

The Discrimination between Magnesium and Manganese by Serum Proteins

A. C. FORADORI, ALBERT BERTINCHAMPS,
JANE M. GULIBON, and GEORGE C. COTZIAS

From the Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973. Dr. Bertinchamps' present address is Direction Générale, Recherche et Enseignement, Services de Biologie, Brussels, Belgium

ABSTRACT Magnesium and manganese have proved physically and functionally interchangeable in many isolated biological systems investigated *in vitro*. This lack of discrimination contrasts sharply with the high biological specificity exhibited by intact mammals under a large variety of conditions. The dichotomy between intact animals and their isolated systems might be due at least partially to presence vs. absence of an intact circulation. Hence the capability of mammalian plasma to discriminate between the alkaline earth and the transition metal was investigated by means of equilibrium dialysis, exchange, ultrafiltration, ultracentrifugation, and zone electrophoresis. The states of the respective elements are thus contrasted as follows: (*a*) Magnesium is partially bound, manganese totally. (*b*) Magnesium is nonselectively bound by serum proteins, manganese selectively by a β_1 -globulin. (*c*) Under conditions approaching physiological, the two metals do not interchange. This is interpreted as indicating that the plasma proteins contribute to biological specificity by discriminating between a trace metal and a macronutrient.

INTRODUCTION

Magnesium and manganese are physically and functionally interchangeable in many isolated biological systems studied *in vitro*. This interchangeability has been found in studies of antibiotic effects (1); of enzymatic activities (2-4); of metal deficiency (5, 6); of oxidative phosphorylation (7, 9); of fatty acid synthesis (8); of metal transport (10); and of mutagenic effects (11). It was even proposed that such results could be expected from the similar complexing tendencies and crystal chemistry of these metals (12-14). Yet, a striking biological specificity characterizes these metals in mammalian organisms, as exemplified by the entirely different consequences of manganese vs. magnesium deficiency (15). These differences suggest that the trace amounts of tissue manganese exert essential functions in the presence of the comparatively large concentrations of magnesium. Indeed, in one mammal, manganese-mag-

nesium exchanges could not be demonstrated, whereas exchanges of ^{54}Mn with ^{55}Mn could be readily induced (16). This led to the postulation that "there exists a segment in the pathway of manganese through the body, the properties of which permit the passage of that metal only" (16).

The present paper pertains to a search for such a segment. Normal circulation of blood was lacking in the isolated biological systems in which the respective alkaline earth and transition metal substituted for each other (17). Therefore, the blood plasma might be capable of discriminating between magnesium and manganese. The experiments reported here indicate that a β_1 -globulin binds manganese in a manner which permits its exchange with manganese while it essentially prevents its exchange with magnesium.

MATERIALS AND METHODS

Human serum or plasma was obtained from hospitalized patients with normal blood cytology and chemistry, and from healthy laboratory workers. The blood was collected without anticoagulant with "Peelaway"[®] disposable syringes (18) in which it was centrifuged at 800 *g* (19) for 30 min. Bovine and rat serum were obtained in a similar manner.

Carrier-free manganous chloride ($^{54}\text{MnCl}_2$) half-life 314 days decaying by K electron capture and 0.84 Mev γ -emission (20), was obtained from the Nuclear Science and Engineering Corporation (Pittsburgh, Pa.) (500 μc per ml in 0.1 N HCl). Aliquots were added to the samples to obtain a final activity of 1 $\mu\text{c}/\text{ml}$. The pH was not changed by this addition.

Magnesium chloride ($^{28}\text{MgCl}_2$) specific activity $> 30 \mu\text{c}$ per g, decaying by a γ -cascade and β -emission (20) was obtained from the Hot Laboratory Division at Brookhaven.

The radioactivities were assayed in a well-type sodium iodide scintillation counter with 23 % efficiency (about 5×10^5 cpm/ μc) for both radioisotopes. Irradiation dose rate to the samples, estimated through the use of a nomogram (21), never exceeded 100 millirad per hr.

The manganese concentration (^{55}Mn) in the samples was determined by neutron activation analysis with a method presented earlier (22). Magnesium concentrations were determined by atomic absorption using a Perkin Elmer 303 spectrophotometer with magnesium sulfate as standard and 0.25 % aqueous strontium chloride as diluent (23). pH determinations were carried out with a Beckman Zeromatic glass electrode assembly.

