The Determination of ^{65}Zn in Tissues with Geiger and Scintillation Counters

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^{65}Zn has been used in several biological investigations (see, for example, Sheline, Chaikoff, Jones & Montgomery, 1943*a, b*; Heath, 1949; Andersson-Kottö & Hevesy, 1949; Banks, Tupper & Wormal, 1950; Heath & Liquier-Milward, 1950; Rosenfeld & Tobias, 1951; Tupper, Watts & Wormal, 1951, 1952, 1954), and since radioactive zinc of high specific activity is now more readily available, it is probable that this isotope will be more widely used in biological studies. As far as we are aware, all the previous workers using ^{65}Zn for biological experiments have made their radioactivity measurements with some type of Geiger-Müller counter. A suitable scintillation counter system, employing a thallium-activated sodium iodide crystal as the phosphor, has been developed, and found to possess several advantages over Geiger-Müller counters for this type of work.

The present paper describes simplified methods for determining ^{65}Zn in tissues using the Veall (1948) type Geiger-Müller counter for liquids, and the scintillation counter. A preliminary note on some of this work has been published elsewhere (Banks, Tupper, Watts & Wormal, 1954).

MATERIALS AND METHODS

Radioactive zinc (^{65}Zn). This was obtained either in the form of neutron-bombarded zinc metal or from copper which had been irradiated in a cyclotron. Improved methods for separating ^{65}Zn from the latter source have been described elsewhere (Tupper & Watts, 1954; Tupper *et al.* 1954).

Geiger-Müller counters. These were of the type described by Veall (1948) for measurements on liquid samples (M6, M6H or M6M; 20th Century Electronics Ltd.).

Scintillation counter. The arrangement and housing of the radioactive sample, the phosphor (a thallium-activated NaI crystal, supplied by Atomic Energy Research Establishment, Harwell), and the photomultiplier tube (EMI 6260) are shown in Fig. 1. It is essential to have good optical contact between the base of the phosphor mounting and the photocathode of the electron multiplier, and this is effected with a thin film of liquid paraffin. The voltage pulses set up in the output resistor of the photomultiplier are fed to a two-stage amplifier (giving a 50-fold gain) situated in the base of the instrument. The output pulses from the amplifier are fed to a decade-type scaling and recording unit (Panax Equipment Ltd., 'Model 100 C'). The 'dead time' of this

system is only of the order of a few microseconds, and consequently dead time corrections are rarely needed in this work. Aluminium filters (with a total thickness equivalent to 480 mg./cm.²) are incorporated into the crystal holder, and the base of the sliding tray which holds the glass dish containing the radioactive sample. This ensures that only the γ -rays produced by the radioactive decay of ^{65}Zn reach the activated NaI crystal.

Fig. 1. Scintillation counter.

The high-voltage power supply for the photomultiplier is drawn from an oscillator type 'power-pack' incorporated in the above-mentioned 'Model 100 C' unit.

After preliminary experiments to determine the optimum pulse discrimination, we were able to obtain reproducible counting rate/voltage characteristic curves, with plateaux having a gradient of about 1%/100 v. From considerations of the stability of the background, stability of E.H.T. (extra high tension) supply, and counting rate/voltage characteristic, an operating voltage of 1500 was chosen for routine work.

METHODS

A shortened wet combustion procedure. A weighed portion (0.1–5 g.) of the material to be investigated is heated with conc. H_2SO_4 or HNO_3 (2 ml./g. tissue, with a minimum of 1 ml.) until a clear solution is obtained. Further portions of acid are added if necessary. When H_2SO_4 is used, SeO_2 (about 5 mg./ml.) should be added and the heating continued for 0.5 hr. after the solution turns clear; part of this digest can then be used for a total nitrogen determination. The acid digest is diluted to a standard volume (e.g. 15 or 20 ml.) with water, and a portion of this solution used for radioactivity measurements with either a liquid counter or the scintillation counter. Digests prepared in this way from normal tissues showed no significant radioactivity under these conditions.

Extraction method. It may be necessary, under certain circumstances, to separate the zinc chemically from other tissue components before its radioactivity is measured. The method of Banks *et al.* (1950) is suitable for this purpose.

The use of the scintillation counter. Tissues (or other biological materials, e.g. faeces) are homogenized, or are digested with H_2SO_4 or HNO_3 as described above. A portion (e.g. 5.0 ml.) of the homogenate or acid digest is placed in a circular glass dish (depth 1 cm., diameter 3 cm.), and the dish is moved to a predetermined and reproducible position

above the phosphor by means of a sliding metal carrier (see Fig. 1).

