Separation on alumina showed that the substance had an absorption curve in ethanolic HCl, with a peak at 275, and inflexions at 300, 330 and 375 m μ . It gave a positive cyanidin reaction with magnesium and hydrochloric acid, and a green colour with ferric chloride solution. It was always associated with the tannins of the leaf, and though it has not yet been isolated in a state of purity, it appears to be a flavanone, related to epicatechin (3:5:7:3':4'-penta-hydroxyflavan). Although this new factor was found in the leaf throughout the whole of its growing period, it was not detected in any other tissue. In contrast, hydrojuglone glucoside was found in all other tissues exhibiting 'apparent vitamin C' activity. Hence it would appear that what has been referred to in the past as the 'apparent vitamin C' of the walnut is hydrojuglone glucoside.

SUMMARY

1. The dye-titration values of hydrojuglone glucoside solutions and of walnut extracts are similarly affected by changes in pH and the time interval between the additions of the dye.

2. Seasonal variations in concentration of the glucoside are similar to those of the 'apparent vitamin C' in buds, catkins and fruits.

3. These facts suggest that most of the 'apparent vitamin C' in the buds, catkins and fruits of the walnut is hydrojuglone glucoside.

4. A second non-specific dye reductant, probably a flavanone, has been found in the leaf.

I wish to thank Dr F. Wokes, the Director of the Laboratories, for affording the opportunity to publish this work, and Miss Nora Baxter for technical help in the preparation of the many extracts used.

REFERENCES

- Daglish, C. (1950*a*). *Biochem. J.* **47**, 452.
 Daglish, C. (1950*b*). *Biochem. J.* **47**, 458.
 Glasstone, S. (1940). *Text Book of Physical Chemistry*, p. 935. New York: van Nostrand.
 Lugg, J. W. H. (1942). *Aust. J. exp. Biol. med. Sci.* **20**, 273.
 Morton, R. A. & Stubbs, A. L. (1946). *Analyst*, **71**, 348.
 Wokes, F. (1946). *Nature, Lond.*, **158**, 133.
 Wokes, F. & Melville, R. (1949). *Biochem. J.* **45**, 343.
 Wokes, F., Organ, J., Duncan, J. & Jacoby, F. (1943). *Nature, Lond.*, **152**, 14.
 Wokes, F., Organ, J. & Jacoby, F. (1943). *J. Soc. Chem. Ind., Lond.*, **62**, 233.
 Wokes, F., Organ, J., James, E. & Melville, R. (1944). *Nature, Lond.*, **154**, 669.

The Fate of Some Intravenously Injected Zinc Compounds

1. THE DETERMINATION OF ⁶⁵Zn IN TISSUES 2. THE FATE OF INJECTED ZINC CARBONATE AND PHOSPHATE AND A ZINC DITHIZONE COMPLEX

By T. E. BANKS, R. L. F. TUPPER AND A. WORMALL

Departments of Biochemistry and Physics, Medical College of St Bartholomew's Hospital, London, E.C. 1

(Received 25 February 1950)

The biological importance of zinc is now well established. Its presence in the pancreas and its role in connexion with the crystallization of insulin (Scott, 1934) and its influence on the hypoglycaemic action of the hormone (Scott & Fisher, 1935) are well recognized. Keilin & Mann (1939, 1940) found that purified carbonic anhydrase contains about 0.33 % zinc, and the role of the metal as an active component of this enzyme has been fully established (cf. the review by Vallee & Altschule, 1949). Zinc has also been found in uricase (Holmberg, 1939; Davidson, 1942; cf. however, Praetorius, 1948) and alkaline phosphatase (Cloetens, 1941, 1944; Nguyen-Van-Thoi, Roche & Roger, 1947).

Various authors have reported that some cancer tissues differ from normal tissues in zinc content

(Carruthers & Suntzeff, 1945, 1946; Cowdrey, Carruthers & Suntzeff, 1948; Heath, 1948, 1949), and it has also been suggested that there is a marked difference between cancer patients and normal individuals with regard to the effect of zinc salts on the serum alkaline phosphomonoesterase (Roche, Nguyen-Van-Thoi, Marcelet, Desruisseaux & Durand, 1946; Roche, Cornil, Desruisseaux, Baudoin & Long, 1947; cf. however, Bodansky & Blumenfeld, 1949).