Protein concentration was determined by a modified biuret method (24) using an automated Autoanalyzer[®] system at 40 samples per hr. The biuret analyses were standardized for each batch of plasma with a micro-Kjeldahl method (25).

One of the systems used for ultrafiltration is shown in Fig. 1 A. Ultrafiltrates of plasma or serum (1.0 ml) were obtained by using a centrifugal field of 300 *g* for four to five periods, each lasting about 1 hr. The membrane used is described below under equilibrium dialysis. The gas phase was (5 % CO_2 -95 % O_2). The initial output of the ultrafiltrate was about 0.1 ml/hr, but the output decreased exponentially with time.

The ultrafiltrate was weighed and its radioactivity determined. At the end of each ultrafiltration the concentrated plasma solution was assayed for radioactivity and occasionally also analyzed for protein by zone electrophoresis.

An alternative procedure (Fig. 1 B) permitted ultrafiltration through the use of gas pressure instead of centrifugal force. The air spaces were flushed with the 5% CO₂-95% O₂ gas mixture and the pressure in the system was raised to 300 mm Hg. This method avoided contact of the plasma with glass, while allowing ready sampling and constant inspection.

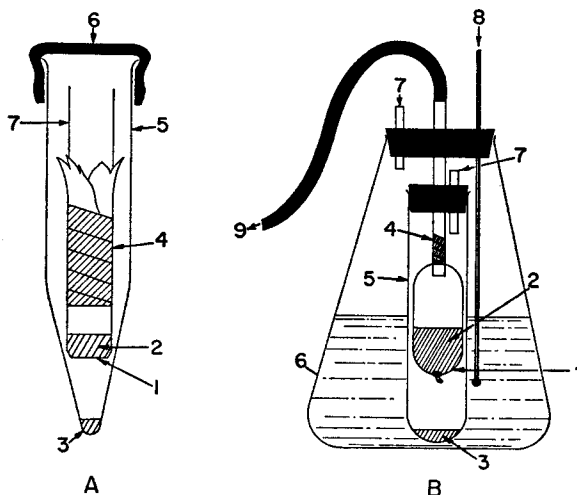


FIGURE 1 A. Ultrafiltration by means of centrifugation: 1 = membrane; 2 = serum; 3 = ultrafiltrate; 4 = electric tape; 5 = 15 ml. Pyrex centrifuge tube; 6 = rubber cap; 7 = cylindrical Pyrex tube.

FIGURE 1 B. Ultrafiltration by means of gas pressure. 1-4 are as on Fig. 1 A. 5 = cylindrical test tube (OD—38 mm, length = 200 mm); 6 = Erlenmeyer flask containing water; 7 = vents; 8 = thermometer; 9 = rubber tube connecting with barometer and gas tank.

Equilibrium dialysis was usually performed according to Hughes and Klotz (26) using a 36/100 ft dialysis tubing from Union Carbide Corp., Visking Div., Chicago, Ill. The manganese content of a batch of this membrane was 116 $\mu\text{g/g}$ dry weight, but after it was immersed overnight at room temperature in an alkaline EDTA solution (Radiacwash¹) and rinsed in triple distilled water (27), its manganese concentration was halved. Whenever indicated, equilibrium dialysis was performed in an all-plastic assembly,² using the same membrane.

For ultracentrifugation samples of plasma were submitted to centrifugal fields of 70,000 *g* for 16–20 hr at 4°C using a Spinco Model L ultracentrifuge with rotor S-40.

¹ Atomic Product Corp., Center Moriches, Long Island, N.Y.

² Equilibrium dialysis cells, Model A, Catalogue No. 425/64, The Chemical Rubber Co., Cleveland, Ohio.

The resulting protein gradient was fractionated with a minimum of mechanical disturbance in the cold room using an all-plastic assembly. Contamination with manganese was thus minimized (28). 1 ml fractions were collected from each tube using a drop-controlled fraction collector.³ Each fraction was diluted to a known volume; radioactivity and protein concentrations were determined in each.