Sufficient material to cover the whole of the bottom of the dish of the scintillation counter should always be used, and it is advisable to maintain an approximately constant volume for any one series of measurements.

RESULTS

Recovery of added ^{65}Zn

Measured amounts of ^{65}Zn were added to samples of normal rabbit tissues. These samples were then digested by sulphuric acid containing selenium dioxide, and the radioactivity of the diluted digests was determined in some experiments with the Veall-type liquid counter and in others with the scintillation counter described above. In all these experiments the recovery of added ^{65}Zn was essentially complete (Table 1).

In many of our investigations with ^{65}Zn it has been found necessary or more convenient to make radioactivity measurements on unhydrolysed tissue homogenates, and tests have therefore been made to determine whether, using the scintillation counter, an accurate measure of the ^{65}Zn content of a tissue can be obtained in this way. The results have shown that determinations made in this simple way are completely reliable.

In a few experiments, samples of normal rabbit liver, kidney and muscle, containing added ^{65}Zn , were digested with conc. HNO_3 , and the radioactivity of the diluted digests was measured in a Veall-type Geiger-Müller counter. The recovery of added ^{65}Zn in a typical experiment was 97, 97, 98,

Table 1. *Recovery of ^{65}Zn added to normal tissues*

For Expts. 1 and 2 a solution containing ^{65}Zn (amounts giving count rates of 2184 and 1226/min. with the counters used in Expts. 1 and 2, respectively) was added to about 5 g. of rabbit tissue. For Expt. 3 normal rabbit liver was mixed with about an equal volume of 0.9% NaCl and the mixture was disintegrated in a Waring Blender and a solution containing ^{65}Zn (giving a count rate of 22176/min. with the appropriate scintillation counter) was added to each 5 ml. sample. All the samples were digested with H_2SO_4 containing SeO_2 .

Expt.	Counter	Tissue	Density of the diluted acid digest	^{65}Zn recovered
			(g./ml.)	(%)
Expt. 1	Geiger-Müller counter for liquids	Liver	1.15	100
			1.19	99
		Kidney	1.23	99.5
			1.23	99
		Muscle	1.24	101
			1.22	96
Expt. 2	Scintillation counter	Liver	1.22	100
			1.23	97
		Kidney	1.19	101
			1.23	100
		Muscle	1.15	103
			1.24	101
Expt. 3	Scintillation counter	Liver	—	98
			—	98
			—	100

98, 98 and 101 %, with the diluted digests having, respectively, densities of 1.26, 1.30, 1.13, 1.34, 1.26 and 1.20 g./ml.

The linearity of the response of the scintillation counter was investigated by comparing the known activity of a series of ^{65}Zn solutions with the count rates obtained using the scintillation counter. The known radioactivity of the solution and the observed count rates were directly proportional to one another at count rates not exceeding 30 000 counts/min. Above this count rate the observed value was slightly less than the calculated value, and this small deviation from a linear relationship at higher count rates is probably due to the inertia of the mechanical register in the scaler employed.

Self-absorption effects

As we wished to make direct radioactivity determinations on the acid digests containing varying amounts of solid matter, and often having relatively high densities, a series of experiments was undertaken to determine whether 'self-absorption' effects were likely to vitiate the results obtained with the liquid counter. Various ^{65}Zn -containing solutions having densities as high as 1.83 g./ml. were studied, including solutions of sulphuric and nitric acids, zinc sulphate, and sodium iodide, and a concentrated casein solution digested with trypsin, and in no case was there any appreciable 'self-absorption'. These results are in agreement with those obtained in recovery experiments (cf. Table 1). Similar results were obtained with the scintillation counter.

Direct comparison of the results obtained by the shortened wet combustion procedure and those obtained by the method of Banks et al. (1950)

Having established that the shortened wet combustion method gives complete recovery of ^{65}Zn added to normal tissues, we made further tests with tissues into which ^{65}Zn had been biologically incorporated, to compare the results with those

obtained by the longer method (Banks *et al.* 1950) which involves complete digestion of the tissue by long heating with various acids and subsequent extraction of the zinc by dithizone (diphenylthiocarbazone).

The results obtained with various tissues (liver, kidney and lung) from a rabbit which had received an intravenous injection of ^{65}Zn (as a zinc-glycine complex) 5 days previously, showed that the simplified method gives reasonably reliable results with ^{65}Zn -containing tissues, even when, as in this particular experiment, the amount of ^{65}Zn present is small.

Comparative count rates for ^{65}Zn using (a) the Veall-type Geiger-Müller counter and (b) the scintillation counter

The radioactivity of a number of ^{65}Zn -containing solutions was measured with the Geiger-Müller counter for liquids and with the scintillation counter, the samples used for the scintillation counter measurements being suitably diluted. The results of these determinations are given in Table 2.

