IRI-rapport: 133-76-02

BINDERS OF INTRAVENOUSLY ADMINISTERED 65-ZINC IN RAT LIVER CYTOPLASM.

by

A.J. Stortenbeek,
C.J.A. van den Hamer

Interuniversity Reactor Institute,
Delft, The Netherlands.

Accepted for publication in Bioinorganic Chemistry.

CYTOPLASMATIC ZN BINDERS

ABSTRACT

The fate of an i.v. injected trace dose of $^{65}Zn^{2+}$ in the rat, was studied over a period of 10 days after injection. Tissue distributions were determined and a special study was made of ^{65}Zn binders in liver cytoplasm. A total of six ^{65}Zn-binding fractions were observed in liver cytoplasm with apparent molecular weights of about 113,000, 66,400, 47,400, 29,000 23,000 and 11,400. A time study showed that 4 h after the injection, the most prominent cytoplasmatic 65-zinc binders are the 133,000, 66,400 and 23,000 molecular weight fractions. A tentative identification of the main Zn binders in the six ^{65}Zn fractions is given, using the collected data regarding their apparent molecular weight, time dependent prominence and content of stable Zn.

INTRODUCTION

The importance of Zn as an essential trace metal and the effects of Zn-poisoning and -deficiency are well documented [1-10]. Hereditary disorders of Zn metabolism in man have never been observed but a congenital disturbance of Zn absorption has been found in cows [11]. In the course of our investigations on heavy metal metabolism, it became evident that knowledge at molecular level is a prerequisite for the good understanding and interpretation of total body metabolism. However, uptill now the research on Zn metabolism at this level has not received much attention.

In this paper the results of investigations on ^{65}Zn metabolism in the rat are reported. Our attention was particularly focussed on the molecular weight determination of the ^{65}Zn-binding fractions in liver cytoplasm and on their ^{65}Zn content as a function of time. Moreover, we have attempted to present a qualitative description of the main Zn binders in the various fractions. The fate of ^{65}Zn given to rats has been studied extensively [12-22], using different routes of administration and doses varying from a trace amount to several hundreds of µg. The intravenous route was chosen by us to avoid a variable resorption time, such as may occur with intraperitonal or oral administration, and we used a trace dose to minimize the disturbance of the physiological concentrations.

The work presented here forms an extension of earlier work by one of us on the Cu-binding proteins in the liver [23,24].

MATERIALS AND METHODS

Sephadex G-100, Sephadex G-75 and Blue Dextran 2000 were obtained from Pharmica (Uppsala, Sweden). Bovine serum albumin, Cohn fraction V was obtained from Calbiochem (La Jolla, Calif., USA); ovalbumin from Fluka AG (Buchs, Swiss); chymotrypsinogen A, analytical grade, from Boehringer Mannheim (Mannheim, BRD); and horse heart cytochrome c, practical grade, from Sigma (St. Louis, Mo., USA).

Carrier free $^{65}Zn^{2+}$ in 10 mM HCl (4 µg Zn and 1.15 - 1.75 mCi per ml), was purchased from the Radiochemical Centre (Amersham, UK). ^{64}Cu was prepared at our institute by activation of metallic Cu (≥ 99.999 % obtained from Ventron Alfa Products, Berverly, Mass., U.S.A.) with thermal neutrons. After activation the metal was dissolved in 70 % HNO_3 (4 µl/mg Cu) and diluted with 130 mM NaCl, 50 mM Na-acetate buffer (pH 5.6) to a final concentration of 0.3 mM Cu. The specific activity at time of injection was about 3 mCi/mg.

Male Wistar-derived rats, Cpb: WU (Central Institute for the breeding of Laboratory Animals, Zeist, The Netherlands), weighing 200-250 g at the time of injection, were used in all experiments. They were housed in plastic cages covered with stainless steel lids and maintained on a diet of standard laboratory food (Hope Farms N.V. Woerden, The Netherlands) and deionized water ad libitum.

$^{65}Zn^{2+}$ (dose: 11 - 35 µCi, 0.04 - 0.08 µg) in 0.5 ml 130 mM NaCl, 50 mM Na-acetate buffer (pH 5.6) was injected via a caudal vein. For simultaneous injection of $^{64}Cu^{2+}$ and $^{65}Zn^{2+}$, 1 ml of this injection solvent was used, but containing 10 µg Cu (i.e. app. 30 µCi) as well as the $^{65}Zn^{2+}$. Rats were killed by decapitation at various time intervals post injection and samples of body tissues and fluids were collected and assayed for ^{65}Zn.

A portion of the liver was homogenized at 0°C in twice its weight 170 mM NaCl and centrifuged at 105,000 g for 1 h. After centrifugation a sample of the supernatant was transferred to a 2.5 x 110 cm Sephadex G-100 or a 1.8 x 108 cm Sephadex G-75 chromatography column, prepared essentially as described by Whitaker [25]. Elution was carried out at

4°C with 50 mM Tris-HCl, 120 mM NaCl, 3 mM NaN_3 (pH 7.0), at a constant rate of 15-20 ml/h. The eluate was continuously monitored at 280 nm and collected in 3-4 ml fractions.

The ^{64}Cu and ^{65}Zn activities were measured as photo peak count rates, determined by means of NaI well-type detectors. Only ^{64}Cu count rates were corrected for decay. To facilitate the comparison between the different column elutions the count rates of the eluate fractions were normalized by multiplication with a factor f, where

$$f = \frac{1}{\Sigma \text{ count rate all fractions}} \times \frac{\text{count rate/g cytoplasm}}{\text{count rate/g homogenate}} \times \text{\% of injected dose found in whole liver}$$

Total Zn concentrations were determined by atomic absorption spectrophotometry. Samples were aspirated directly in an air-acetylene flame and no corrections were made for aspecific absorption.

The molecular weights of gel chromatographic fractions were estimated by means of a calibration curve, a representative example of which is shown in figure 1. These calibration curves were constructed by plotting the relative elution volumes (elution volume of a substance divided by void volume) found for bovine serum albumin, ovalbumin, chymotrypsinogen A and cytochrome c, against the log of their molecular weights using the values 67,000, 45,000, 25,000 and 13,000 resp. Blue Dextran 2000 served as void volume marker. The average void volumes observed for Sephadex G-100 and G-75 columns were 217 ml and 105 ml resp. The slopes of the calibration lines for the different columns showed minor variations, therefore a calibration was performed for every new column and repeated regularly. The hemoglobin in rat liver cytoplasm was eluted as a 43,000 molecular weight protein, in good agreement with ref. 25.

RESULTS

In figure 2 the ^{65}Zn content of a number of tissues is plotted against time post injection of the trace dose. The results show that intravenously injected $^{65}Zn^{2+}$ was rapidly removed from the circulation and accumulated mainly in the liver which contained maximally about one third of the dose. ^{65}Zn activity in whole blood decreased slower than in plasma, probably due to the incorporation of ^{65}Zn in newly synthesized erythrocyte carbonic anhydrase [26].

