Binding of the Mercury of an Organic Mercurial Diuretic by Plasma Proteins.* (18101)

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During investigations of the time-course of various elements in the body, certain differences were noted between the behavior of Na^{22} and Na^{24}, introduced as a mercurial diuretic (Mercuhydrin‡)(1-5). With comparable concentrations in the serum, there were differences in both time of appearance and concentration of the isotopes in various fluids of the body(1,2,4). These physiologic data suggested a tendency for the radiomercury to bind to plasma proteins, thus influencing the transfer of the isotope around the body. To investigate the possible relationship of protein binding of the radiomercury upon its transfer across vascular membranes *in vivo,* observations were made on the diffusion of the mercury of the mercurial diuretic across a semipermeable membrane *in vitro.*

Materials and methods. Test solutions containing the labeled mercurial diuretic consisted of human Ringer's solution, reconstituted dried human plasma, fresh human plasma and human urine. Dialyzing bags were made of cellophane sausage casing, impermeable to plasma proteins. All dialyses were performed in duplicate. Samples of test solutions and bathing solutions were taken before and after dialysis and were prepared and counted according to methods previously described(6,7). Two minims of a solution containing 50,000 units of penicillin per cc was added to each bag and bath to prevent bacterial growth. The reconstituted plasma was found to have a total protein of 7.3 g per 100 cc and albumin of 4.9 g per 100 cc.

Results. Results of many experiments are summarized in Tables I and II. The dialysis of Mercuhydrin in Ringer's solution (Table I, Exp. 2) showed a decrease in transfer of mercury across the membrane. This suggests that the mercury was bound to the plasma proteins. From the dialysis of Mercuhydrin in Ringer's solution placed inside the cellophane bag against plasma as the bath (Table I, Exp. 3) it is apparent that the plasma proteins bound the mercury which diffused into it. At equilibrium, the mercury in the Ringer's solution was "free" and was probably in equilibrium with a similar concentration of free mercury in the bag. The concentration of "bound" mercury in the plasma is equal to the total concentration of mercury minus the concentration of "free" mercury. Thus, the mercury was respectively 96% and 94% protein-bound (Table I, Exp. B and C).

When relatively large concentrations of mercury were present, the mercury diffused across the membrane in quantity despite the

* Aided by grants from a War Department Contract No. WD-49-007-MD-389, Life Insurance Medical Research Fund, a Public Health Service Grant and the Mrs. E. J. Caire Fund for Research in Heart Disease.

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‡ The sodium salt of methoxy-oximercuripropylsuccinylurea with theophylline, prepared with radiomercury in this laboratory by Messrs. Harold Krahnke, Darwin Kaestner and Edwin Sprengler through the courtesy of Dr. H. L. Daiell, Director of Research, Lakeside Laboratories, Milwaukee.

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TABLE I. Dialysis of 10 cc Volumes of 1:2000 Dilution of Mercuhydrin for 120 Hr at 7°C.

Exp. No.	Solution in: Dialyzing bag	Solution in: Bath	Conc. of radiomercury in: Bag, CPM/cc	Conc. of radiomercury in: Bath, CPM/cc	Mercury in plasma: Bound, %	Mercury in plasma: Free, %
1	Ringer's	30 cc vol. Ringer's	1177	1173		
2	Plasma	Ringer's	4213	183	96	4
3	Ringer's	Plasma	96	1690	94	6

CPM = Counts per minute.

TABLE II. Dialysis of 10 cc Volumes of Various Dilutions of Mercuhydrin in Plasma in Dialyzing Bags.

Exp. No.	Solution in: Dialyzing, bag	Solution in: Bath, Ringer's	Initial dilution of mercuhydrin	Final conc. Hg, μg/cc	Conc. of sol. in: Bag, CPM/cc	Conc. of sol. in: Bath, CPM/cc	Mercury in plasma: Bound, %	Mercury in plasma: Free, %
4a*	Plasma	30 cc	1:1000	28.9	8566	340	96	4
b	,,	,,	1:500	52.7	15623	1813	88	12
c	,,	,,	1:100	162.5	48766	18926	61	39
5a†	,,	10 cc	1:800	45.9	238	22	92	8
b	,,	,,	1:400	78.0	468	94	80	20
c	,,	,,	1:200	144.4	836	314	63	37
d	,,	,,	1:100	260.0	1487	612	45	55
e	,,	,,	1:50	487.5	2750	1916	30	70

* 120 hr, 70°C. † 5 hr, 37°C, with constant agitation.

presence of plasma (Table II, Exp. 4c, 5c, d and e). Thus, the quantity of mercury bound by the plasma appears to be a function of mercurial concentration.

