Arh. hig. rada, *21* (1970) 23.

^{65}Zn IN SERUM PROTEINS IN PERSONS EXPOSED TO ZINC. INVESTIGATION IN VITRO

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(Received for publication February 23, 1970)

Human sera of the workers exposed to zinc labelled with ^{65}Zn (10^{-7}M) in vitro, were investigated by two-dimensional electrochromatography on filter paper. It was found that immediately or 3 hours after labelling the sera with ^{65}Zn almost all ^{65}Zn was in the region of albumin.

Metal fume fever, according to its symptoms, looks as if being caused by parenteral foreign proteins. It is well known that some metals are bound to serum proteins. But for some metals the normally existent proteins in blood have a function of a physiological carrier. The syndrom of metal fume fever can be caused by: Zn, Cu, Mg, but Al, Ni, Cd, Se, Ag, and even Fe is mentioned (1). However metal fume fever is mostly caused by inhalation of zinc oxide fumes.

Gurd (2) has shown that zinc is bound to imidazole groups of the human serum albumin. Zinc in plazma exists in at least two fractions, – firmly bound zinc amounting to about 34% and loosely bound amouting to 66% of the total zinc content (3).

Wolff (4) has found that in dogs, after an oral dose of ^{65}Zn 36% ^{65}Zn is associated with serum albumin as loosely bound zinc, 24% with alpha$_1$-globulin and 27% with alpha$_2$-globulin as firmly bound zinc, while the rest is bound to beta$_1$ and gamma-globulin. This loosely bound zinc seems to represent the transport form of zinc. *Vesell* and *Bearn* (5) and later *Dennes* et al. (6) have observed a preponderance of radiozinc localised in the alpha-globulin region. The former two authors used electrophoresis at pH 8.6 for the separation of plasma proteins labelled in vitro with ^{65}Zn. Similar results have also been reported by *Dennes* et al. utilizing the Cohn fractionation. *Okunewick* et al. (7) used ultracentrifugation and electrophoresis on filter paper. They have found that after ultracentrifugation the percentrage of ^{65}Zn is nearly identical with the percentage of the proteins contained in the respective fractions.

Fritz and *Geitz* (8) have found by gch filtration on columns of Bio-Gel P 150, that the eluation peaks of copper, gallium and zinc all lie within the limits of albumin peak but are in distinctly different positions.

One-dimensional electrophoresis on the supporting medium does not allow an exact determination of the fractions to which ^{65}Zn is bound (9). It was therefore of special interest to examine the bounding of ^{65}Zn by two-dimensional electrochromatography, because the separation of proteins by this technique gives no overlapping of protein fractions.

MATERIAL AND METHODS

The examinations were carried out in a group of workers (N = 6) working in a brass foundry in Zagreb. Only one worker from the group worked there for about one year; the other five worked therefor about ten or more years. From each worker 5 ml of blood were taken at the end of work hours. The sera were prepared by usual procedure.

Serum proteins were labelled with the carrier-free ^{65}Zn (the Radiochemical Centre Amersham) in 1 N HCl. After hydrochloric acid was evaporated, 0.2 ml serum was added into the test tube, so that the concentration of ^{65}Zn was 10^{-7} M.

Two-dimensional electrochromatography was performed in a barbituric buffer, pH 8.6, which is a standard buffer for protein separations, and also proved convenient for this kind of investigation. In a previous work (9) it was demonstrated that the results depend on the time of incubation of sera with ^{65}Zn, on the pH range and on the buffer used.

Separations of serum proteins were performed in an apparatus after *Pučar* (10).

The separation conditions were: 420 V, 25 mA, barbituric buffer pH 8.6 $\mu = 0.05$, the time of separation was about 3 hours and it was performed on the filter paper Munktel 20/150.

RESULTS AND DISCUSSION

Immediately after the labelling of sera ^{65}Zn almost all ^{65}Zn was in the region of albumin (Fig 1). When the electropherogram, stained to proteins, was carefully covered with the radioautogram it was seen that a part of albumin was without ^{65}Zn. This small part of albumin had the highest electrophoretic mobility. In the region of beta and gama-globulins there was a faint trail of ^{65}Zn half way the migration of the above mentioned globulins. A trail few centimeters long observed at the start was similar to the trail of ^{65}Zn incubated in the same buffer (Fig. 2). This ^{65}Zn is not bound to proteins and migrates as a complex of diethyl-barbituric acid (11) with a pronounced chromatographic effect.