All tissues tested showed a rapid initial ^{65}Zn uptake. In bone this initial uptake was followed by a slower second phase as was earlier shown by Bergman and Wing [22]. ^{65}Zn decrease was rapid in the viscera, while little release was observed in brain and muscle. Replacing the trace dose of 0.04 - 0.08 µg by 10 µg ^{65}Zn did not significantly change the tissue distribution at ½, 1, 2, 4 and 24 h p.i.

In the liver cell ^{65}Zn was mainly located in the cytoplasm, i.e. about 80 % at 15 min p.i., followed by a gradual increase to almost 100 % at 30 h p.i. Typical examples of elution patterns of Sephadex G-100 fractionated liver cytoplasm at ½, 8 and 239 h p.i. are present in figure 3. A total of six ^{65}Zn-binding fractions could be distinguished (indicated as I-VI), although not all at the same time p.i. For fractions I-VI molecular weights of 100 - 145,000, 63 - 68,000, 47 - 48,000, 28 - 30,000, 21 - 25,000 and 10 - 12,000 resp. were estimated from their relative elution volumes in several experiments.

In a time study the cytoplasmatic ^{65}Zn-binding was more extensively studied between 15 min and 10 days p.i. Figure 3 shows that at 15 min after injection fraction I was the main ^{65}Zn-binding fraction, while the others, except fraction VI, were poorly resolved. Fractions III and IV were only clearly visible during the first hour p.i. and later diminished, though they were probably still present as shoulders of peaks II and V. Fractions II and V on the other hand became gradually more distinct and from 4 h p.i. onwards always showed as well-separated peaks. For this reason fractions II and V were, together with fraction I, considered to contain the main ^{65}Zn binders.

The poor resolution shortly p.i. - and the shoulders later on -

made it impossible to measure reliably the total count rates of the most prominent fractions (I, II and V). For these reasons a (semi) quantitative analysis of the time dependance was made by using maximum count rates of these fractions, corrected for the estimated contributions of adjacent fractions (see fig. 4a). Fraction VI was always clearly separated so a total count rate vs time p.i. plot could be made, see figure 4b. This figure shows that fraction VI was never detected beyond 30 h p.i. and before that time its presence was erratic.

Measurements of total Zn content of Sephadex G-100 fractionated liver cytoplasm also showed several Zn-containing fractions, see fig. 5. Five of them probably correspond to the ^{65}Zn-containing fractions I-V, as indicated in this figure. Besides these, two more total Zn fractions were found, one in the void volume and the other with an apparent molecular weight of 35,000. In the region of the ^{65}Zn fraction VI, no total Zn could be detected (3 experiments). In one of these experiments total Zn was detected in a ^{65}Zn-labeled cytoplasm; again no Zn was detected in the region of fraction VI, although ^{65}Zn was present in this fraction.

Elution diagrams of successive Sephadex G-75 fractionations of ^{64}Cu and ^{65}Zn liver cytoplasm are presented in figure 6. Molecular weight estimates for the ^{65}Zn fractions found this time were 80,000, 62,000, 30,000 and 25,000 and were taken to correspond to the Sephadex G-100 fractions I, II, IV and V resp. (fig. 3). The apparent molecular weights of the ^{64}Cu fractions were 85,000, 43,000, 30,000 and 10,000, indicating that the 30,000 molecular weight fraction, probably being cytocuprein [23], coincided with ^{65}Zn fraction IV.

In one case a rat was injected simultaneously with $^{65}Zn^{2+}$ and $^{64}Cu^{2-}$. The animal was decapitated after 2 hours and the liver cytoplasm was isolated. This cytoplasm was carried through the first two steps of an isolation of rat liver cytocuprein [23]. It was first subjected to fractional precipitation with ethanol-chloroform (9:1, v/v). The resulting crude cytocuprein fraction, which appeared to contain both ^{65}Zn and ^{64}Cu, was then passed through a Sephadex G-75 column. The resulting elution patterns of both radionuclides covered each other remarkably well.

DISCUSSION

a. Tissue distribution of i.v. injected $^{65}Zn^{2+}$

Rat plasma contains about 1.5 µg Zn per ml [27], of which about 80 % - the albumin bound Zn [28]- is readily exchangeable [29,30]. Taking the plasma volume as 4 % of the body weight, the exchangeable intravascular Zn in a 200 g rat can be estimated to be 9.6 µg. A dose of 0.04 - 0.08 µg, as used in our experiments, will therefore come close to truly tracering the Zn in the plasma and will disturb this pool only minimally.

Different Zn doses and routes of administration have been used in the literature. Nevertheless, when comparing our observations with the ^{65}Zn tissue distributions reported by others, we often found a reasonable agreement [14, 16, 17, 20]. Therefore route of application or amount of Zn given, does not necessarily appear to influence the tissue ^{65}Zn distribution. This is also shown by the similar distributions we observed after injecting a trace dose of 10 µg Zn.

In all investigations the liver contained the highest percentage of the ^{65}Zn dose shortly after administration which underlines the central role of this organ in trace metal metabolism.

b. Cytoplasmatic distribution of ^{65}Zn in the liver

Most of the ^{65}Zn in the liver after i.v. injection was found in the cytoplasm: about 80 % at 15 min p.i., rising to almost 100 % at 30 h p.i. and beyond. These values are somewhat higher than those (e.g. 70 - 80 % at 24 h after subcutanous injection [31]), reported elsewhere, and still higher than the values observed for the cytoplasmatic content of total Zn [32-35].

Our molecular weight estimates of cytoplasmatic ^{65}Zn carriers are hard to compare with the results of others, mainly because in the literature calibration data are presented incompletely or not at all. In those cases when besides the ^{65}Zn elution patterns protein diagrams (280 or 254 transmittance) were presented, we were able to interrelate both patterns by determining the positions of ^{65}Zn activity maxima on the protein elution pattern. By comparing these positions to our results, we found reasonable agreement with

the results of Shaikh and Lucis |31| and Günther et al. |36|, except for the absence of our fraction V in the patterns of the latter. A molecular weight of 23,000, as estimated by us for this fraction, has not yet been reported for a cytoplasmatic Zn binder in rat liver.

For the carriers of total Zn similar problems of comparison were solved in the same way in those cases where protein transmittance patterns were available. Then we found fair agreement between our data and those of Webb |37| and Winge et al |38|. One of the total Zn fractions was interpreted by Winge et al |38| to be superoxide dismutase, an enzyme said to be identical with cytocuprein |39|. Using the data from ref.|38| we computed a molecular weight of 21,000 for this fraction, which is in reasonable agreement with the molecular weight of 23,000 we found for the corresponding fraction (V in fig. 5). However, as will be argued below, cytocuprein is in our opinion not present in fraction V but in fraction IV.

c. A qualitative description of the ^{65}Zn binders in liver cytoplasm

Our gelchromatography experiments led to a tentative identification (table 1) of the ^{65}Zn-binding fractions in the liver cytoplasm using three criteria, viz. their apparent molecular weight, their presence as function of time since injection and the presence of stable Zn in the fraction. It must be borne in mind, however, that molecular weight estimations based on gelchromatography can sometimes result in anomalous values |25,40|, one of the reasons why we described in detail (see Materials and Methods) how we arrived at our estimates.