Zone electrophoresis was done in Durrum cells (29) with Whatman No. 3 paper. The buffer was sodium barbital, pH 8.6, 0.05 M. The manganese content of three different batches of this buffer was 1.52, 1.30, and 1.11 μg per liter. Runs were conducted at a voltage gradient of 3 v/cm at 1.5 mamp/cm strip-width intensity during 16 hr at room temperature. After drying, each strip was divided longitudinally into halves. One half was fixed and stained by the aqueous bromophenol blue method (30). The other half was cut into small segments corresponding to each protein zone and to each space between. Radioactivity was individually determined for each segment.

The following reagents were used: Manganous chloride (Allied Chemical Corp., Morristown, N. J.), Magnesium sulfate, Baker analyzed stock No. 2500, lot No. 1265 (contained 640 m μg ^{55}Mn /g on triplicate neutron activation analysis). The barbital buffer used was Beckman Spinco (buffer B-2 contained the amounts of ^{55}Mn indicated above.)

OBSERVATIONS

Ultrafiltration The results of some ultrafiltrations of human sera are shown in Fig. 2. These were obtained at 4°C with both methods. As in all experiments run, the results showed very low concentrations of ^{54}Mn in the ultrafiltrate. Furthermore, progressive ultrafiltration of the same sample showed that the output of radioactivity diminished more rapidly than the output of ultrafiltrate. Hence, these findings contrasted with those obtained by others studying magnesium. These showed that 57–84% of serum magnesium was ultrafilterable and this value seemed independent of the duration of ultrafiltration (35–42).

Equilibrium Dialysis In initial experiments, 1 ml of human serum containing 1–2 μc ^{54}Mn was dialyzed in the cold in a bath consisting of 300 ml of 0.1 M NaCl or KNO_3 (pH 6.8–7.0). The radioactivity lost from the serum was negligible (Fig. 3) unless the bath was 0.02 M MnSO_4 . In the latter case a rapid, prolonged loss of the radioactivity occurred which proceeded at a complex exponential rate. Exhaustive dialysis against various $^{55}\text{MnSO}_4$ solutions failed to remove the last 0.8–1.0% of ^{54}Mn from the serum. Such an experiment is illustrated in Fig. 4. Here the dialysis was continued without changing the bath for 66 hr. In two experiments with different sera, the baths contained the manganese concentrations indicated.

In subsequent experiments the plastic equipment was used to dialyze 1 ml

³ Model 1205 automatic fraction collector, Research Specialties Co., Richmond, Calif.

of serum against 2 ml 0.03 M potassium phosphate buffer pH 7.2 at 23°C for 24 hr. The radioactivity lost from the serum varied between 0.1 and 1.3% and this did not materially change when the dialysis was continued for an additional 42 hr.

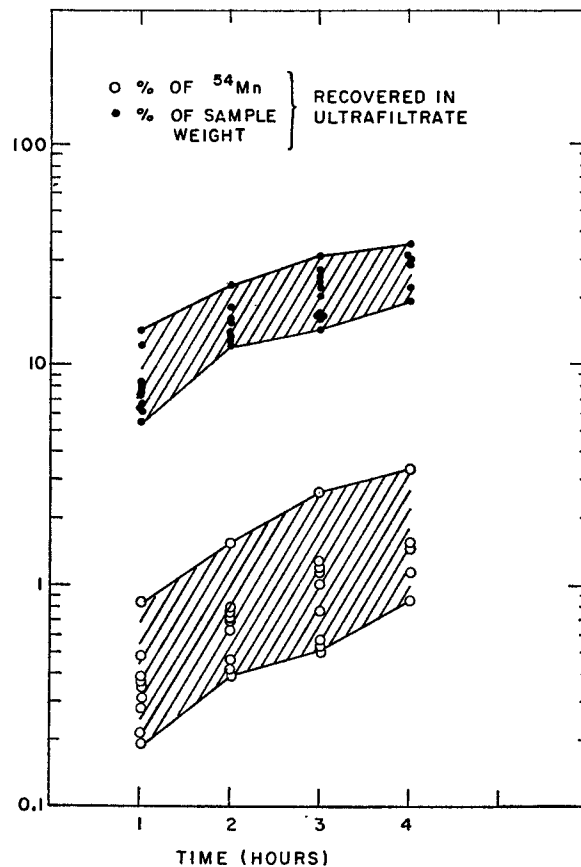


FIGURE 2. Output of ultrafiltrate and of ^{54}Mn during ultrafiltration of serum as per cent of total serum. The solid lines represent the envelope of the data.

