Errors in Plasma Volume Measurement from Adsorption Losses of Albumin-I^{131}.* (22737)

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Recently, when using albumin-I^{131} to measure plasma volume, we obtained unexpectedly low results. Adsorption losses of the albumin-I^{131} to the walls of containers offered a possible explanation, and experiments showed this to occur. The degree of error introduced in plasma volume measurement by this adsorption and methods of preventing it have been studied.

Methods. Various samples of a commercial preparation of human albumin-I^{131},‡ containing 50 to 140 μC I^{131} per mg albumin when received, were used. Ninety-six to 98% of the radioactivity was protein-bound as shown by precipitation with 5% trichloroacetic acid. When received the material containing 1 mC was diluted in 30 ml sterile 0.9% NaCl. This stock solution was stored in the refrigerator and discarded after 3 to 4 weeks. Radioactivity was measured in capped vials in a well scintillation counter. All glassware was cleaned in concentrated chromic acid-sulfuric acid cleaning mixture (100 g K dichromate in 200 ml H_2O, treated with 1000 ml conc. H_2SO_4) for from 0.5 to 24 hours, then rinsed repeatedly with hot water, cold water and distilled water. Of the many solvents tested only chromic acid-sulfuric acid completely removed the adsorbed radioactivity from glass in a few minutes at 25°C. Hence this mixture was used for "extracting" the bound radioactivity. The loss by adsorption to pipettes and containers made special precautions necessary for the accurate preparation and assay of dilute solutions of albumin-I^{131}. Losses proved negligible in a micrometer syringe filled with a solution containing 200 or more μg protein/ml, and more dilute solutions were therefore prepared by delivering a minute volume of a relatively concentrated solution from this syringe into the solvent. To prevent losses in 1 ml transfer pipettes, these were first filled and emptied thrice with a 1 mg/ml solution of plasma albumin or dilute human plasma. Then air was sucked through them till the lower 3 inches of the pipette was almost dry. After this treatment, at most 2% of the radioactive contents was adsorbed.

Results. Table I summarizes the results of a typical experiment in which 27 μg of albumin-I^{131} were transferred to 4 pairs of 50 ml and 4 pairs of 250 ml flasks. To each pair was added one of the 4 solvents—distilled water, 0.9% NaCl, 0.1 N NaOH and 0.9% NaCl containing 1 ml human plasma/100 ml. The flasks were mixed by repeated inversion initially and before removing the samples. It is seen that counts are lost from water and sodium chloride solution and that these losses increase with storage at low temperature overnight and are proportionately greater from the more dilute solutions. Most of the lost counts are regained within a few minutes after adding NaOH. Counts are not lost from stored solutions which contain the carrier protein. A number of similar experiments showed: a) that KI in 1% concentration or less did not prevent loss of counts from water solutions; b) 3 and 5% NaCl, 1 and 3% $NaHCO_3$, and 3% Na Acetate did not prevent loss of counts; c) irregular small losses of counts occurred from 0.1 N NaOH and 1% Na_2CO_3 solutions; d) the 1% detergent solutions used by Sear *et al.*(6)§ completely blocked the loss of counts for several days at 4°C; e) addition of sufficient human serum albumin or human plasma, with or without KI, blocked the loss of counts from 0.9%

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§ One percent Alconox cleaning solution.

TABLE I. Loss of Counts from Albumin-I^{131} Solutions in Volumetric Flasks.

	Distilled H_2O	.9% NaCl	.1 N NaOH	Carrier protein in .9% NaCl
A. 27 μg Albumin-I^{131} in 50 ml of solvent				
Counts/min./ml				
After 90 min. at 25°C	18240 (85%)	19160 (89 %)	21740 (101 %)	21560 (100 %)
After 20 hr at 4°C	13700 (64%)	17100 (80 %)	21000 (97.5%)	21360 (99 %)
B. 27 μg Albumin-I^{131} in 250 ml of solvent				
Counts/min./ml				
After 90 min. at 25°C	3190 (75%)	3760 (88.5%)	4390 (103 %)	4250 (100 %)
After 20 hr at 4°C	1960 (46%)	3050 (72 %)	4160 (98 %)	4350 (102.5%)
After adding 300 mg NaOH	4160 (98%)	4040 (95 %)		

NaCl completely for as long as 2 weeks at 4°C.