Because the binding of ^{65}Zn in fraction I is very fast and involves a large amount (fig. 3), this fraction is likely to contain the initial binder of freshly arriving Zn, which it will subsequently transfer to other binders, e.g. enzymes. A high molecular weight initial binder of Zn in rat liver cytoplasm is in striking contrast to the 10,000 molecular weight protein found to be the first binder in case of Cu |24|.

In the 63 - 68,000 molecular weight fraction II, albumin could be the main Zn binder. Immunologic evidence for this assumption will be presented elsewhere |A.J. Stortenbeek and C.J.A. van den Hamer, in preparation|.

Fractions III and IV both bind ^{65}Zn rapidly and seem to play a minor role in ^{65}Zn-binding at longer times p.i., as indicated by the shoulders on peaks II and V (fig. 3). Neither of these fractions point to any of the known cytoplasmatic zinc-enzymes [3] and it may be suggested that fraction III mainly contains a nonspecific Zn binder. In view of the following observations it may be assumed that fraction IV consists of cytocuprein:

- a 30,000 molecular weight was found on basis of gelchromatography
- identical positions were observed for the main ^{64}Cu fraction and ^{65}Zn fraction IV after Sephadex G-75 separation of liver cytoplasm (fig. 6)
- on isolation of cytocuprein from a ^{64}Cu, ^{65}Zn double labeled rat liver cytoplasm, some of the ^{65}Zn was probably bound to the partly purified cytocuprein, as shown by its appearance in the same ethanol-chloroform fraction as ^{64}Cu and by the almost identical elution diagrams of both radionuclides after Sephadex G-75 chromatography of this fraction
- the stable Zn detected in this fraction (see fig. 5) is in accordance with its reported presence in cytocuprein [39].

The increase in concentration of ^{65}Zn in fraction V with time seems characteristic for a metalloprotein [3], which does not exchange its endogenous Zn, but can incorporate it only during synthesis.

The results of figure 4b show that shortly after injection ^{65}Zn was occasionally absent from fraction VI. When, however, ^{65}Zn was present in this fraction, the binding seemed to be very fast while beyond 30 h p.i. no ^{65}Zn was ever detected. The accidental presence of ^{65}Zn in fraction VI indicates that the presence of a Zn binder here is not a normal feature but depends on uncontrolled factors. This Zn binder may be metallothionein, a protein involved in heavy metal detoxification [31, 41, 43] and which is known to contain Zn [44] The synthesis of metallothionein is reported to be induced by Cd [41,42,45], Hg [46] or large doses of Zn [35, 37, 47]. Since the amount of Zn injected in our experiments was far too little to induce synthesis of metallothionein [37, 48], its presence should have accidental reasons to be sought in environmental and nutritional factors. Because the animals in our experiments did not belong to one group, a variable exposure to heavy metals cannot be ruled out.

If the main ^{65}Zn binder of fraction VI is indeed metallothionein, it may be concluded from the results shown in fig. 4b that the in vivo

^{65}Zn incorporation in metallothionein is very rapid. The disappearance of ^{65}Zn from fraction VI is also rather rapid, in contrast to the reported behaviour of Cd in metallothionein [31]. However, one has to keep in mind that probably more than one form of metallothionein or metallothionein-like materials exist as already suggested by Chen et al [49].

We were unable to detect stable Zn in the region of fraction VI in three experiments. It is not possible to decide whether this absence is due to an accidental absence of a Zn binder or that the concentration of stable Zn was under our detection limit. One experiment in which ^{65}Zn - but no stable Zn - was found in fraction VI seems to point to the second possibility, because if metallothionein were the main Zn binder in fraction VI one would indeed expect the presence of stable Zn.

Finally it seems worthwhile to point out that the three most prominent ^{65}Zn-containing fractions I, II and V correspond to the three fractions that also contain most of the total Zn (fig. 5). Moreover, the less prominent fractions III and IV also contain some stable Zn. Therefore it may be said that the various cytoplasmatic Zn binders are indicated by the gelchromatographic distribution of the ^{65}Zn fractions, found at various times after injection.

ACKNOWLEDGEMENTS

The authors wish to thank drs. H. Nederbragt, Zoötechnical Institute, Utrecht, The Netherlands, for his assistance in performing the atomic absorption analyses and drs. M.P. de Haas, Interuniversitair Reactor Instituut, Delft, The Netherlands, for his help in writing a program for computing the normalized count rates.

REFERENCES.

1. L.S. Hurley, Am. J. Clin. Nutr. 22, 1332 (1969).
2. W.J. Miller, Am. J. Clin. Nutr. 22, 1323 (1969).
3. A.F. Parisi and B.L. Vallee, Am. J. Clin. Nutr. 22, 1222 (1969).
4. A.S. Prasad, D. Oberleas and G. Rajasekaran, Am. J. Clin. Nutr. 23, 581 (1970).
5. A.S. Prasad and D. Oberleas, J. Appl. Physiol. 31, 842 (1971).
6. H.A. Schroeder and A.P. Nason, Clin. Chem. 17, 461 (1971).
7. M. Chvapil, Life Sci. 13, 1041 (1973).
8. S. Elias and M. Chvapil, J. Surg. Res. 15, 59 (1973).
9. B. Jaremin, Bull. Inst. Mar. Med. Gdansk 24, 85 (1973).
10. H.H. Sandstead, Am. J. Clin. Nutr. 26, 1251 (1973).
11. J. Kroneman, G.J.W. v.d. Mey and A. Helder, Zentralbl. Veterinaermed., Reihe A 22, 201 (1975).
12. I.G.F. Gilbert and D.M. Taylor, Biochim. Biophys. Acta. 21, 545 (1956).
13. J.C.N. Wakely, B. Moffatt, A. Crook and J.R. Mallard, Int. J. Appl. Radiat. Isot. 7, 225 (1960).
14. J.E. Ballou and R.C. Thompson, Health Phys. 6, 6 (1961).
15. M.E. Rubini, G. Montalvo, C.P. Lockhart and C.R. Johnson, Am. J. Physiol. 200, 1345 (1961).
16. P. Czerniak, A. Naharin and N. Alexander, Int. J. Appl. Radiat. Isot. 13, 547 (1962).
17. S. Br .manandam, B. Nagarajan and V.M. Sivaramakrishnan, Indian J. Exp. Biol. 3, 88 (1965).
18. F. Dorn and Th. Gürther, Z. Klin. Chem. Klin. Biochem. 8, 618 (1970).
19. V. Eybl and J. Sýkora, Z. Gesamte Exp. Med. 152, 274 (1970).
20. U. Auth, Strahlentherapie 4, 490 (1973).
21. A.H. Methfessel and H. Spencer, J. Appl. Physiol. 34, 58 (1973).
22. B. Bergman and K.R. Wing, Acta Physiol. SCand. 92, 451 (1974).
23. I. Sternlieb, C.J.A. van den Hamer, A.G. Morell, S. Alpert, G. Gregoriadis and I.H. Scheinberg, Gastroenterology 64, 99 (1973).
24. C.J.A. van den Hamer, A.G. Morell and I.H. Scheinberg, Symposium on Protides of the Biological Fluids, Bruges, 1975.
25. J.R. Whitaker, Anal. Chem. 35, 1950 (1963).
26. J.E.A. McIntosh and C. Lutwak-Mann, Biochem. J. 126, 869 (1972).
27. B. Bergman, R. Sjöström and K.R. Wing, Acta Physiol. Scand. 92, 440 (1974).
28. E.L. Giroux, Biochem. Med. 12, 258 (1975).
29. F.A. Suso and H.M. Edwards, Jr., Proc. Soc. Exp. Biol. Med. 137, 306 (1971).
30. E.L. Giroux and R.I. Henkin, Bioinorg. Chem. 2, 125 (1972).