In view of these suggested differences between cancer and normal tissue in respect to zinc 'metabolism', we have investigated the distribution of zinc following the intravenous injection of various zinc compounds, using ⁶⁵Zn (of half-life period 250 days) as isotopic tracer. The present paper describes

(1) a method for the determination of ^{65}Zn in animal tissues, and (2) the fate of suspensions of zinc carbonate and phosphate, and a solution of a zinc diphenylthiocarbazonate (dithizone) complex, after their intravenous injection into normal rabbits. These experiments were designed to give information about the way in which inorganic particles are removed from the blood stream in comparison with the fate of injected protein antigens and to obtain, if possible, specific deposition of radioactive compounds in certain organs.

1. The estimation of radioactive zinc in tissues

Diphenylthiocarbazonate (dithizone) forms coloured complexes with zinc and many other metals. These metals can be extracted from solution by dithizone dissolved in chloroform (or carbon tetrachloride) and this extraction is selective depending on the pH. For the investigations described here, it was unnecessary, however, to remove other metals present as non-radioactive isotopes; only complete extraction of the zinc is important.

The method finally adopted by us was a modification of that of Gettler & Bastian (1947); the acid digestion process is essentially that of these authors, but we used about 1 g. of tissue, as against 25 g. employed by Gettler & Bastian, with an equivalent reduction in the amounts of the various reagents. The tissue was digested with nitric acid, followed by further digestion with sulphuric acid and a mixture of nitric and perchloric acids. The zinc was then extracted from the aqueous solution by dithizone at pH 8.5–9.0; we have found that extraction at this pH is more complete than that at pH 5.5 used by Gettler & Bastian. Extraction at pH 8.5–9.0 certainly removes traces of other metals in addition to zinc, but at this pH there is maximum extraction of zinc (95–100%). Furthermore, we used a carbon tetrachloride solution of dithizone, instead of one in chloroform, for the former appears to give better extraction of the zinc, and the reagent undergoes less decomposition on keeping. Other modifications are included in the description given below.

EXPERIMENTAL

Reagents

All reagents should be of A.R. grade.

Digestion mixture. 60% (w/v) HClO_4 (2 vol.) plus conc. HNO_3 (1 vol.).

Citrate buffer (solution A; cf. Association of Official Agricultural Chemists, 1945). A mixture of 1 l. of 0.5M-ammonium citrate and 140 ml. of N-NH_3 is diluted to 4 l. The 0.5M-ammonium citrate solution is made by dissolving 226 g. of $(\text{NH}_4)_2\text{HC}_6\text{H}_5\text{O}_7$ in 2 l. of water, and adding NH_3 until the pH is 8.5–8.7.

Dithizone solution. Diphenylthiocarbazonate (dithizone) (40 mg.) is dissolved in CCl_4 (1 l.). The solution is well shaken and allowed to stand overnight. It is then filtered and stored in a dark bottle away from the light.

Method

Large organs, such as liver, lungs, heart and kidneys are dried in an air oven at 120–130°. If the dried organ weighs more than 2 g. it is powdered, and samples (0.6–1.0 g.) are weighed out for digestion. Small organs, or pieces of skin and muscle, with a wet weight of less than 3 g., are cut into small pieces and digested without drying.

Digestion. The tissue (usually 1 g. of dried tissue or 1–3 g. of wet tissue, blood or plasma) is weighed into a 150 × 25 mm. Pyrex boiling tube. Conc. HNO_3 (2 ml.) is added and the tube left to stand for 1 hr. in a warm room with occasional shaking. With tissues containing much fat, e.g. liver, it is advisable to allow the tube to stand longer until frothing has ceased. The tube is then 'stoppered' with a glass bulb and heated in a boiling-water bath until all the tissue has dissolved and the evolution of oxides of N_2 has virtually ceased. The tube is then cooled to room temperature and the contents filtered through glass wool into a 50 ml. Kjeldahl flask. This filtration removes any fat and decreases the risk of explosions during the HClO_4 digestion. The glass wool is washed thoroughly with dilute HNO_3 (1 vol. of conc. HNO_3 plus 9 vol. of water) and the washings collected in the Kjeldahl flask.

Conc. H_2SO_4 (12 drops) and two small glass beads are then added to the contents of the flask. The mixture is heated over a free flame until all the HNO_3 has boiled off, and charring, usually accompanied by frothing, begins. The heating is then increased and digestion mixture is added dropwise until the black liquid has become yellow or colourless. Heating is continued until SO_3 is evolved. The solution is allowed to cool to room temperature, and water (1 ml.) is added; the mixture is again boiled until SO_3 is evolved. The addition of water and boiling is repeated once more to ensure the removal of all traces of oxides of N_2 .

