Distribution of CO and Radiochromium in Blood and Tissues of Rabbit and Dog. II. Radiochromium.* (23530)

REIDAR WENNESLAND,† RODGER SHEPHERD, NORMAN NOMOF, ELLEN BROWN, JAMES HOPPER, JR., AND BARBARA BRADLEY

Department of Medicine, University of California School of Medicine, San Francisco

In animal experiments designed to determine the localization of extravascular carbon monoxide (CO), excess of CO over radiochromium (Cr^{51}) in tissue homogenates as compared to their contents in blood was used as an index of localization. Cr^{51} in excess of CO was found in the lungs and spleen, which suggested that Cr^{51}-tagged red cells may be sequestered from the circulating blood in these organs(1). Because of the possibility that loss of tag from the blood might influence the results of blood volume determinations done with the Cr^{51} method, this phenomenon has been studied more fully.

Methods. The procedures used for tagging with Cr^{51}, *in vitro* handling of the blood, simultaneous administration of tagged cells and CO to the rabbits and dogs, and analysis of the blood and tissues have been described (1,2). In 10 dogs the sites of injection of the tagged cells and of sampling and the mode of death varied, as shown in Fig. 1. To test the effects of the *in vitro* handling of blood on the sequestration phenomenon, 25 rabbits were injected with cell suspensions that had been treated in various ways. Usually the cells were resuspended in saline after tagging and were stored overnight at 5°C, but in 12 experiments they were stored for 1 to 3 weeks. Half of these specimens were suspended in saline, the other half in their own plasma. Hemolysis was seen only in saline suspensions stored for 1 week or more. The cells in these preparations were washed until the supernatant appeared clear before they were injected into their respective donors. Otherwise the experiments were conducted as usual. The CO:Cr^{51} ratio of each tissue homogenate (R_t) was compared to the ratio in the animal's blood (R_b) to obtain an R_t:B_b ratio(1). When the R_t:R_b ratio of the tissue homogenate was less than one, it could be assumed that Cr^{51} had accumulated in that tissue(1).

Results. The results of the analyses of tissue homogenates and blood from the 10 dogs, expressed as R_t:R_b ratios, appear in Fig. 1. Ratios for spleen were less than one in all dogs killed by bleeding. The exsanguinated animals had small, pale spleens, which gave homogenates consisting mostly of gray pulp. No evidence of Cr^{51} accumulation in the spleen appeared in the dogs killed with sodium pentobarbital (Nembutal). Here, the spleens were large, filled with blood, and produced homogenates consisting mostly of blood. The lung ratios were also less than one in all but 2 experiments where the tagged cells had been injected into branches of the portal vein. Here, the livers showed an excess of Cr^{51}, while the lungs did not. Two animals were perfused with saline after bleeding. This accentuated the differences between tissue and blood ratios; the lowest values for spleen,

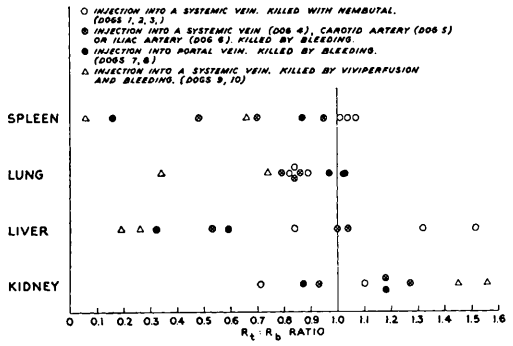

FIG. 1. $R_t : R_b$ ratios of tissue homogenates from dogs killed 20 minutes after receiving CO and Cr^{51}-tagged cells simultaneously. R_t = ratio in tissue homogenate of CO content (volume %) to Cr^{51} content (counts per second). R_b = ratio of CO to Cr^{51} content of the corresponding blood sample.

* This study was aided by grants from Edwards and Hertzstein Funds, allocated by Committee on Research, University of California School of Medicine, and from the U. S. Public Health Service and the San Francisco and Monterey County Heart Assns.

† Research Fellow, Am. Heart Assn., while this work was in progress.

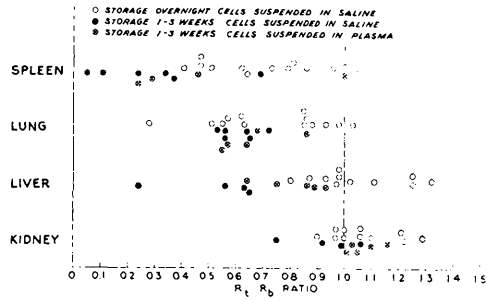

FIG. 2. $R_t : R_b$ ratios of tissue homogenates from 25 rabbits given autogenous red cells tagged with Cr^{51}, after storage for various intervals before injection, and CO. R_t and R_b are derived from tissue and blood analyses (see text and Fig. 1).

lung and liver were found in the experiment where the perfusion was particularly complete. After injection of the tagged cells into a common carotid artery the brain showed no retention of tag, but the usual excess was found in spleen and lung. When the injection was into the right external iliac artery, no excess of Cr^{51} was found in muscle or spongy bone of the right as compared to the left leg, and the usual retention occurred in lung and spleen. There was no evidence of chromium retention in the kidneys.

Fig. 2 shows the results of studies relating to the *in vitro* handling of the blood in 25 rabbits. The longer the storage time, the greater the excess of Cr^{51} found in lung, liver and spleen, particularly when the cells had been stored in saline.