31. Z.A. Shaikh and O.J. Lucis, Arch. Environ. Health 24, 419 (1972).

32. Y. Suzuki and H. Yoshikawa, Ind. Health 9, 198 (1971).

33. I. Bermmer, N.T. Davies and C.F. Mills, Biochem. Soc. Trans 1, 982 (1973).

34. F. Alfaro and F.W. Heaton, Br. J. Nutr. 32, 435 (1974).

35. R.W. Chen, D.J. Eakin and P.D. Whanger, Nutr. Rep. Int. 10, 195 (1974).

36. T. Günther, B. Ruhe, J. Schmalbeck and N. Tehrani, Z. Klin. Chem. Klin. Biochem. 12, 327 (1974).

37. M. Webb, Biochem. Pharmacol. 21, 2751 (1972).

38. D.R. Winge and K.V. Rajagopalan, Arch. Biochem. Biophys. 153, 755 (1972).

39. G.W. Evans, Nutr. Rev. 29, 195 (1971).

40. P. Andrews, Biochem. J. 96, 595 (1965).

41. Z.A. Shaikh and O.J. Lucis, Fed. Proc. 29 Abs., 298 (1970).

42. M. Webb, Biochem. J. 124, 17 P (1971).

43. Y. Suzuki, Ind. Health 10, 52 (1972).

44. J.H.R. Kägi and B.L. Vallee, J. Biol. Chem. 235, 3460 (1960).

45. M. Webb, J. Reprod. Fert. 30, 83 (1972).

46. Y. Suzuki, Ind. Health 10, 56 (1972).

47. N.T. Davies, I. Bermmer and C.F. Mills, Biochem. Soc. Trans. 1, 985 (1973).

48. M.P. Richards and R.J. Cousins, Bioinorg. Chem. 4, 215 (1975).

49. R.W. Chen, P.D. Whanger and P.H. Weswig, Biochem. Med. 12, 95 (1975).

TABLE I Qualitative description of the ^{65}Zn-containing fractions of rat liver cytoplasm after gel chromatography.

fraction number *)	molecular weight x 10^{-3}	T_{max} **) (h)	period of prominence p.i.	tentative identification
I	113	¼	always	"first Zn binder"
II	66	8	> 1 h	cell albumin
III	47	¼	< 1 h later: shoulder of fraction II?	nonspecific Zn binder
IV	29	¼	< 1 h later: shoulder of fraction V?	cytocuprein
V	23	30	> 2 h	Zn metalloprotein
VI	11	¼	< 1 day	metallothionein

*) see figure 3

**) time p.i. when highest count rate was observed, see fig. 3 and 4

FIG. 1. Sephadex G-100 calibration curve.

Column dimensions: 2.5 x 110 cm. Elution was carried out at 4°C with 50 mM Tris-HCl, 120 mM NaCl, 3 mM NaN_3 (pH 7.0), at a rate of 14.6 ml/h. Concentrations of cytochrome c (A), chymotrypsinogen A (B), ovalbumin (C), bovine serum albumin (D) and Blue Dextran 2000, were 7.5, 5, 15, 15 and 5 mg/ml, resp. Sample volume was about 2 ml.

FIG. 2. ^{65}Zn content in rat tissues after i.v. injection of a trace dose.

0.04 - 0.08 µg, 11 - 35 µCi $^{65}Zn^{2+}$ in 0.5 ml 130 mM NaCl, 50 mM Na-acetate (pH 5.6), was injected via a caudal vein in male Wistar-derived rats, weighing 200 - 250 g at time of injection. The first 16 hours the points represent the averages of 2 - 4 experiments; from 30 hours on, each point represents one experiment. (A) ^{65}Zn content in % of dose per organ. (B,C) ^{65}Zn content in % of dose per g tissue or, in case of spleen and brain, per organ. The bone samples consisted of femur and tibia of a hind leg; muscle tissue was also taken from a hind leg.

FIG. 3. ^{65}Zn elution patterns of Sephadex G-100 fractionated rat liver cytoplasm at ½, 8 and 239 h after i.v. injection of a trace dose.

Chromatographic conditions as described in fig. 1; rats and injection as described in fig. 2. The arrows indicate the average peak positions of the ^{65}Zn-containing fractions I - VI.

FIG. 4. Time dependence of the ^{65}Zn content of fractions I, II, V and VI (see fig. 3).

(A) Maxima of normalized count rates of fractions I, II and V. (B) Total normalized count rate of fraction VI. The dots indicate the results of single experiments; the graph is drawn through the averages.

FIG. 5. Total Zn content in fractions of rat liver cytoplasm after Sephadex G-100 chromatography.

Zn content was determined by atomic absorption spectrophotometry. Chromatographic conditions as described in fig. 3, except for the elution rate which was 18.5 ml/h. The arrows indicate the peak positions of the total Zn-containing regions with apparent molecular weights of 112,000, 68,000, 46,000, 35,000, 29,000 and 23,000. The Roman numerals indicate the ^{65}Zn-containing fractions (fig. 3) to which the total Zn regions are assumed to correspond. The dashed line shows the transmittance at 280 nm in arbitrary units.

FIG. 6. $^{64}Cu^{2+}$ and $^{65}Zn^{2+}$ elution patterns of Sephadex G-75 fractionated rat liver cytoplasm after i.v. injection of either of the radionuclides.

$^{64}Cu^{2+}$ (10 µg) or $^{65}Zn^{2+}$ (0.08 µg) in 0.5 ml mM NaCl, 50 mM Na-acetate (pH 5.6), were injected via a caudal vein in 200 g male Wistar-derived rats. The animals were decapitated 2 h p.i. Column dimensions: 1.8 x 108 cm. Elution was carried out at 4°C with 50 mM Tris-HCl, 120 mM NaCl, 3 mM NaN_3 (pH 7.0), at a rate of 15 ml/h. The Roman numerals correspond to the ^{65}Zn-containing fractions as indicated in fig. 3. N.B. Because only the peak positions of both elution patterns were of interest and the count rates of the radionuclides differed widely, no units are indicated on the ordinate.

