In vitro Transfer of Radiocadmium, CD^{115}, from Plasma to Human Erythrocytes.* (25242)

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A finding incidental to earlier studies(1,2) in this laboratory was *in vivo* transfer of radiocadmium, Cd^{115M}, from plasma to canine erythrocytes following intravenous administration of the isotope. This report concerns further investigation of the kinetics of cadmium metabolism, specifically with respect to *in vitro* transfer of the metal from plasma to erythrocytes. Such studies are particularly appropriate since cadmium is related to enzymatic phenomena(3,4), is found in trace amounts in man(5,6), and has been reported to occur naturally in the equine kidney cortex(7).

Materials and methods. Cd^{115} (half life, 53 hours) was obtained from Oak Ridge National Laboratories as cadmium nitrate in excess nitric acid and neutralized with sodium hydroxide before use. Absorption studies indicated the presence of a small amount of Cd^{115M} (half life, 43 days) as a contaminant. The latter did not interfere with the present investigation, however. Heparinized blood was obtained from donors, and after 30 minutes, 30 ml were placed in paraffinized flasks, suspended in a constant temperature bath (38°C) with continuous agitation. Approximately 10 μc of Cd^{115} were added to each flask and thoroughly mixed with the contents. After one, 2, 4, 8, 12, and 24 hours, samples were withdrawn from the flasks. The blood samples were centrifuged in Wintrobe hematocrit tubes, and supernatant plasma was removed for counting. The buffy coat was removed, and residual erythrocytes washed 3 times with human Ringer's solution before counting to determine rate of transfer of Cd^{115} to the erythrocytes. To detect hemolysis, the serum potassium was determined by the flame photometric technic in each sample. All counting was done with a thin mica end window Geiger-Muller counter, using methods previously described(8).

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Results. The results are illustrated in Fig. 1, in which black circles represent specimens in which hemolysis occurred, and white circles represent non-hemolyzed specimens. Except for subject 6, the erythrocytes generally behaved in like manner. Samples collected through the eighth hour were free from detectable hemolysis. Thereafter varying degrees of hemolytic activity were noted in all specimens. Thus, the similarity of results obtained in later specimens may represent incomplete erythrocyte binding of cadmium due to loss of erythrocytic binding substance to the serum. Conversely maximal metal uptake might have occurred prior to hemolysis with subsequent release of bound cadmium. Further studies are needed to determine these points.

In a single subject (No. 6) prompt and marked binding of cadmium occurred. Degree of binding persisted at essentially the same level in spite of later hemolysis, suggesting that such bonds, once formed are relatively stable. The patient from whom this sample was obtained was the only member of the group with a hematologic abnormality. During the 2 weeks prior to sampling, he suffered from considerable gastrointestinal bleeding, secondary to a malignant process. It is inviting to postulate that increased cadmium uptake was due to the presence of an increased number of circulating immature red blood cells as a result of the bone marrow stimulation arising from hemorrhage. The latter have a higher concentration of nucleic acid, and a stickier surface, but less globin. At this time there was no obvious explanation for the increased affinity of this subject's erythrocytes for cadmium.

Discussion. The erythrocyte is rich in constituents with which cadmium is capable of forming compounds of varying stability(9,10). Since there exists a broad spectrum of similar affinities, the qualitative and quantitative aspects of such unions are complex and largely

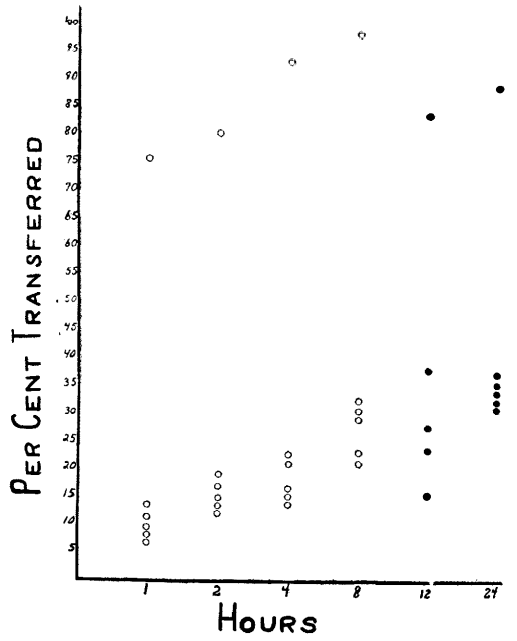

FIG. 1. % Cd^{115} transferred from plasma to human erythrocytes.

unknown. Without the application of more sophisticated technics, it is impossible to determine whether or not cadmium formed a stable complex with the constituents of the red cell surface or entered the erythrocyte proper. The former seems unlikely, particularly since similar studies with radioactive sodium chromate have indicated that most of the test isotope was bound to intracorpuscular globin, and only insignificantly to hematin (11). Nevertheless, the numerous metabolic transfer systems of the erythrocyte require localization to the cell surface of enzymes which may bind cadmium to a variable degree. Many intracorpuscular substances and enzymes are capable of uniting with cadmium. Perhaps, as suggested for mercury, cadmium principally combines with thiols. Other reactions may involve amino, hydroxy, imidazole, or carboxyl groups.

Summary. The 24-hour in vitro transfer of radiocadmium, Cd^{115}, from plasma to human erythrocytes was investigated. Most of the isotope became erythrocyte-bound during the first 8 hours of incubation although subsequent kinetics were somewhat obscured by hemolysis. The magnitude of the shift approximated 30% of total cadmium, although the erythrocytes of one subject, who had recently hemorrhaged, exhibited a much greater affinity for the metal. These findings suggest the desirability of further studies to determine the value of cadmium as an indicator of erythrocyte survival.

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Comparison of Physiological Properties of Certain Phage-typable Coagulase-positive Staphylococci.* (25243)

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Although the literature on physiological activities of Staphylococci is extensive, little has been published concerning a comparison of these properties between different phage-typable groups. The purpose of our studies

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