DISCUSSION

The method for determining the ^{65}Zn content of tissues which we have hitherto used (Banks *et al.* 1950) is laborious and it involves considerable manipulation and transference of solutions, with a loss of 3–5 % of the ^{65}Zn . The present paper describes much simpler methods which can satisfactorily be used for most biological investigations with ^{65}Zn . Where the tissue samples contain significant amounts of other radioactive isotopes, however, the longer procedure of Banks *et al.*, involving complete digestion of the tissue by acid and subsequent extraction of the zinc, should be followed.

Our first aim was to modify the original method by omitting the time-consuming extraction of the zinc, but since acid digests of tissues contain varying amounts of solid matter it was first necessary to establish that there was no appreciable self-absorption when the radioactivity of these ^{65}Zn -containing acid digests was measured in a Veall-type liquid counter. Veall (1948) has shown that significant self-absorption occurs, with solutions having densities as low as 1.03 g./ml., when this counter is used for measuring the radioactivity of ^{40}K and ^{32}P , but our investigations have shown that there is no similar effect with ^{65}Zn , even when the density of the solution was considerably greater than that of the most concentrated tissue digest. It was, therefore, considered justifiable to use simple acid digests of tissues for ^{65}Zn determinations, and the results presented in Table 1 confirmed this view.

Table 2. *Comparative count rates for ^{65}Zn sources measured with a Geiger-Müller liquid counter and with the scintillation counter*

Sample	Radioactivity (net counts/min./10 ml. solution)		Ratio (B/A)
	Geiger-Müller counter (A)	Scintillation counter (B)	
1	814	19 176	23.8
2	1 604	37 136	23.1
3	3 269	75 300	23.0
4	6 600	158 834	24.0
5	8 130	193 240	23.8
6	11 040	250 300	22.8

For experimental details see this page.

The absence of any detectable self-absorption effect in these experiments with the Veall-type liquid counters indicates that these counters were functioning as γ -ray counters, the soft positrons (β^+) and K -capture X-rays emitted during the radioactive decay of ^{65}Zn being completely absorbed by the solution itself and the glass wall of the counter tube (25–30 mg./cm.²).

For the preparation of the acid digest of a tissue, either sulphuric acid (plus selenium dioxide) or nitric acid may be used, but the former has several advantages over the latter; the sulphuric acid digest can be used also for a total nitrogen determination, and this is particularly helpful in many biological investigations with ^{65}Zn . Digestion of the tissue with nitric acid to give a homogeneous solution is appreciably quicker than digestion with sulphuric acid and may be preferred when large tissue samples are being analysed, but with nitric acid there is a much greater risk of corrosion of the radioactivity measuring apparatus by acid fumes. Furthermore, material is frequently precipitated when the nitric acid digests of tissues are diluted.

A scintillation counter has a high efficiency for the detection of γ -rays and would therefore be expected to be a more sensitive instrument than the Geiger-Müller counters used for the detection and measurement of ^{65}Zn , 45 % of the disintegrations of which results in γ -ray emission (Hollander, Perlman & Seaborg, 1953). The prediction was confirmed by the results of the many parallel observations, typical examples of which are recorded in Table 2, the scintillation counter being about 23 times as sensitive as the Veall-type Geiger-Müller counter.

The accuracy which can be attained in a radioactivity measurement depends on the ratio of the (net count rate of the radioactive source)/(background count rate). Although the background count rate obtained with the scintillation counter is considerably larger than that obtained with the Geiger-Müller tubes used, these being about 150 and 12 counts/min. respectively, the (net count rate of sample)/(background count rate) ratio is larger, and therefore more favourable, than the corresponding ratio for the Geiger-Müller counters. Also, since larger counting rates are given with the scintillation counter, it is often possible to obtain a desired statistical accuracy in a shorter time.

A further advantage of the scintillation counter method is that the glass dishes in which the final solution is placed for radioactivity measurements are of simple design and are easily cleaned. They are less likely to become seriously contaminated than are the Geiger-Müller counters for liquids, and since a separate dish can be used for each sample, many

radioactivity measurements can be made in a short time. The method is of general application and has given satisfactory results with unhydrolysed samples of ^{65}Zn -containing egg yolk, blood and homogenized faeces.

SUMMARY

1. A simplified method of determining the ^{65}Zn content of tissues, using the Veall-type liquid counter, is described.

2. A scintillation counter suitable for the measurement of ^{65}Zn present in fluids or tissue homogenates is described.

3. The special advantages of the scintillation counter in biological work with radioactive isotopes are briefly discussed.

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