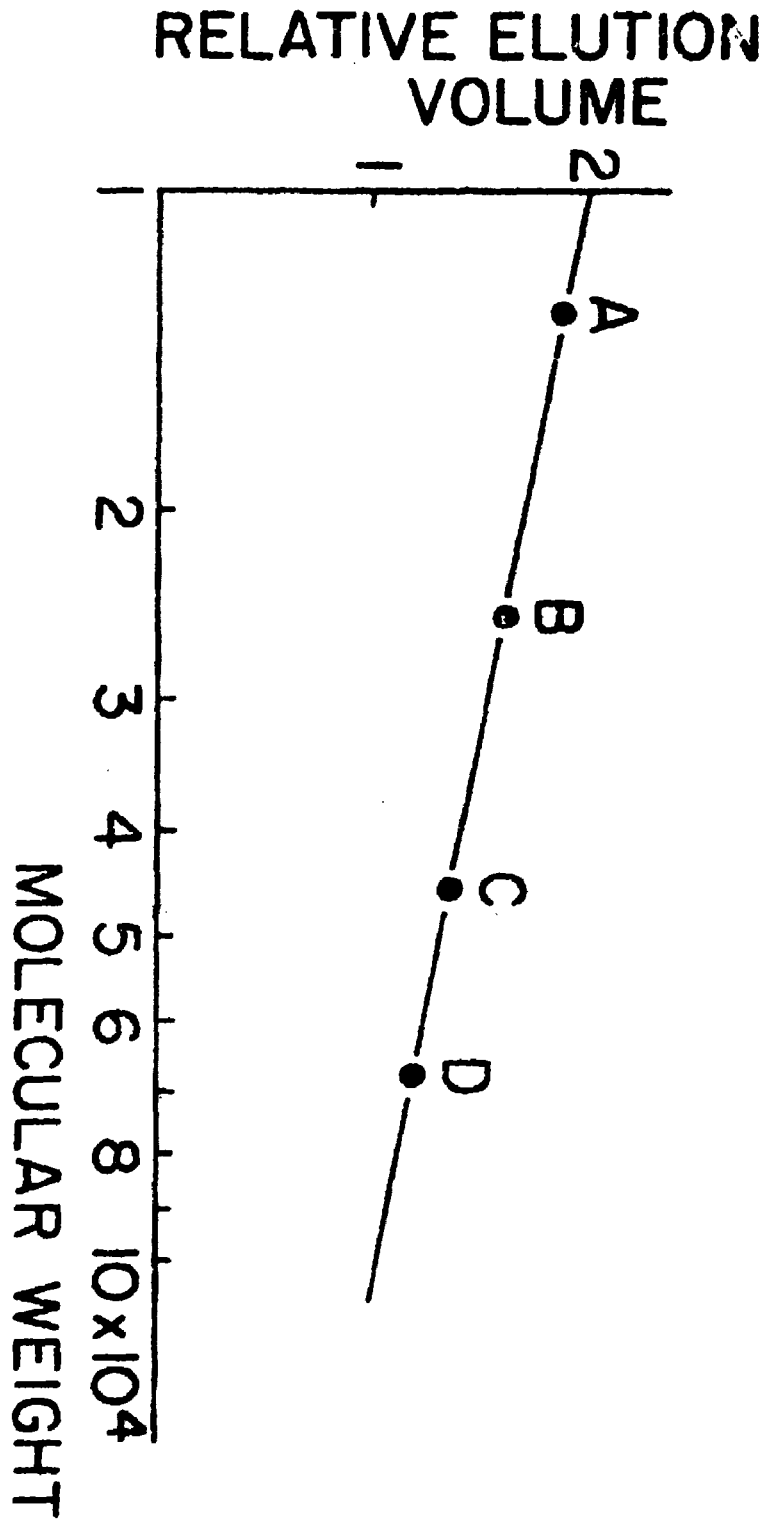
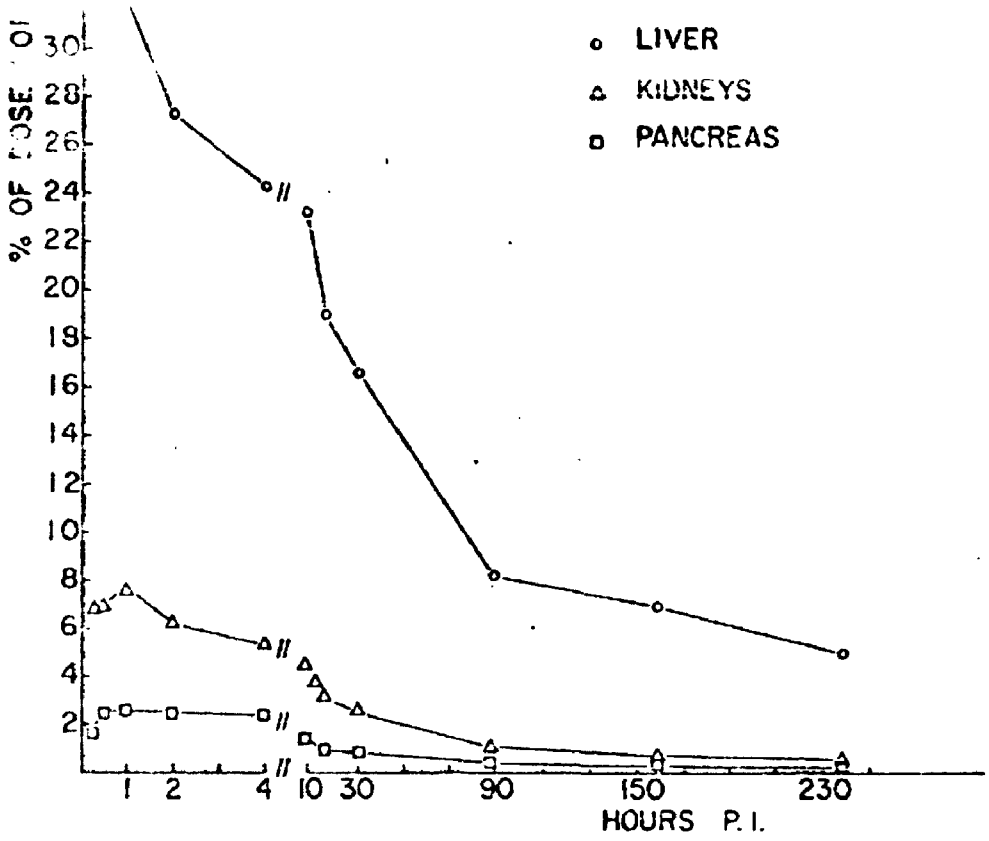
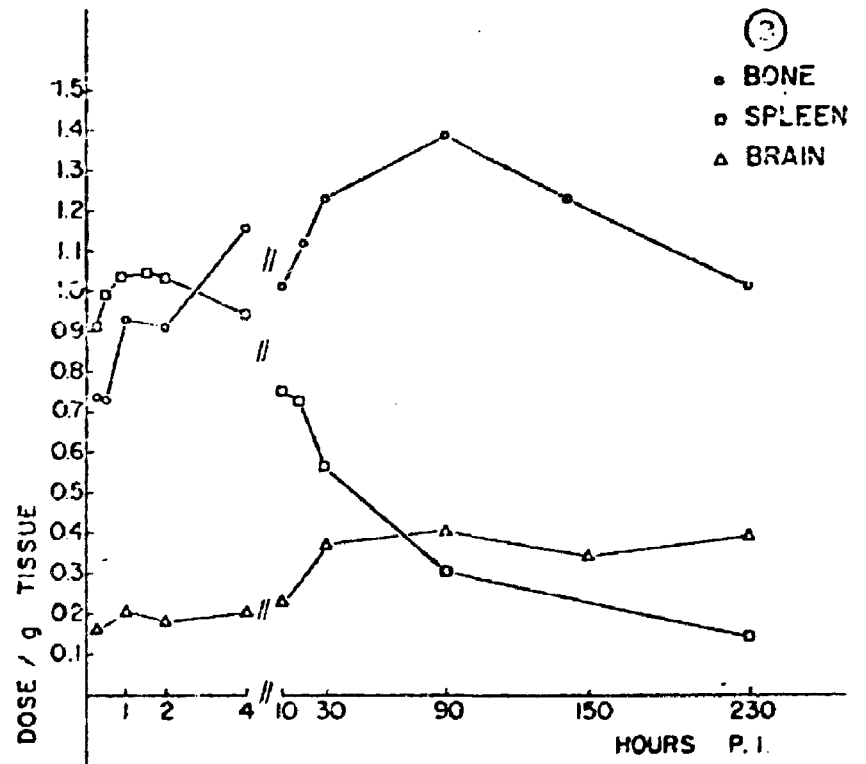