Figure 1 shows the results of 2 of a number of experiments designed to measure the adsorption of protein to glass from 0.9% NaCl solution at room temperature. In Exp. A, 3 ml protein solution was exposed to the inner surface of 10 × 75 mm Pyrex test tubes capped with Parafilm and mixed by inversion initially and at 10 minute intervals thereafter; in Exp. B, 15 ml protein solution was exposed to the inner surface of 15 × 125 mm Pyrex test tubes with mixing only initially. At concentrations of protein of the order of 1 μg ml or less, 10% to 50% of the protein is adsorbed to the walls of the test tubes. As the protein concentration increases the proportion of the total adsorbed decreases, until at concentrations of between 10 and 30 μg ml, only 2 to 5% of the total is adsorbed. In Fig. 2 the μg protein adsorbed per cm^2 glass in Experiments A and B are plotted against the *final* concentrations of protein in solution. (The *final* concentrations were obtained by subtracting the quantity adsorbed from the quantity added initially to the test tube). The quantity of protein adsorbed by unit area of surface increases over the whole range of concentrations studied, but most rapidly over the range from 0.01 to 5 μg ml (Fig. 2 insert). A few experiments showed that when 3 ml solution of 2 μg albumin-I^{131}/ml saline is mixed by repeated inversion in 10 × 75 mm test tubes, one half the equilibrium value is adsorbed in from 1 to 5 minutes and equilibrium appears to be reached in from 5 to 20 minutes. Albumin-I^{131} adsorbs to surfaces other than glass. It adsorbs vigorously to polyethylene, as noted by Sear, Allen and Gregersen(6). We have observed that albumin-I^{131} also adsorbs to celluloid and other transparent plastics, to glass tubes coated with silicone and histological paraffin, and to stainless steel and rubber.

Discussion. Different samples of human albumin-I^{131} show some variation in adsorption but in general the adsorption isotherms

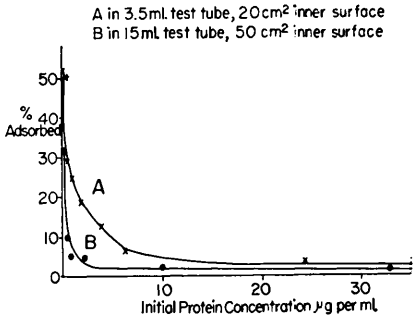

FIG. 1. % of total albumin-I^{131} adsorbed in 1 hr plotted against initial conc. (μg/ml).

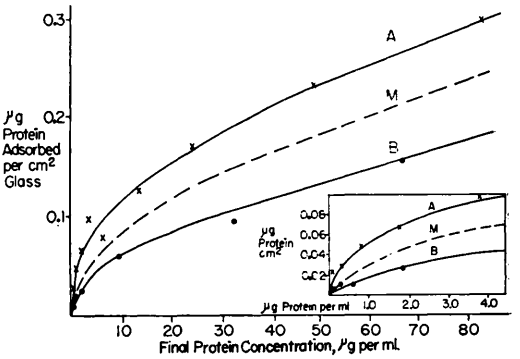

FIG. 2. Relation between protein conc. and quantity of protein adsorbed to glass.