Aliquots of human serum (1.0 ml) containing ^{54}Mn were dialyzed either against 300 ml of neutralized 0.1 M KCl; or this solution with added MnSO_4 (6.1×10^{-5} M); or MgSO_4 (9.9×10^{-5} M). The pH of all the baths was 7.0. After 24 hr of dialysis at 4°C the per cent of radioactivity lost by these aliquots was 12–19% of the original in the control baths, 75% in the serum dialyzed against the manganese solution, and 19% in the serum dialyzed against the magnesium-containing bath. These experiments were repeated with magnesium concentrations in the dialysis baths grossly exceeding physiological limits. When the MgSO_4 concentration was 4.0×10^{-3} M, 76% of the ^{54}Mn

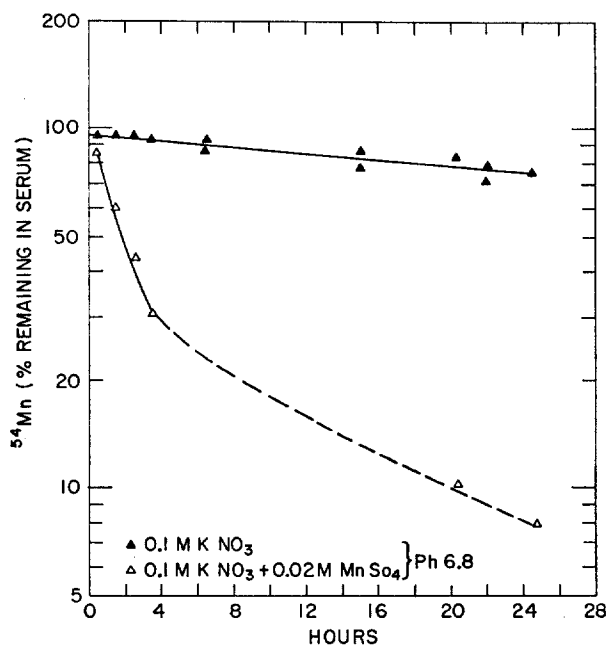


FIGURE 3. Dialysis of serum containing ^{54}Mn in the presence and absence of $^{55}\text{MnSO}_4$.

was lost from the plasma, and this figure increased to 94% of the original radioactivity when the MgSO_4 concentration in the bath was raised to 40.6×10^{-3} M. Furthermore, it should be recalled that the magnesium salt contained manganese.

Ultracentrifugation When human or bovine plasma containing ^{54}Mn was submitted to high speed centrifugation, gradients of both radioactivity and protein concentrations were produced and defined by analyses of the fractions collected. One would expect that plots of the radioactivity against the protein

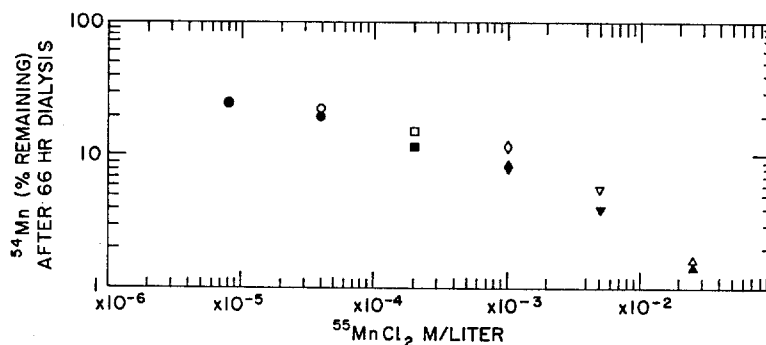


FIGURE 4. Exhaustive dialysis of serum containing ^{54}Mn against baths containing $^{55}\text{MnSO}_4$. In the absence of MnSO_4 only 10–15% of the radioactivity left the serum.