Extraction with dithizone. The cooled contents of the flask are transferred to a 250 ml. pear-shaped separating funnel, and the flask is washed with approx. N-HCl to ensure complete transference of Zn. Phenol-red solution (2 or 3 drops) is added to the contents of the funnel and NH_3 (sp.gr. 0.880) added dropwise until the solution is just alkaline to phenol red. Citrate buffer (solution A, 10 ml.) is then added.

Dithizone solution (20 ml.) is run into the funnel from a burette, the stoppered funnel is shaken for 30 sec., and the two phases allowed to separate. The lower phase which will be bright red, if sufficient Zn is present, is separated and retained, the last drop in the stem of the funnel being carefully washed into the collecting vessel with CCl_4 . The extraction with dithizone (10 or 20 ml. each time) is repeated until the characteristic red colour of the Zn-dithizone complex no longer appears and the dithizone extract remains green; the aqueous phase at this stage should be orange, due to the presence of excess dithizone. In practically all the experiments described here no extra Zn had to be added; where at this stage less than 30 ml. of the dithizone solution is required (corresponding to about 45 μg . of Zn or equivalent amounts of certain other metals) we recommend the addition of about 40 μg . of Zn, as ZnCl_2 solution, to act as 'carrier'; in experiments involving the use of radioactive Zn of high specific activity 'carrier' Zn should be added in all cases

where determinations are made on less than 1 g. of liver, or 1.5 g. of other tissues or blood. Any drops of dithizone solution on the surface of the aqueous phase are shaken down and the aqueous phase rinsed with 1 or 2 ml. of CCl_4 . The washings are added to the contents of the collecting vessel. CCl_4 (10 ml.) is then added to the funnel and the aqueous phase extracted for 30 sec.; the CCl_4 layer should be green if all the Zn has been extracted. The CCl_4 layer is run into the collecting vessel, the last drops in the stem of the funnel being washed into the vessel with CCl_4 .

Acid extraction of the zinc. The Zn-dithizone extract is shaken with 0.2N-HCl (5 ml.) for 1.5 min. in a 250 ml. separating funnel, whereupon the red dithizone solution

transferred to a graduated 12.5 ml. flask. The dithizone solution (*a* plus *b*) is again extracted with 0.2N-HCl (5 ml.) and the separation and washing with CCl_4 carried out as before; the acid extract is added to the contents of the graduated flask. Finally the funnel is washed with 0.2N-HCl (2 ml.) and the washings added to the contents of the graduated flask. Distilled water is then added to bring to the 12.5 ml. mark; this volume was selected to allow 1–2 ml. of the extract to be used for washing out the 'liquid counter' before the introduction of exactly 10 ml. of the solution (i.e. 80 % of the whole) for radioactivity measurements. An outline of this method for the extraction of Zn from tissues is given in Fig. 1.

Determination of radioactivity. In some of the earlier experiments a small measured sample (1–2 ml.) of the final ^{65}Zn -containing solution was dried on a small nickel holder and counts were made with a thin mica side-window counter (Banks, 1946). Subsequently counts were made, on 10 ml. of the solution, in the counter described by Veall (1948), and this method was found to give the most satisfactory results. These ^{65}Zn determinations were made by comparing the radioactivity of the unknown with that of the ^{65}Zn -containing solution used for the injection or for addition to tissue samples. Control determinations were frequently made on normal tissues and they invariably gave zero readings.

RESULTS

Recovery of ^{65}Zn added to normal tissues

To test the efficiency of the method, varying amounts of ^{65}Zn -containing zinc chloride solutions were added to normal rabbit tissues (lung, skin, liver and blood) and ^{65}Zn determinations were then made as described above. Some of the experiments were made with ^{65}Zn solutions of fairly high specific activity, to avoid appreciable increases in the total zinc content of the tissue samples.

The tissues were cut into small pieces and weighed amounts (1–3 g. of the wet tissue) introduced into 150 × 25 mm. Pyrex tubes. Measured amounts of a solution of zinc chloride containing ^{65}Zn were added to the tubes, and the mixtures were dried, with the tubes closed by glass bulbs, at 120–130° for 2 days; the digestion and ^{65}Zn determination were then carried out as described above. Duplicate determinations were made and the individual results are given in Table 1.

