Effect of Red-Cell-Bound Mercury on Measurements of Tissue Mercury Distribution*

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Blood is the transport vehicle of methylmercury (MeHg) in the body (GIBLIN and MASSARO, 1974; NORDBERG and SKERFVING, 1972; PASSOW, 1970; WHITE and ROTHSTEIN, 1973), with almost complete binding to the hemoglobin molecule (GARCIA *et al.*, 1974; ROTHSTEIN, 1973). Although some variation in the percentage of mercury contained in red cells has been reported among species, most data indicate that red cells contain over 90% of the total blood MeHg (LUNDGREN *et al.*, 1967; NORDBERG and SKERFVING, 1972; SUZUKI *et al.*, 1971; SWENNSON *et al.*, 1959). Because of the high affinity of the red cell for MeHg, gross errors in tissue content of MeHg could result unless corrections are made for the MeHg in red cells retained in the tissues. This may be particularly true if tissues are examined within a few hours or days after dosing (before equilibration between red cells and tissues occurs). Results from most studies using isotope techniques to show distribution and tissue content of MeHg do not reflect residual red cell MeHg (ANSARI *et al.*, 1973; IVERSON *et al.*, 1973; REYNOLDS and PITKIN, 1975; ULFVARSON, 1969). Few studies (MOFFITT and CLARY, 1974; SOMJEN *et al.*, 1973) have been performed in which such corrections were made for MeHg bound to the hemoglobin of red cells.

The purpose of this experiment was to determine whether the relative contribution of red cell MeHg to total tissue MeHg is sufficiently great to affect the measurement of total tissue MeHg.

Materials and Methods

Thirteen female Sprague-Dawley rats (averaging 230 g in weight) from a closed colony at the

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Comparative Animal Research Laboratory were injected in the tail vein with 5 μCi (0.55 μg) of MeHg (New England Nuclear) 24 hours before killing. The animals, divided into a control group containing five animals and a perfused group containing eight animals, were anesthetized with sodium pentobarbital. In control animals a blood sample was taken by cardiac puncture, and the animals were decapitated. In the perfused group, a jugular vein and common carotid artery were cannulated with polyethylene tubing (ID 0.023 inch, OD 0.038 inch), and a blood sample was collected. The perfusate was passed through Tygon tubing from a reservoir of heparinized Ringer's solution warmed to 39°C, through a perfusion pump, and into the carotid cannula. The open end of the jugular cannula was placed in a collection beaker. Perfusing solution was pumped into the animal by means of the carotid cannula, and outflow was collected from the jugular cannula. Each rat was perfused for approximately 50 minutes (at which time no red cells were visible in the solution passing from the jugular cannula) at a rate of 1 ml/minute.

Sixty to ninety percent of the red cells were removed from the perfused rats. The percentage of red cells removed was calculated for each animal by using the concentration change of MeHg that occurred at the end of the perfusion.

Organs were counted for ^{203}Hg on a Nuclear Chicago NaI (TI) 2.5-inch crystal detector connected to a Model 1185 pulse-height analyzer and sample changer. The following tissues from controls and perfused groups were counted for radioactivity: blood, heart, lungs, kidney, brain, pituitary, liver, and the remaining carcass (excluding spleen and gastrointestinal tract). Results were expressed both as % dose/g tissue and as % dose/organ so that any effects of edema induced by the perfusion could be detected. The mean differences between control and perfused data were tested for significance by an unpaired *t*-test.

Results

Initially, MeHg was taken up by red cells with relatively little remaining in the plasma. Whole blood contained 1.35 ± 0.14% (mean ± 1 SD) dose/ml, and plasma contained 0.03 ± 0.05% dose ml. Therefore, approximately 97% of the MeHg in blood was found in the red cells, which agrees with an earlier report by GARCIA *et al.* (1974).

Assuming a blood volume of 5.5%, the red cells of the control group contained approximately 16.0 ± 1.7% (mean ± 1 SD) of the injected dose. In the perfused group, 9.5 ± 2.2% of the dose was recovered by the perfusing process.

Distribution of MeHg in five control and five perfused rats is listed in Table I as % dose/g tissue and in Table II as % dose/organ. There was a significant decrease in observed MeHg in all tissues when expressed as % dose/g except for liver. Significant decreases also occurred in all organs (on a total-organ basis) except for kidney and liver.