concentration in each fraction would yield straight lines (39). Figs. 5 A and 5 B illustrate such plots for bovine serum containing ^{54}Mn or ^{28}Mg . It is noteworthy that the lines obtained with ^{54}Mn had essentially a zero intercept; whereas those obtained with ^{28}Mg had intercepts of about 30. Zero intercepts were also obtained with human sera containing ^{54}Mn , but the slope was less steep than with bovine sera. Analyses of identical fractions of bovine sera for the natural, nonradioactive ^{55}Mn and the natural, nonradioactive magnesium are shown in Figs. 5 C and 5 D. The intercept of the lines obtained on the axis of the metal concentration represents the "nonprotein-bound"

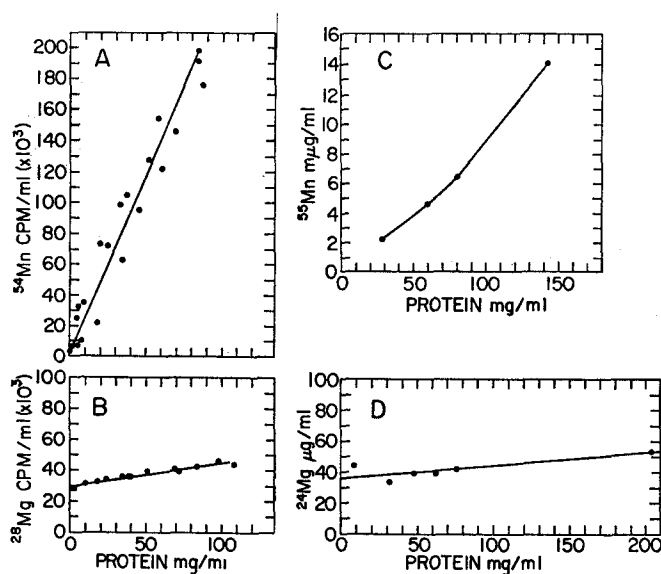


FIGURE 5. Centrifugal fractionation of bovine plasma. A, correlation between ^{54}Mn and protein. B, same for ^{28}Mg . C, same for ^{55}Mn . D, same for ^{24}Mg .

metal, namely the sum of the "free" manganese plus that bound to micro-molecules. Whereas manganese was overwhelmingly protein-bound, protein-bound magnesium was only about 30% of the total. This was calculated from the following: total Mg = 65 $\mu\text{g}/\text{ml}$; nonprotein-bound Mg = 42 $\mu\text{g}/\text{ml}$.

Electrophoresis Solutions of carrier-free ^{54}Mn and mixtures of $^{54}\text{Mn} + ^{55}\text{MnSO}_4$ showed on electrophoresis a rapid migration of the radioactivity towards the negative electrode in the form of a single component. In spite of the alkaline pH and the presence of oxygen, no measurable radioactivity remained at the origin, suggesting that manganic oxides did not precipitate significantly. In contrast to these protein-free systems, electrophoresis of plasma containing ^{54}Mn yielded results such as those shown in Fig. 6. In this figure there are presented the means and ranges of 10 separate analyses on 10

different human sera. It is evident that the protein-bound radioactivity was overwhelmingly associated with the β -globulin fraction. Experiments were also performed with sera from four heparinized Sprague-Dawley rats each of which had been injected intraperitoneally with 200 μC of the $^{54}\text{MnCl}_2$,

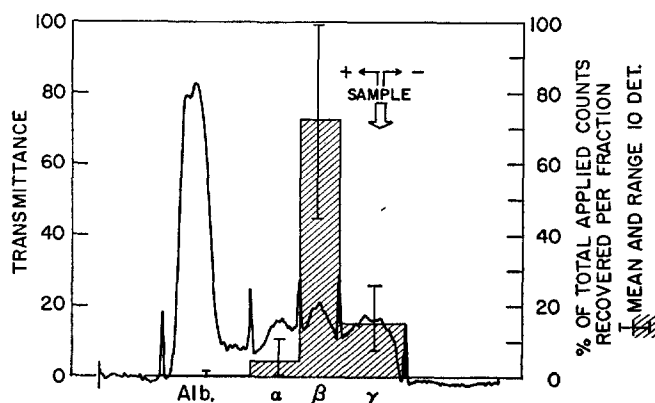


FIGURE 6. Electrophoresis of human sera containing ^{54}Mn .