The recovery obtained was very satisfactory for a long digestion and extraction process involving considerable manipulation. In twenty-two of the twenty-nine determinations made in this series the recovery was 95–100 %, and the mean value for the whole series referred to in Table 1 was 96.5 % with a s.d. of ± 2.6 %. There was no significant difference between the results with the low and those with the relatively high specific activity zinc, and in the latter group the increase in the total zinc content of the tissue was sometimes as little as 0.4 %. Thus the method is satisfactory for tissues which contain their normal amount of total zinc.

* Additional ordinary zinc salt added, if necessary, as a carrier (see Text).

Fig. 1. Outline of method of extraction of ^{65}Zn from tissues.

should turn green. The lower layer (*a*) is run out and retained, and the aqueous phase is washed with CCl_4 (2–3 ml.) and the CCl_4 phase (*b*) separated. The aqueous acid solution is then

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

Tissue	Wt. (g.)	Zinc added*		^{65}Zn recovered (%)	
		($\mu\text{g.}$)	Radioactivity (counts/min.)		
None	—	92	280	99.2	95.4
		365	1120	97.4	99.4
Lung	1	92	280	87.9	94.6
		365	1120	96.5	101.2
Skin	1	92	280	96.5	97.0
		365	1120	96.5	98.3
Liver	3	365	1120	97.0	—
	2	0.5	332	94.4	98.3
	2	1.8	1328	98.5	98.2
	2	92	220	95.4	91.4
	2	365	880	96.4	98.8
Blood	(1.5 ml.)	0.5	332	97.9	95.2
		1.8	1328	98.7	97.0
		92	220	94.9	94.0
		365	880	95.2	98.7

* Owing to the many different methods of determination used, it is difficult to give precise values for the Zn content of normal tissues. According to Vallee & Altschule (1949) most normal organs, including the pancreas, of man and other vertebrates, contain 20–30 $\mu\text{g.}$ of Zn/g. of wet tissue, and liver contains about twice this amount. Human blood contains $8.8 \pm 2 \mu\text{g./ml.}$ (Vallee & Gibson, 1948).

In the results recorded in this paper, no correction has been made for the slight loss of zinc in the digestion and extraction process, since we are primarily concerned with relative values. Furthermore, it should be remembered that this loss is frequently less than the corresponding s.d. of the radioactivity 'count'.

2. The fate of intravenously injected zinc carbonate and phosphate and zinc dithizone complex

EXPERIMENTAL

Zinc carbonate suspension. The radioactive Zn used for this preparation was part of a sample of powdered Zn metal of low specific activity supplied to us by the Medical Research Council Tracer Element Sub-Committee in 1948; it gave 4390 counts/min./mg. on our sets at the time these injection experiments were made. The Zn (46 mg.) was dissolved in 10N-HCl (0.2 ml.) and the solution was diluted with water (1.8 ml.). A mixture of 0.75M- NaHCO_3 - H_2CO_3 buffer pH 7.5 (1 ml.) and 2N- Na_2CO_3 (0.2 ml.) was then added dropwise with shaking. The suspension of ZnCO_3 was shaken and immediately injected into rabbit no. Z1 (1.95 kg.); a similar suspension was injected into rabbit no. Z2 (1.65 kg.).

Zinc phosphate suspension. A sample (46 mg.) of the above-mentioned ^{65}Zn -containing zinc metal was dissolved in 10N-HCl (0.2 ml.) and water (1.8 ml.) added. A saturated Na_2HPO_4 solution (0.5 ml.) was then added dropwise with shaking, and the solution brought to pH 7.5 (final volume 3.2 ml.). The suspension was shaken and immediately injected into rabbit no. Z3.