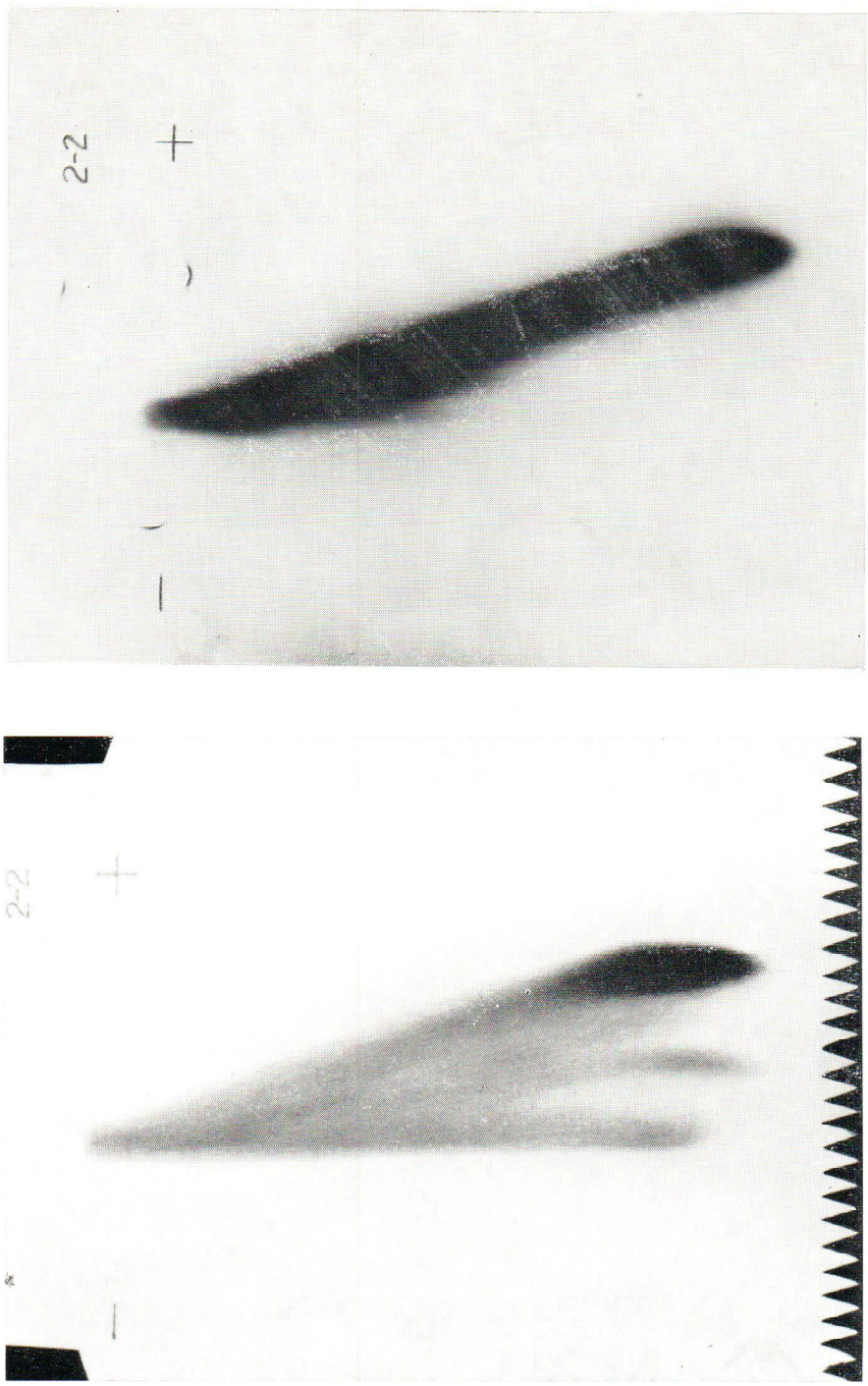

Fig. 1. *Two-dimensional electrochromatography of human serum of the workers exposed to zinc, labelled with ^{65}Zn in vitro. Buffer: barbituric pH 8.6. $\mu = 0.05$. Left: electropherogram dyed to proteins, right: radioautogram*

Fig. 2. *Radioautogram of the two-dimensional electrochromatography of the* ^{65}Zn *incubated in the same barbituric buffer pH 8.6,* $\mu = 0.05$

The continuous electrophoretic separation of the sera of nonexposed subjects labelled with ^{65}Zn in vitro, has shown that 89,7% ^{65}Zn was in the region of albumin and alpha$_1$-globulin, 10% in the region of alpha$_2$ and beta-globulins, and only 0.2% in the region of gama-globulin (9).

It seems that qualitatively there are no differences in the binding of ^{65}Zn to serum proteins between exposed and nonexposed subjects.

However the obtained results show differences with regard to the reports from literature. According to our investigations ^{65}Zn is mostly found in the region of albumin and only a negligible amount in the globulin region.

These results cannot be connected with the investigations of those who have found that percentage of ^{65}Zn in plasma fractions is nearly identical with the percentage of proteins in the same fractions. They cannot be connected with the data showing that ^{65}Zn is preponderately localised in the alpha-globulin region either.

Disferences in the results of the bounding of ^{65}Zn to different protein fractions are likely to derive from the use of different methods for protein separations.

CONCLUSION

Human sera of the workers exposed to zinc, labelled with ^{65}Zn (10^{-7}M) in vitro, were investigated by two-dimensional electrohromatography on filter paper. The supporting electrolyte as barbituric buffer pH 8.6 ionic strength 0.05. Almost all ^{65}Zn was found to be bound to serum proteins while only a negligible amount seemed to exist in a free form, i.e. not bound to proteins. It was found that immediately or 3 hours after labelling the sera with ^{65}Zn almost all ^{65}Zn was in the region of albumin, whereas only a trace amount was associated with globulins.

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Sadržaj

DISTRIBUCIJA ^{65}Zn U SERUMSKIM PROTEINIMA ISPITANIKA EKSPONIRANIH PARAMA CINKA. ISPITIVANJA IN VITRO

Ispitana je distribucija ^{65}Zn u serumskim proteinima ispitanika, koji su profesionalno bili izloženi parama cinka. Serumi ispitanika su obilježeni sa ^{65}Zn ($10^{-7}M$) in vitro. Separacija bjelančevina je izvršena dvodimenzionalnom elektrokromatografijom. Kako se migracioni putevi bjelančevina separiranih ovom metodom ne preklapaju nema mogućnosti da se inaktivne frakcije kontaminiraju sa ^{65}Zn. Odmah nakon obilježavanja seruma sa ^{65}Zn, skoro sav ^{65}Zn se vezao na bjelančevine seruma. Gotovo sav vezani ^{65}Zn se nalazi u albuminskom području. Elektroforetski najbrži dio albuminske zone nema radioaktivnosti. ^{65}Zn inkubiran u istom barbituratnom puferu, pH 8,6 putuje s velikim kromatografskim efektom, kao kompleks cinka s dietil-barbiturnom kiselinom.

Primljeno 23. II 1970.

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