RELATIVE ELUTION VOLUME
1
2
A
B
C
D
1
2
3
4
5
6
8
10x10⁴
MOLECULAR WEIGHT

FIG. 1


LIVER
KIDNEYS
PANCREAS
% OF DOSE
HOURS P.I.


BONE
SPLEEN
BRAIN
% OF DOSE / g TISSUE
HOURS P.I.

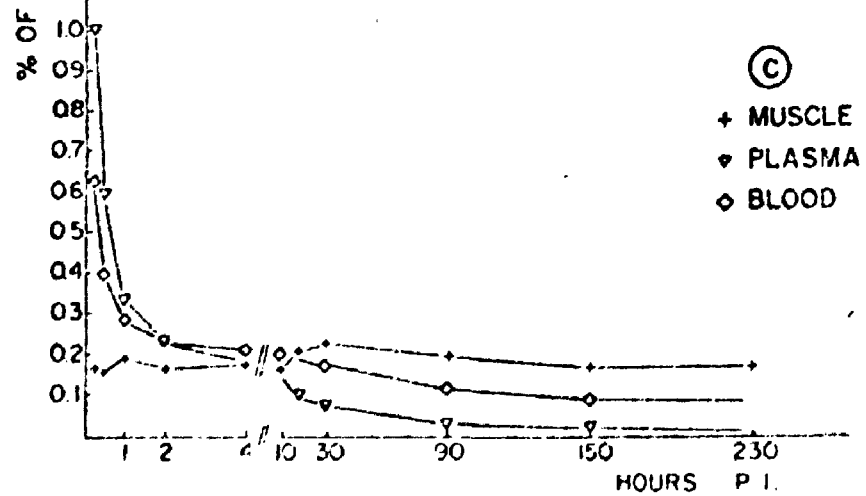

MUSCLE
PLASMA
BLOOD
HOURS P.I.

FIG. 2

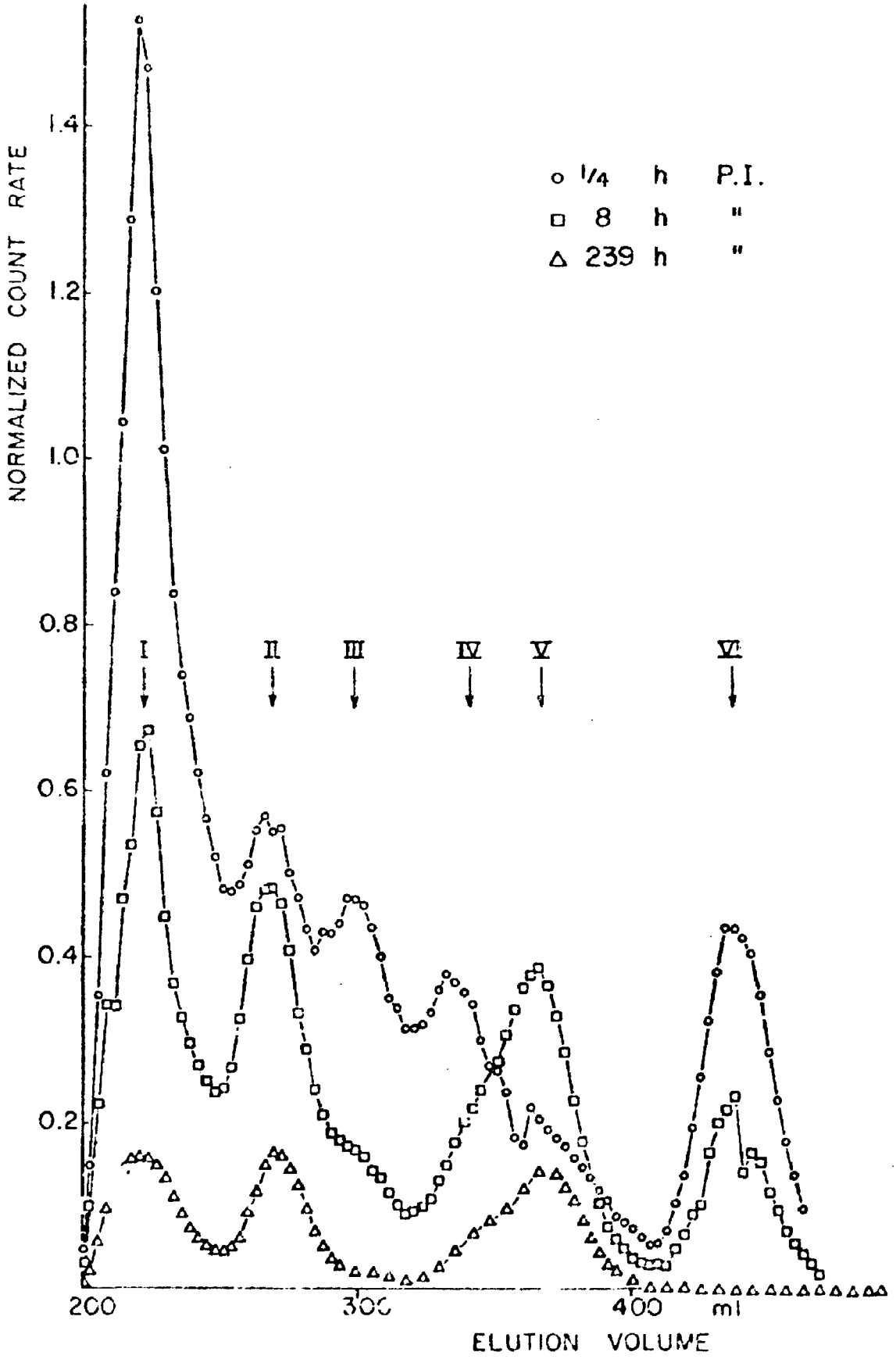
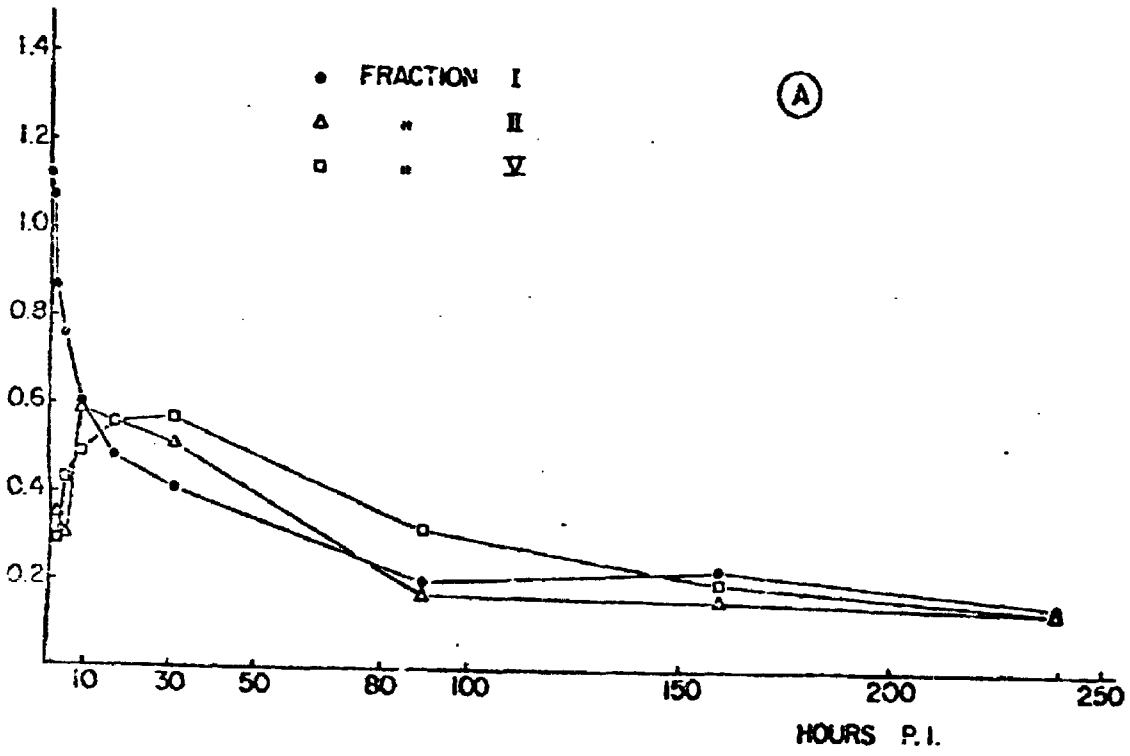