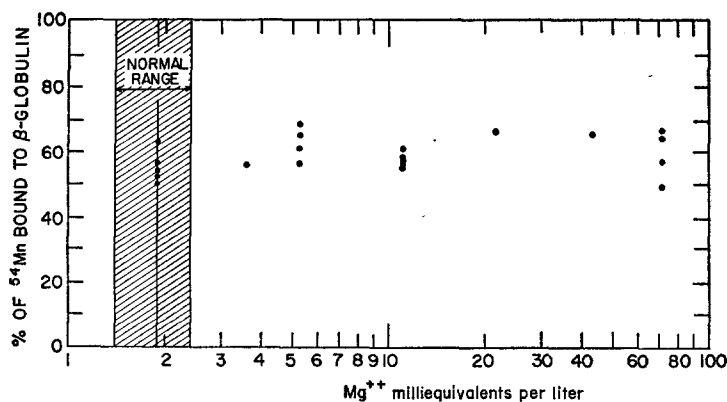


FIGURE 7. Electrophoresis of human sera containing ^{54}Mn plus the indicated concentrations of Mg as MgSO_4 .

4 hr prior to rapid exsanguination. Both electrophoresis and ultrafiltration of these sera gave results identical to the respective earlier ones in which human or bovine sera were studied. When the β -globulins of human sera were split electrophoretically into β_1 and β_2 by the addition of 1.2 mM Ca lactate to the buffer (40), the protein-bound radioactivity migrated almost exclusively with the β_1 -group of globulins. Experiments with bovine and with rat serum yielded identical results. In all these experiments a trace of radioactivity was associated with the albumin fraction.

A similar distribution of the radioactivity was encountered after addition of

MgSO₄ to human sera (Fig. 7). The apparent failure of magnesium to exchange with the β_1 -bound ⁵⁴Mn was confirmed with bovine sera. When the magnesium concentrations were extended over an additional log unit, little if any diminution of the β -bound radioactivity became evident, whereas the radioactivity associated with albumin (0.5% of total) declined significantly.

DISCUSSION

The proportion of total serum magnesium bound to protein has been extensively investigated by others as is shown in Table I. These values are similar in magnitude to those calculated from our ultracentrifugal experiments. By

TABLE I
PROTEIN-BOUND MAGNESIUM OF NORMAL SERA

Investigator(s)	Year	Total serum magnesium bound to protein	
		Mean	Range
		%	%
Stary and Winternitz	1929 (31)	28	
Watchorn and McCance	1930-31 (32)	27	24-36
Soffer et al.	1941 (33)	24	3-22
Lavietes and Dine	1942 (34)	22	12-31
Bissel	1945 (35)	30	17-42
Cope and Wolf	1942 (36)	27	14-54
Copeland and Sunderman	1952 (37)	35	23-48
Walser	1961 (38)	65	

contrast, manganese was shown to be almost totally protein-bound by all four methods used here.

With regard to protein-bound magnesium, it is reported that: "There are as many undissociated magnesium proteinates as there are discrete proteins in plasma . . ." (41). The electrophoretic experiments reported here with ⁵⁴Mn showed essentially a single protein fraction, determined by paper electrophoresis, as binding manganese. The latter finding held true regardless of whether the tracer was administered in vivo or in vitro. These observations seem credible on both experimental and theoretical grounds. Experimentally, the proportion of the radioisotope added that was recovered with the β_1 -globulin fraction was sufficient to support the hypothesis of a single protein as the manganese-binding site in plasma. Theoretically, the electromotive force could possibly dissociate progressively a given metal-protein complex, but there is no evidence that the relatively small forces used could induce the formation of artificial complexes (29). Furthermore, the experiments presented here agree with the corresponding ones of Himmelhoch et al. (42).

Both dialysis and electrophoresis did show exchanges between magnesium

and manganese, but only with magnesium concentrations grossly exceeding physiological limits. Exchanges between ^{54}Mn and ^{55}Mn , on the other hand, could be demonstrated with much smaller concentrations of manganese. This indicated that the binding sites of the β_1 -globulin fraction of serum had a much higher affinity for the transition group metal than for the alkaline earth.

The sum of this evidence contrasts the respective states of these metals in serum with regard to the proportion of protein-bound metal; with regard to their receptor proteins; and with regard to their mutual interchange. Subject to appropriate confirmation, these marked differences between these two metals can be assumed to indicate that their respective carrier systems in serum contribute to their biological specificity.