Zinc dithizone complex. This preparation was made from a radioactive Zn source of considerably higher specific activity than that used for the preparation of the zinc

carbonate and phosphate suspensions. NH_3 (sp.gr. 0.880) was added to a ^{65}Zn -containing ZnCl_2 solution (20 ml.) until the pH was 8.5–9.0 and the solution was extracted with a solution of dithizone in CCl_4 (40 mg./l.). The CCl_4 was removed from the extract by distillation *in vacuo*, and the residue of Zn dithizone complex was dissolved in triacetin (3 ml.); this solution was then shaken well with normal rabbit serum (3 ml.) and the emulsion used immediately for the injections. Six rabbits (nos. 618, 788, 728, 701, 553 and 648; weights respectively 3.5, 2.0, 2.7, 3.1, 3.5 and 2.1 kg.) received intravenous injections of the emulsion (which contained 0.207 mg. of total Zn/ml., and gave 2.4×10^6 counts/min./ml. on our apparatus on the day when the injections were made) in amounts of 0.25 ml./kg. Two rabbits were killed 24 hr., two 72 hr. and two 10 days after the injections. Samples of blood were taken, and the rabbits were killed by a blow on the back of the neck; the animals were quickly cut open and the appropriate tissues removed for determination of their ^{65}Zn contents. The radioactivity standards for this experiment were prepared by digesting 0.10 ml. of the Zn dithizone-serum mixture with conc. HNO_3 (2 ml.) and adding water to give 500 ml.

Injections and the collection of the tissue samples. The suspension or the Zn dithizone emulsion was injected into the marginal ear vein of a rabbit, and blood samples were taken from the ear not used for the injection; heparin was added to these blood samples as anticoagulant. The animals were killed by a stunning blow at the back of the neck, and various organs and tissues removed as quickly as possible, including a sample of muscle taken from the thigh. When possible, the whole of each of the organs examined was removed, weighed and analysed, so that the total amount of ^{65}Zn in these organs could be expressed as a percentage of that injected into the animal. The tubes containing the radioactive zinc suspension or emulsion, and the syringes used for the injections, were subsequently washed with 0.1N-HCl, and an allowance was made for the amount of ^{65}Zn remaining in them.

RESULTS

Intravenously injected suspensions of zinc carbonate and phosphate

The rabbits injected with these suspensions appeared to suffer no serious ill effect up to the time they were killed (45 min. after the injection). The results of the ^{65}Zn determinations on the tissues (Table 2) showed that there was no significant difference between the distribution of the zinc carbonate and that of the zinc phosphate. The results with the two zinc salts are, therefore, grouped together, and discussed as a whole.

The small amounts of ^{65}Zn present in the hearts and spleens of the injected rabbits can most probably be attributed to the radioactive zinc in the blood present in these organs, but the amount present in the lungs, per g. of wet tissue, was 100–150 times that in the heart, spleen or kidney. In fact, half or nearly half the injected ^{65}Zn was recovered from the lungs, the values being 44 and 48 % for the rabbits injected with zinc carbonate and 53 % for that injected with zinc phosphate. Although the livers of these rabbits contained very little of the injected ^{65}Zn , in two cases out of three the amount present was appreciably greater than that present, shortly before the animal was killed, in an equal weight of blood. The total amount in the liver was, however, only a small part (1.6–5.4 %) of the injected ^{65}Zn .

Intravenously injected zinc dithizone complex

It was thought that organic zinc compounds were more likely to give preferential deposition of zinc in certain tissues, than were suspensions of insoluble inorganic zinc salts. After a fairly extensive search

for a suitable organic zinc compound, i.e. for one having a marked stability, and preferably a low solubility, in water and a low toxicity for the rabbit, we ultimately decided to use a zinc dithizone complex. This compound is practically insoluble in water, but it is readily soluble in triacetin, and we have found that if this triacetin solution is shaken well with normal serum, it forms an emulsion suitable for injection.

After the intravenous injection of this emulsion into rabbits the bulk of the injected zinc was removed from the blood by the lungs (Table 3). For example, the lungs of the two rabbits killed 24 hr. after the injection contained 78.5 and 80.6 % of the injected zinc, and 52.7 and 56.0 % was present in the lungs of the two rabbits killed 10 days after the injection. The livers of these rabbits contained small but significant amounts of the injected zinc, but other organs and tissues contained very small amounts only. Determinations on the separated plasma and the unwashed cell fraction of the blood showed that at least half and sometimes three-quarters of the ^{65}Zn in the blood was associated with the cells. No value is recorded for the total ^{65}Zn content of the pancreas, since it was found difficult to be certain that the whole of this organ had been collected.