An attempt was made to assay the total amounts of Cr^{51} lost from the cells after they had been handled and stored in various ways. Table I shows the amounts of Cr^{51} lost to the supernatant in experiments on blood taken from 8 rabbits and stored in saline or plasma for 1 day to 3 weeks. Table II shows the total amount of Cr^{51} retained in lungs, liver and spleen combined as % of the dose of Cr^{51} given to each of the 8 rabbits. The Cr^{51} content of the organs was determined by analysis of the entire organ (lung and spleen) or of duplicate aliquot samples (liver). The CO content of the homogenates and of the blood provided a means of correcting for the blood content of the organs.

Discussion. The exact mechanism responsible for sequestration of Cr^{51} is not shown by these studies, but must relate to physicochemical changes in the red cells caused by *in vitro* handling and to the functions of the reticuloendothelial system. Tompkins has described changes in erythrocytes when suspended in saline that are reversed by addition of plasma(3). Some of the injected erythrocytes were retained in the lung or liver, depending on which of these organs was traversed first; Cr^{51} accumulated in the spleen even after the cells had passed through the lung or liver. In contrast, no retention was found in the brain or extremities when their capillary beds were the first ones reached by the tagged cells. Sequestration of cells in the lung may be analogous to the trapping in this organ of leukocytes(4) or of macromolecules (5).

The more roughly the blood was handled, the greater the sequestration of Cr^{51}. With prolonged storage of tagged cells, more and more Cr^{51} was lost, particularly when the cells were resuspended in saline. However, even with 3 weeks of storage, the total loss of tag

TABLE I. Loss of Cr^{51} to the Supernatant after Storage of Tagged Rabbit Erythrocytes in Plasma or Saline at 5°C for 1 Day to 3 Weeks.

	Storage time	A Radioactivity of cell suspension	B Radioactivity of supernatant	B as % of A
		Counts/sec./ml		
Cells suspended in plasma	1 day	4,490	5.5	.1
	1 wk	2,743	5.5	.2
	2 "	2,380	7.0	.3
	3 "	1,905	11.2	.6
Cells suspended in saline	1 day	4,170	4.5	.1
	1 wk	5,420	37.0	.7
	2 "	2,130	21.4	1.0
	3 "	2,180	32.7	1.5

TABLE II. Combined Loss of Cr^{51} by Sequestration in Lung, Spleen and Liver of Rabbits Killed 20 Min. after Injection of Tagged Cells Stored in Plasma or Saline at 5°C for 1 Day to 3 Weeks.

	Storage time	A Radioactivity of inj. 10 ml of cell suspension (Counts/sec.)	B Radioactivity of total Cr^{51} retained in lungs, liver and spleen (Counts/sec.)	B as % of A
Cells suspended in plasma	1 day	33,750	35	.1
	1 wk	21,750	82	.3
	2 "	lost		
	3 "	14,500	232	1.6
Cells suspended in saline	1 day	30,000	55	.2
	1 wk	38,750	1,010	2.6
	2 "	16,500	471	2.8
	3 "	18,250	609	3.3

from the blood by leakage *in vitro* and by early sequestration was not more than 2 to 4% (Tables I and II).

Errors caused by *in vitro* leakage and sequestration would be inconsequential in the ordinary blood volume determination. If cells are stored only 1 day, even in saline, the combined loss amounts to 0.3% (Tables I and II), which would be equivalent to an error of 15 ml in the measurement of a 5,000 ml blood volume. Hence it seems unnecessary to add plasma to the suspending medium as recommended by Chaplin(6). Reported studies of red cell survival(2,7,8) have indicated that the losses of Cr^{51} which occur at longer intervals after injection of tagged cells are of much greater magnitude than were observed here (6% during the first 24 hours, then about 1% per day).

Summary. 1. Carbon monoxide and autogenous red cells labeled with Cr^{51} were administered simultaneously to dogs and rabbits; tissue homogenates and blood were then analyzed for radioactivity and CO content. 2. Small amounts of Cr^{51} accumulated regularly in spleen and lung and, when the cells were delivered via the portal vein, in the liver. No sequestration occurred in brain, kidneys or extremities. 3. Prolonged storage of tagged cells, particularly when suspended in saline rather than plasma, increased the loss of Cr^{51} from the cells *in vitro* and by early sequestration after injection of the cells into their donors. 4. When cells were stored in saline for only 1 day, combined losses caused by *in vitro* leakage and sequestration were too small to influence the results of blood volume determinations.

1. Wennesland, R., Nomof, N., Hopper, J., Jr., Brown, E., and Bradley, B., PROC. SOC. EXP. BIOL. AND MED., 1957, v96,

2. Nomof, N., Hopper, J., Jr., Brown, E., Scott, K., and Wennesland, R., *J. Clin. Invest.*, 1954, v33, 1382.

3. Tompkins, E. H., *J. Lab. and Clin. Med.*, 1954, v44, 554.

4. Bierman, H. R., Kelly, K. H., and Cordes, F. L., *Ann. New York Acad. Sc.*, 1956, v59, 850.

5. Waser, P., and Hunzinger, W., *Experientia*, 1951, v7, 6.

6. Chaplin, H., *J. Physiol.*, 1954, v123, 22.

7. Ebaugh, F. G., Jr., Emerson, C. P., and Ross, J. F., *J. Clin. Invest.*, 1953, v32, 1260.

8. Mollison, P. L., and Veall, N., *Brit. J. Haematol.*, 1955, v1, 62.

Received August 19, 1957. P.S.E.B.M., 1957, v96.