NORMALIZED COUNT RATE
1.4
1.2
1.0
0.8
0.6
0.4
0.2
o 1/4 h P.I.
□ 8 h "
Δ 239 h "
I
II
III
IV
V
VI
200
300
400
ml
ELUTION VOLUME

FIG. 3


FRACTION I
" II
" V
A
1.4
1.2
1.0
0.8
0.6
0.4
0.2
10
30
50
80
100
150
200
250
HOURS P.I.

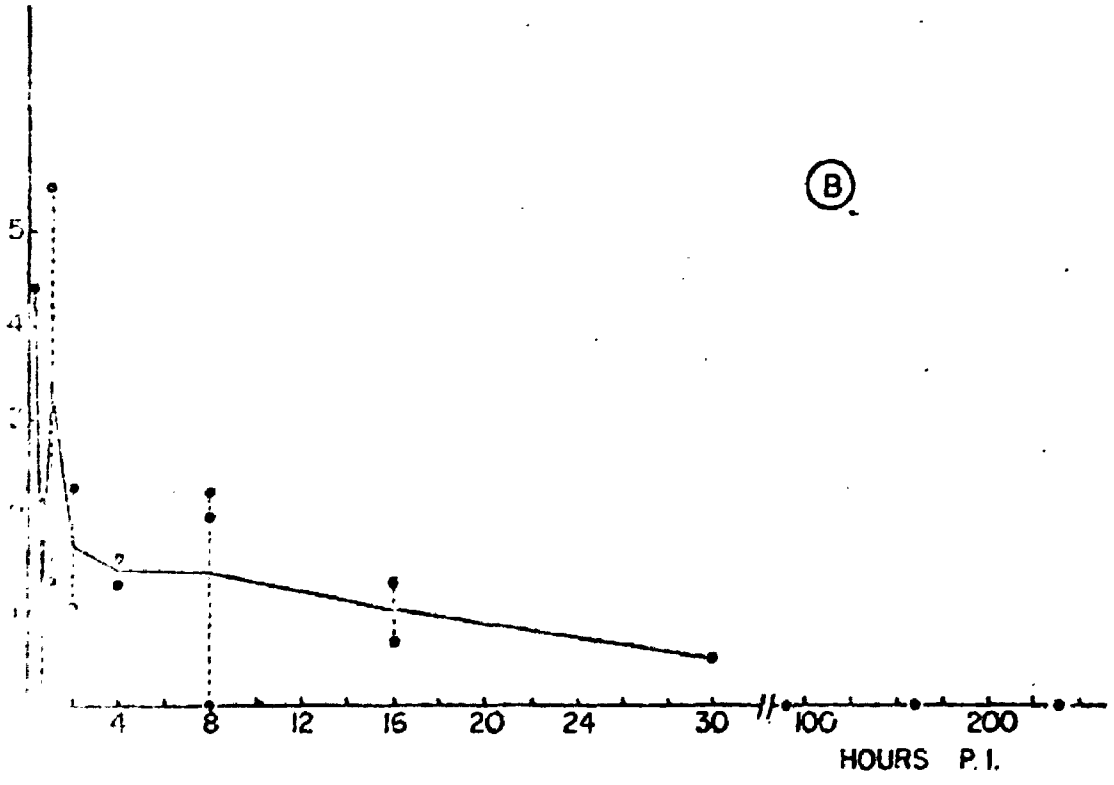
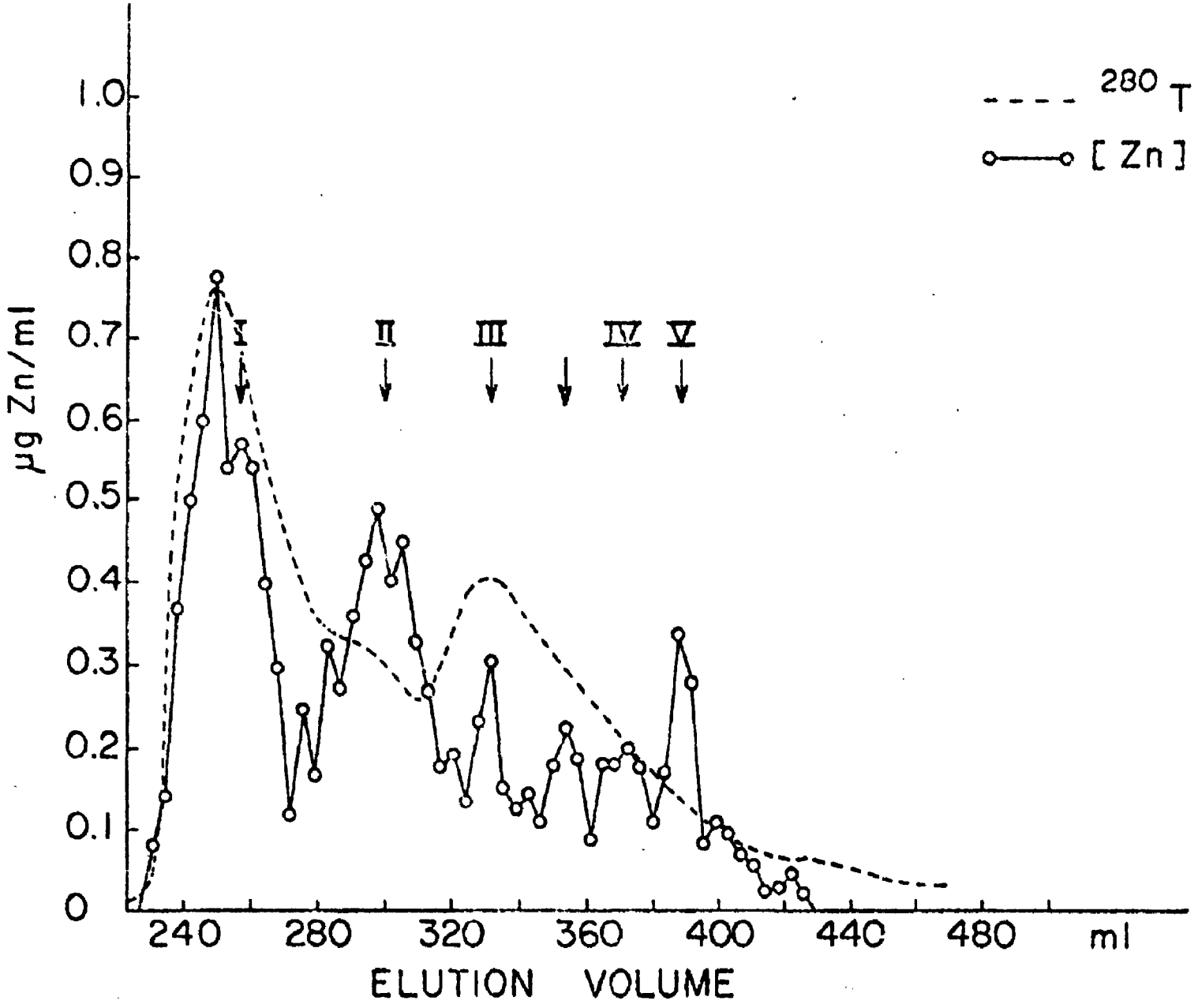

