Short Communication

CONTAMINATION PROBLEMS IN THE TRACE ANALYSIS FOR PROTEIN BOUND METALS

K. FRITZE, R. J. GIEZ

Department of Chemistry, McMaster University, Hamilton, Ontario (Canada)

(Received January 3, 1968)

Introduction

The most serious problem in the analysis of blood for trace metals appears to be contamination, particularly during the sampling stage. Many elements occur in concentrations around the part per billion level which means that 10^{-8} g of contamination leads to entirely erroneous results in a 1 ml sample. Once a sample has been contaminated the question arises whether the metal ion forms a complex with any of the serum proteins. If this were the case the metal would be found in a particular protein fraction and could then be mistakenly identified as a bio-synthesized metal protein.

Experimental

Serum samples were “contaminated” with microgram quantities of radioactive tracers of lanthanum, gallium, copper, zinc, chromium, manganese, and iron. The choice was rather arbitrary, based essentially on nuclear properties. The serum samples were then fractionated by gel filtration with 0.15 M ammonium acetate on columns of Bio-Gel P 150. This type of gel-filtration chromatography leads to a separation of the serum proteins into three major fractions which are indicated by three peaks in the elution curve. The first peak contains β_{2M}^- (19 Sγ-) and α_{2M} globulins, and α- and β-lipoproteins, the second peak 7 S γ-globulins and the third peak albumin. Protein elution curves were obtained using an LKB Uvicord ultraviolet absorptiometer and recorder. In order to obtain the trace metal elution curves, the effluent was collected in small fractions which were in most cases counted with a 7.5 · 7.5 cm NaI(Tl) detector coupled to a Nuclear Data 256 channel analyser.

Copper

A number of experiments were done using copper which had been irradiated in the McMaster Reactor to produce ^{64}Cu. A typical elution curve is shown
in Fig. 1 and was obtained from 0.3 ml of pig serum containing 50 µg of copper. When the metal concentration is much lower (i.e. ~ 10 µg/ml) it was found in other experiments that the ratio of albumin copper to "ionic" copper becomes much larger. The small copper peak in the first protein peak was observed in each experiment and amounted to ~ 1% of the "albumin-bound" copper.

Fig. 1. Elution curves obtained in a chromatographic separation on Bio-Gel P 150 of pig serum which had been spiked with radioactive tracers of copper, chromium and manganese.

The drawing shows the combined results of two experiments

Manganese and chromium

"Carry-free" $^{54}$Mn was produced by the reaction $^{54}$Fe(n, p)$^{54}$Mn on specpure iron in the McMaster Reactor. The manganese was separated from iron and $^{51}$Cr (produced by $^{54}$Fe(n, x)$^{51}$Cr) using anion exchange methods. In the γ-ray spectrum of the manganese fraction no residual $^{59}$Fe or $^{51}$Cr could be detected. A sample was then prepared containing $^{54}$Mn (~ 280,000 cpm) 50 µg of manganese

J. Radioanal. Chem. 1 (1968) 265–268