Mr. S. T. Miller performed the neutron activation analyses reported here. Mrs. Lily Tang performed several of the electrophoretic experiments. Mr. Alexander Nicholson standardized the biuret method.

Dr. Foradori is an International Fellow from Catholic University, Santiago, Chile.

This work was supported by the United States Atomic Energy Commission and by United States Public Health Service Grant No. 2 FO5 TW 881 02.

Received for publication 5 April 1967.

REFERENCES

1. ALBERT, A., and C. W. REES. 1956. Avidity of the tetracyclines for the cations of the metals. *Nature*. **177**:433.
2. BOYER, P. D., H. LARDY, and K. MYRBÄCK, editors. 1959. *The Enzymes*. Academic Press, Inc., New York. 2nd edition.
3. WACKER, E. C., and B. L. VALLEE. 1958. Magnesium metabolism. *New Engl. J. Med.* **259**:431, 475.
4. MOHAMED, M. S., and D. M. GREENBERG. 1945. Liver arginase. I. Preparation of extracts of high potency, chemical properties, activation, inhibition and pH activity. *Arch. Biochem.* **8**:349.
5. SHILS, M. E., and E. V. MCCOLLUM. 1943. Further studies on the symptoms of manganese deficiency in the rat and mouse. *J. Nutr.* **26**:1.
6. BOYER, P. J., H. SHAW, and P. H. PHILLIPS. 1942. Studies on manganese deficiency in the rat. *J. Biol. Chem.* **143**:417.
7. BRONK, J. R., and W. W. KIELLY. 1957. Ionic requirements for oxidative phosphorylation, $\text{ATP}^{252}/\text{P}$ exchange and ATPase. *Biochim. Biophys. Acta.* **24**:440.
8. TIETZ, A. 1957. Studies on the mechanism of fatty acid synthesis. *Biochem. Biophys. Acta.* **25**:303.
9. LINDBERG, O., and L. ERNSTER. 1954. Manganese a cofactor of oxidative phosphorylation. *Nature*. **173**:1038.
10. JENNINGS, D. H., D. C. HOOPER, and A. ROTHSTEIN. 1958. The participation of phosphate in the formation of a "carrier" for the transport of Mg^{++} and Mn^{++} ions into yeast cell. *J. Gen. Physiol.* **41**:1019.
11. SARACHEK, A. 1959. The induction by Mn^{++} of heritable respiratory deficiency in non-dividing populations of *Saccharomyces*. *Biochim. Biophys. Acta.* **33**:227.