B
5
4
4
8
12
16
20
24
30
100
200
HOURS P.I.

FIG. 4

FIG. 5


280 T
[Zn]
μg Zn/ml
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
I
II
III
IV
V
240
280
320
360
400
440
480
ml
ELUTION VOLUME

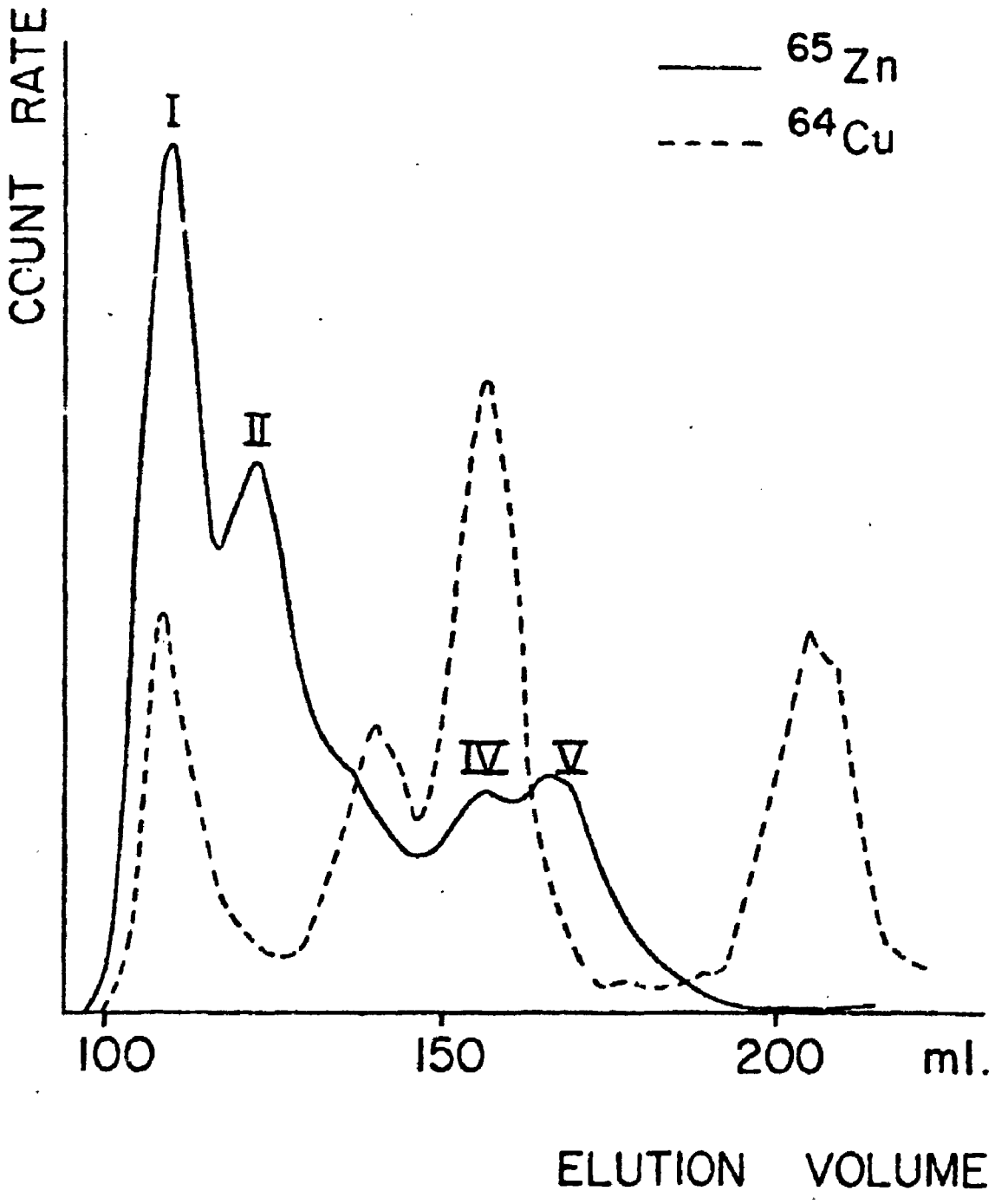


FIG. 6