convenable de préparation fût appliquée.

ZUSAMMENFASSUNG

Die Aufnahme und Verteilung von ^{65}Zn in normalen und bösartigen Geweben und in daraus hergestellten Fraktionen wurde nach Injektion dieses Elementes in Form von Chlorid in Mäuse, welche einen Tumor hatten, untersucht. Sowohl in dem untersuchten Fall von Sarkom, wie von Carcinom, nahm das bösartige Gewebe mehr ^{65}Zn pro Gewichtseinheit in der gegebenen Zeit von 18 Stunden auf, als das normale, das Wachstum des Tumors unterstützende Gewebe. Desoxyribose-nukleoprotein aus Tumorzellkernen wies einen bedeutenden Gehalt an ^{65}Zn auf. Dasselbe wurde bei Leberzellkernen beobachtet, wenn eine geeignete Methode zu ihrer Herstellung gewählt wurde.

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