lie in the area bounded by curves A and B, Fig. 2. We find also that rabbit albumin-I^{131} and human gamma globulin-I^{131} adsorb similarly. Recently Bull(2) has reported quantities of unlabelled bovine albumin adsorbed to glass from buffer of pH 4.66 that would fit well with a projection of curve A, Fig. 2. The errors due to adsorption in a plasma volume measurement depend on the fraction of the total albumin-I^{131} adsorbed to the glassware. This depends on the solvent, protein concentration, area of surface, time of exposure to surface, temperature and the effectiveness of mixing in the solution. At room temperature with 0.9% NaCl as solvent the approximate errors resulting from adsorption can be calculated from Curve M, the mean of curves A and B, Fig. 2, provided that the surface area of the glassware and the protein concentrations of the solutions are known. Calculations show that the surface volume relationships are of great importance. Thus, volumetric glassware with a surface of less than 1 cm^2 per ml contained solution adsorbs less than 1% of the contained protein at concentrations of 10 μg ml. and less than 5% at concentrations of 1 μg ml. whereas, glassware with a surface of 20 cm^2 per ml may adsorb 15% of the contained protein at 10 μg protein/ml, and 35% at 1 μg ml. Examples of the former glassware are volumetric flasks of 100 ml capacity or greater, and of the latter, 1 ml Tuberculin syringes and straight 1 ml pipettes. (Pipettes would be a very grave source of error were it not that they are filled and emptied rapidly, and, since albumin diffuses slowly, time is insufficient for equilibrium to be reached. Experiment shows that with each filling a few per cent of the contents is adsorbed until the surface becomes saturated.) The calculated percentages of protein that become adsorbed during a plasma volume measurement, in which 10 ml of 0.9% NaCl solution of albumin-I^{131} containing 10 to 40 μg protein ml are injected, and the radioactivity in 1 ml of plasma separated from the blood samples is compared with that of 1 ml of a 1 in 100 dilution of the injection solution are: In the syringe, 3 to 6; in the glassware containing the separated plasma, nil; in the glassware used for the standards (a) 100 ml volumetric flask, 8 to 10; (b) two 1 ml transfer pipettes, 2 to 3 each. Since losses in the syringe lead to an overestimate whereas those occurring during the preparation of the standards lead to an underestimate of plasma volume, these errors partially cancel out. As a result, plasma volume is underestimated by 8 to 12% depending on the protein concentration of the injected solution. In unfavorable circumstances such as storage of standards in the cold and preparation of standards with distilled water, greater errors would arise. Schultz *et al.*(5) who measured radio activity with G-M counters, added plasma to their standards to equalize adsorption losses of B rays; Sear *et al.*(6) prepared their standards in 1% detergent. These two groups of workers showed good agreement between plasma volume measurements made in man and dog with T1824 and albumin-I^{131}. Other workers (*e.g.*1,7) have failed to find such good agreement. Recently, technics using scintillation counters have been described(3) in which losses by adsorption would be anticipated; Gamble *et al.*(4) have already noted such losses. It is clear that to make precise measurements these losses must be avoided. This may be achieved as follows: When the albumin-I^{131} is prepared for injection at least 1 mg carrier protein per ml solution is added. For carrier protein either the patient's plasma or a preparation of human plasma albumin is used. To make the standards a measured volume of the solution prepared for injection is diluted in a volumetric flask, either with 0.9% NaCl containing 0.5 mg plasma protein/ml, or with 1% detergent solution.

Summary. Adsorption losses of albumin-I^{131} to volumetric glassware may be sufficient to cause significant underestimates of plasma volume. Some factors affecting these losses are examined and methods of minimizing their effects are described.

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Erythropoietic Stimulating Effects of Plasma Extracts from Anemic Human Subjects. (22738)

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It is now a well documented finding that various types of anoxia evoke the production, in laboratory animals, of circulatory factors capable of stimulating erythropoiesis(1-11). These observations raise the question as to whether such activity is also demonstrable in the blood of patients with various forms of anemia. Few studies have attempted to assess the erythropoietic (EP) properties of body fluids of human subjects. Some of the experiments have been performed on umbilical cord blood of normal newborn babies and have indicated EP activity in such material when tested in rodents and man(12-16). EP activity is also stated to be present in the serum of a man sojourning at altitude for 8 days (12), in the plasma of patients with chronic heart disease(1), in human subjects with anemia following hemorrhage(17) and in infants with erythroblastosis fetalis(16). Plasma from subjects with congenital spherocytosis or pernicious anemia in reticulocyte crisis was ineffective(18). These studies with human material fail to evaluate the response of the blood-forming organs in the test animals. The criteria used by the investigators, namely red cell (RBC) counts and/or reticulocyte response, cannot always be depended upon as reflecting the degree of EP stimulation(19,20).

The present studies are concerned with the effects exerted by extracts of plasma and one of urine from patients with certain types of anemia. Since previous experiments(5-8,10) indicate that more of the EP factor is recoverable in hemolytic than in other types of experimentally induced anemias, possibly because the hemolytic varieties are usually more severe, it was felt that an examination of the effects of body fluids secured from patients with Cooley's and sickle cell anemia might be rewarding.

Materials and methods. Plasma was obtained from 7 authenticated cases of Cooley's anemia (6 children and 1 adult) and 2 patients with sickle cell anemia. One case of chronic hypoplastic anemia in a child is also included. Four normal subjects served as donors for control plasma. A 24 hour sample of urine from one of the Cooley's subjects was also tested. In Table I are indicated the hemoglobin concentration, reticulocyte percentage and nucleated RBC numbers in the peripheral blood of each of these individuals at the time of blood withdrawal.

Because of the effectiveness of an acidification-boiling procedure in revealing the EP factor in the blood and urine of anoxic rodents (5-7,10,11,21), it was decided to utilize this extraction technic for the plasmas of the anemic patients. Blood collected from each of the patients was heparinized (10 mg/50 ml blood). The plasmas were acidified to pH 5.5 with lN HCl and boiled for 10 minutes. Following filtration, the coagulated residues were discarded and the filtrates neu-