DISCUSSION

Several investigators have studied, in recent years, the fate of intravenously injected radioactive colloidal particles in attempts to obtain preferential retention of the radioactive material by the lymphoid tissue. Several radioactive inorganic colloids have already been given a therapeutic trial. Investigations on small animals, and in some cases on

Table 2. *The distribution of ^{65}Zn in the tissues of rabbits 45 min. after the intravenous injection of suspensions of ^{65}Zn -containing zinc carbonate or phosphate*

(Rabbits Z1 and Z2 were injected with the ZnCO_3 suspension and rabbit Z3 with zinc phosphate. See the text for experimental details.)

Tissue:	Rabbit no.	...	^{65}Zn content (as % of that injected)					
			Per g. of wet tissue			In whole organ or tissue		
			Z1	Z2	Z3	Z1	Z2	Z3
Blood (a)			0.096	0.107	0.022	9.4*	8.8*	1.8*
Blood (b)			0.071	0.091	0.036	6.9*	7.5*	3.0*
Liver			0.025	0.081	0.138	1.6	3.1	5.4
Lungs			6.77	5.90	7.06	44.0	48.4	52.9
Heart			0.013	0.001	0.038	0.05	0.01	0.15
Kidney (right)			0.041	0.063	0.028	0.20	0.40	0.14
Kidney (left)			0.048	0.042	0.042	0.25	0.32	0.22
Spleen			0.017	0.048	0.051	0.01	0.04	0.03
Gall bladder (plus contents)			0.004	0.007	0.017	0.01	0.02	0.03
Muscle			0.001	0.003	0.008	—	—	—
Skin			0.010	0.011	—	2.9†	3.0†	—

(a) and (b) refer to blood samples taken 1 and 27 min. respectively after the injection.

* Assuming a blood volume of 50 ml./kg. of body wt.

† Assuming that the whole skin is 15 % of the body wt., the approximate value recorded for man (Muchow, 1925).

Our results with an organic zinc compound, the zinc-dithizone complex, are of special interest in that the complex is insoluble in water but is soluble in triacetin, olive oil, lard and presumably in the body fats generally. One of our objects in these investigations was to try to obtain deposition of this radioactive zinc complex in certain tissues of the body without significant increases in the concentration of zinc ions or water-soluble zinc compounds in the blood and tissue fluids. In some of our (unpublished experiments we found that when a

solution of zinc-dithizone complex in triacetin is kept in contact with normal serum there is a gradual decrease in the amount of zinc in the triacetin solution and a corresponding increase in zinc in the aqueous phase. Thus it is probable that when 'zinc dithizone' is deposited in the tissues, gradual liberation and removal of the zinc will occur. This loss is, however, very slow with the zinc complex deposited in the lungs (Table 3). Since our supply of radioactive zinc of high specific activity was small, we had to limit our experiments to determinations on six rabbits, covering the period 1-10 days after the injections. It is our intention, however, to study more fully the deposition of injected radioactive zinc-dithizone complex in the lungs and other tissues and also in various carcinomas. In addition, histological examinations will be made to determine whether the zinc dithizone complex has caused any significant structural changes in these tissues.

The results of the present investigations show that about four-fifths of the ^{65}Zn of the injected zinc dithizone complex was present in the lungs of the rabbits 24 hr. after the injection, and that the deposited ^{65}Zn was only slowly removed from that tissue. In general, the other tissues examined contained only a small amount of ^{65}Zn , but 3-10 days after the injection the liver, pancreas and duodenum usually contained, per g. of wet tissue, a little more than did muscle, heart or kidney. The higher concentrations in the pancreas and duodenum can be attributed to the important role of pancreatic and

duodenal juices in the excretion of zinc (Montgomery, Sheline & Chaikoff, 1943).

SUMMARY

1. A method for the determination of ^{65}Zn in animal tissues, based on that described by Gettler & Bastian (1947) for the determination of zinc in normal human tissues, is described. The recovery of added ^{65}Zn is usually 95-100%, the mean of twenty-nine determinations being 96.5% (S.D., $\pm 2.6\%$).

2. A study has been made of the distribution of radioactive zinc in the tissues of rabbits following the intravenous injection of ^{65}Zn -containing zinc carbonate or phosphate, or a zinc dithizone complex.

3. Half or nearly half the injected zinc was found in the lungs 45 min. after the injection of the zinc carbonate or phosphate. Small amounts only were present in the liver and kidneys.

4. About 80% of the injected zinc dithizone complex was present in the lungs after 24 hr., and over 50% remained there 10 days after the injection. The livers contained small amounts only (1.4-5.3%) of the injected zinc.