After perfusion, distribution of MeHg in the perfusing solution was measured in three rats. In these animals, 95 ± 1% (mean ± 1 SD) of the recovered labeled mercury was in the red cells.

TABLE I

Methylmercuric chloride distribution in control and perfused rats*

	% Dose/g Tissue		
	Control	Perfused	P <
Heart	0.32 ± 0.05	0.19 ± 0.01	.001
Lung	0.53 ± 0.09	0.22 ± 0.02	.001
Muscle	0.13 ± 0.01	0.11 ± 0.01	.001
Kidney	4.05 ± 0.30	2.14 ± 0.17	.001
Brain	0.06 ± 0.01	0.04 ± 0.01	.005
Pituitary	0.26 ± 0.05	0.14 ± 0.03	.005
Carcass**	0.20 ± 0.01	0.10 ± 0.03	.025
Liver	0.55 ± 0.06	0.52 ± 0.05	N.S.

*Mean ± 1 SD.
**Whole animal minus visceral organs and brain.

TABLE II

Methylmercuric chloride distribution in control and perfused rats*

	% Dose/Organ		
	Control	Perfused	P <
Heart	0.25 ± 0.04	0.18 ± 0.03	0.01
Lung	0.57 ± 0.08	0.37 ± 0.04	.001
Kidney	7.06 ± 1.09	5.98 ± 0.83	N.S.
Brain	0.10 ± 0.01	0.07 ± 0.01	.005
Pituitary	0.003 ± 0.001	0.001 ± 0.001	.01
Carcass**	25.13 ± 1.78	21.02 ± 1.77	.005
Liver	4.48 ± 0.44	4.95 ± 0.94	N.S.

*Mean ± 1 SD.
**Whole animal minus visceral organs and brain.

Discussion

The data clearly show that significant quantities of red-cell-bound MeHg were removed from the body tissues when the whole animal was perfused. It is therefore necessary to adjust tissue MeHg content for red cell MeHg when studying MeHg distribution.

Exceptions to this were the kidney and the liver. The reason for lack of an effect in the total kidney was not readily apparent. Apparently there was some edema of the kidney since there was a significant decrease in kidney mercury on a % dose/g basis but not on a whole-organ basis. An increase in the variance of kidney samples expressed as % dose/g over the variance of kidney samples expressed as % dose/organ was at least partially responsible. The coefficients of variance increased from 7.4% and 7.7% (control and perfused, respectively) in the former group of data to 15.5% and 13.8% in the latter. Although there appeared to be a decrease in the mean MeHg content of whole kidneys from 7.06% of the dose to 5.98%, the variance of the samples when considered on an organ basis was too large to allow the difference between means to be significant.

Our results agree with the data of SOMJEN *et al.* (1973) who used ^{51}Cr-labeled red cells or ^{131}I albumin; these investigators found no change in kidney MeHg content when corrected for red cell MeHg. Visual inspection of the liver revealed little blanching, an indication that the perfusion technique did not efficiently remove red cells from the liver (probably because of the unique circulatory properties of this organ).

In our study, more than 95% of the labeled mercury in the perfusate was found in red cells after the perfusing solution was passed through the animal. The distribution of mercury between red cells and perfused solution was not significantly different from the distribution of mercury in whole blood. This supports the hypothesis that tissue-bound labeled mercury was not removed from the animals by the perfusion process and that all of the mercury was bound to red cells.

Our findings, supporting other limited data (MOFFITT and CLARY, 1974; ROTHSTEIN, 1973) on the MeHg content of body tissues, suggest that it is necessary to correct for red cell MeHg of liver, spleen, and brain. Findings in our study clearly demonstrate the need for this correction in most tissues.

There are a number of ways in which this correction can be made. These include the use of estimated residual blood volumes or labeled red cells. Radiolabeling of tracer amounts of red cells in each animal should be the preferred method. *In vivo* labeling with ^{59}Fe and subsequent separation of photopeaks by gamma counting is one method, but the *in vivo* labeling requires an additional donor animal and considerable additional time. *In vitro* labeling of red cells with ^{51}Cr requires less initial effort but necessitates the use of liquid scintillation techniques because the gamma photopeaks of ^{51}Cr and ^{203}Hg are too similar for efficient separation.

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