12. ORGEL, L. E. 1958. Enzyme-metal-substrate complexes as coordination compounds. *Biochem. Soc. Symp. (Cambridge, Eng.)*. **15**:8.
13. Vallee, B. L. 1960. Metal and enzyme interactions. Correlation of composition, function and structure. *In The Enzymes*. P. D. Boyer, H. Lardy, and K. Myrbäck, editors. Academic Press, Inc., New York. **3**(Pt. B):225.
14. WILLIAMS, R. J. P. 1953. Metal ions in biological systems. *Biol. Rev. Cambridge Phil. Soc.* **28**:381.
15. COMAR, C. L., and F. BRONNER, editors. 1964. *Mineral Metabolism: An Advanced Treatise*. Academic Press, Inc., New York.
16. COTZIAS, G. C., and J. J. GREENOUGH. 1958. The high specificity of the manganese pathway through the body. *J. Clin. Invest.* **37**:1298.
17. COTZIAS, G. C. April 10, 1963. Trace metals: Essential or detrimental to life. Brookhaven Lecture Series No. 828 (T-323). 1.
18. COTZIAS, G. C., S. T. MILLER, and J. EDWARDS. 1966. Neutron activation analysis: The stability of manganese concentration in human blood and serum. *J. Lab. Clin. Med.* **67**:836.
19. DOLE, V. P., and G. C. COTZIAS. 1951. A nomogram for the calculation of relative centrifugal force. *Science*. **113**:552.
20. STROMINGER, D., J. M. HOLLAENDER, and G. T. SEABORG. 1958. Table of isotopes. *Rev. Mod. Phys.* **30**:627.
21. BERTINCHAMPS, A₄ J., and G. C. COTZIAS. 1958. Dosimetry of radioisotopes. *Science*. **128**:988.
22. PAPAVALIOU, P. S., and G. C. COTZIAS. 1961. Neutron activation analysis: The determination of manganese. *J. Biol. Chem.* **236**:2365.
23. SUNDERMAN, F. W., and F. W. SUNDERMAN, JR., editors. 1966. The clinical measurement (of magnesium) in biological fluids. *In Clinical Pathology of Serum Electrolytes*. Charles C Thomas, Springfield, Illinois. 56.
24. GORNALL, A. G., C. BARDAWILL, and M. M. DAVID. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**:751.
25. HILLER, A., J. PLAZIN, and D. D. VAN SLYKE. 1948. A study of conditions for Kjeldahl determination of nitrogen in proteins. Description of methods with mercury as catalyst, and titrimetric and gasometric measurements of the ammonia formed. *J. Biol. Chem.* **176**:1401.
26. HUGHES, T. R., and J. M. KLOTZ. 1956. Analysis of metal protein complexes. *In Methods of Biochemical Analysis*. D. Glick, editor. Interscience Publishers, Inc., New York. **3**:265.
27. PETERSON, E. A., and E. A. CHIAZZE. 1962. Some experimental factors in the gradient chromatography of serum proteins. *Arch. Biochem. Biophys.* **99**:136.
28. FORADORI, A. C., and G. C. COTZIAS. 1966. Ligand competition for the essential trace metal manganese in non-contaminated serum. *Federation Proc.* **25**:432.
29. BLOCK, R. J., E. L. DURRUM, and G. ZWEIG. 1958. *A Manual of Paper Chromatography and Paper Electrophoresis*. Academic Press, Inc., New York.
30. JENKS, W. P., M. R. JETTON, and E. L. DURRUM. 1955. Paper electrophoresis as a quantitative method. Serum proteins. *Biochem. J.* **60**:205.
31. STARY, Z., and R. WINTERITZ. 1929. Über das physikalisch-chemische Verhalten des Magnesium im Serum. *Z. Physiol. Chem.* **182**:107.

32. WATCHORN, E., and R. A. McCANCE. 1930-31. Inorganic constituents of cerebrospinal fluid, calcium and magnesium. *Quart. J. Med.* **24**:371.
33. SOFFER, L. J., C. COHN, E. B. GROSMANN, M. JACOBS, and H. SOBOTKA. 1941. Magnesium partition studies in Graves' disease and in clinical and experimental hypothyroidism. *J. Clin. Invest.* **20**:429.
34. LAVIETES, P. H., and R. DINE. 1942. Serum magnesium in thyroid disease. *J. Clin. Invest.* **21**:781.
35. BISSEL, G. W. 1945. The magnesium partitions in hyperthyroidism. *Am. J. Med. Sci.* **210**:195.
36. COPE, C. L., and B. WOLF. 1942. The ultrafilterable serum magnesium in hyperthyroidism. *Biochem. J.* **36**:413.
37. COPELAND, B. E., and F. W. SUNDERMAN. 1952. Studies in serum electrolytes. XVIII. The magnesium binding property of the serum proteins. *J. Biol. Chem.* **197**:331.
38. WALSER, M. 1961. Ion association. VI. Interaction between calcium, magnesium, manganic phosphate, citrate and protein in normal human plasma. *J. Clin. Invest.* **40**:723.
39. MASKET, A. V., A. CHANUTIN, and S. LUDEWIG. 1942. Studies on the calcium protein relationship with the aid of the ultracentrifuge. II. Observations on serum. *J. Biol. Chem.* **143**:763.
40. LAURELL, C. B., S. LAURELL, and N. S. KOOG. 1956. Buffer composition in paper electrophoresis. *Clin. Chem.* **2**:99.
41. PRASAD, A. S., E. B. FLINK, and H. H. ZINNEMAN. 1959. The base binding property of the serum proteins with respect to magnesium. *J. Lab. Clin. Med.* **54**:357.
42. HIMMELHOCH, S. R., H. A. SOBER, B. L. VALLEE, E. A. PETERSON, and K. FUWA. 1966. Spectrographic and chromatographic resolution of metaloprotein in human serum. *Biochemistry.* **5**:2523.