Other similar experiments indicated that the mercury of the mercurial diuretic was similarly bound by the plasma proteins after intravenous administration of the drug. The mercury in normal and protein-free urine, on the other hand, remained dialyzable.

Discussion. Until comparatively recently, observations regarding mercury-protein interactions have been concerned with denaturative phenomena obtained with relatively high concentrations of mercury(8). Hughes(9,10), by ultracentrifugal and chemical methods, noted soluble mercury-albumin complexes consisting of one molecule of mercury to one or two of albumin. On the basis of these studies he postulated a single reactive sulfhydryl group per molecule of the albumin by which the mercury is bound(10). Other reactive sulfhydryl groups are to be found among the globulins(11), and it is probable that mercury is bound by them as well. Studies utilizing the ultracentrifuge have not shown measurable "free" mercury either in a 1:2 molar mixture of a mercuric compound with human serum albumin *in vitro*(11) or in plasma obtained from a human subject 10 to 30 minutes after intravenous injection of the mercurial diuretic(12). The consistent presence of mercury outside the bags in these experiments (Table II) indicates that under these conditions there is some "free" mercury even in relatively dilute solutions.

These observations have many interesting biologic implications. For example, studies on renal extraction and excretion of mercury (5) revealed that 25 to 50% of the mercury could not be accounted for in the circulating blood or in the kidney within 5 minutes after injection of the mercurial diuretic. This loss of mercury cannot be explained by dif-

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fusion out of the vascular system of mercury attached to proteins(13) nor can the disappearance of the mercury be accounted for in extracellular fluid obtained from various sites(4). Within the first minute or so after injection, mercurial dilutions of 1:500 and less exist(1). At these dilutions, there should be appreciable quantities of nonprotein bound mercury. It would appear, therefore, that much of this initially "free" mercury becomes bound to structures of or immediately adjacent to these blood vessels.

Summary and conclusion. The experiments herein reported corroborate the observations of other investigators that the mercury of an organic mercurial diuretic may be bound by the proteins of the plasma. The extent of this binding is a function of mercurial concentration; at dilutions of the diuretic of 1:1000 or higher the mercury is over 90% bound; at dilutions of 1:100 or lower, the mercury is over 50% free. This may well explain certain phenomena observed in the tracer studies of the mercurial diuretics.

The author wishes to express his appreciation for the valuable assistance and guidance of Dr. George E. Burch, who provided the facilities for these studies.

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Received July 21, 1950. P.S.E.B.M., 1950, v75.

Diurnal Variation in the Plasma Iron Level of Man.* (18102)

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During a study of factors affecting the level of iron in the plasma of man, it became apparent that the plasma iron level normally undergoes a regular diurnal variation. Reference to the literature shows that several continental investigators have described such a variation. Anglo-American workers have either failed to observe this, taken their samples at a constant time, or ignored the variation in their studies on plasma iron.

Methods. In this study 19 adult male subjects, without demonstrable organic disease which would affect the plasma iron, were used. Samples of approximately 8.0 ml of blood were taken at intervals of 2 to 4 hours throughout a 24-hour period from 12 subjects beginning at 9:00 a. m. and from 7 subjects beginning at 5:00 p. m. Glassware used for the collection, storage and estimation of plasma iron was carefully cleansed by first rinsing with water and then immersing in potassium dichromate and sulfuric acid cleaning solution for a minimum of one hour. It was then rinsed thoroughly with tap water and this was followed by three washings in double distilled water prepared in an all glass distillation apparatus. The blood was taken with all glass syringes and placed into 15 ml tubes to which 2 drops of 20% potassium oxalate had been added previously. The plasma was then separated by centrifugation and the iron estimation made by a modification of the method of Barkan and Walker(1).

Reagents. 1. Hydrochloric acid, 1.2% solution in double distilled water. 2. Trichloroacetic acid (redistilled), 20% solution in double distilled water. 3. Thioglycolic acid (Eastman Kodak-Practical). 4. O-phenanthroline monohydrate, 0.1% solution in double distilled water. 5. Sodium acetate, saturated solution in double distilled water.

Procedure. To 1.5 ml of plasma or serum, 0.75 ml of 1.2% hydrochloric acid is added. The plasma is then incubated at 37°C for

* This study was aided by a grant from the United States Public Health Service.

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