The authors are pleased to express their indebtedness to the British Empire Cancer Campaign, the Medical Research Council and the University of London Central Research Fund for grants (to A. W.) which have covered the main expenses of this work. Thanks are also tendered to the Medical Research Council Tracer Elements Sub-Committee for supplies of ^{65}Zn .

REFERENCES

- Association of Official Agricultural Chemists (1945). *Methods of Analysis of the Association of Official Agricultural Chemists*, p. 124. Washington: Association of Official Agricultural Chemists.
- Banks, T. E. (1946). *Brit. J. Radiol.* **19**, 333.
- Banks, T. E., Boursnell, J. C., Dewey, H. M., Francis, G. E., Tupper, R. & Wormald, A. (1948). *Biochem. J.* **43**, 518.
- Banks, T. E., Francis, G. E., Franklin, K. J. & Wormald, A. (1950). *Biochem. J.* **47**, 374.
- Bodansky, O. & Blumenfeld, O. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 546.
- Carruthers, C. & Suntzeff, V. (1945). *J. biol. Chem.* **159**, 647.
- Carruthers, C. & Suntzeff, V. (1946). *Cancer Res.* **6**, 296.
- Cloetens, R. (1941). *Biochem. Z.* **310**, 42.
- Cloetens, R. (1944). *Arch. int. Pharmacodyn.* **69**, 386; cited from *Chem. Abstr.* (1946), **40**, 4091.
- Cowdry, E. V., Carruthers, C. & Suntzeff, V. (1948). *J. nat. Cancer Inst.* **8**, 209.
- Davidson, J. N. (1942). *Biochem. J.* **36**, 252.
- Dobson, E., Kelly, L., Jones, H. & Gofman, J. (1947). Communication to the 17th Int. physiol. Congr. Oxford.
- Dougherty, E. C. & Lawrence, J. H. (1948). In *Advances in Biological and Medical Physics*, vol. 1, ed. by Lawrence, J. H. & Hamilton, J. G. New York: Academic Press Inc.
- Gettler, A. O. & Bastian, R. (1947). *Amer. J. clin. Path.* **17**, 244.
- Hahn, P. F. & Sheppard, C. W. (1946). *Sth. med. J., Nashville*, **39**, 558.
- Heath, J. C. (1948). *26th Ann. Rep. Brit. Emp. Cancer Campgn.*, p. 173.
- Heath, J. C. (1949). *Nature, Lond.*, **164**, 1055.
- Holmberg, C. G. (1939). *Biochem. J.* **33**, 1901.
- Jones, H. B., Wrobel, C. J. & Lyons, W. R. (1944). *J. clin. Invest.* **23**, 783.
- Keilin, D. & Mann, T. (1939). *Nature, Lond.*, **144**, 442.
- Keilin, D. & Mann, T. (1940). *Biochem. J.* **34**, 1163.
- Montgomery, M. L., Sheline, G. E. & Chaikoff, I. L. (1943). *J. exp. Med.* **78**, 151.
- Muchow (1925). *Tabul. biol., Berl.*, **2**, 468.
- Nguyen-Van-Thoi, Roche, J. & Roger, M. (1947). *Biochim. Biophys. Acta*, **1**, 61.
- Praetorius, E. (1948). *Biochim. Biophys. Acta*, **2**, 590.
- Roche, J., Cornil, L., Desruisseaux, G., Baudoin, N. & Long, S. (1947). *C.R. Soc. Biol., Paris*, **141**, 1251.
- Roche, J., Nguyen-Van-Thoi, Marcelet, J., Desruisseaux, G. & Durand, S. (1946). *Bull. Acad. Méd.* **130**, 294.
- Scott, D. A. (1934). *Biochem. J.* **28**, 1592.
- Scott, D. A. & Fisher, A. M. (1935). *J. Pharmacol.* **55**, 206.
- Sheppard, C. W. & Hahn, P. F. (1946). *Sth. med. J., Nashville*, **39**, 562.
- Sheppard, C. W., Wells, E. B., Hahn, P. F. & Goodell, J. P. B. (1947). *J. Lab. clin. Med.* **32**, 274.
- Vallee, B. L. & Altschule, M. D. (1949). *Physiol. Rev.* **29**, 370.
- Vallee, B. L. & Gibson, J. G. (1948). *J. biol. Chem.* **176**, 445.
- Veall, N. (1948). *Brit. J. Radiol.* **21**, 347.