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Distribution of CO and Radiochromium in Blood and Tissues of Rabbit and Dog. I. Carbon Monoxide.* (23568)

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Comparative studies on methods for measuring blood volume have shown that the volume of distribution of carbon monoxide (CO) is 12 to 16% greater than that of radioactively tagged red cells in splenectomized dogs(1) and human subjects(2). The discrepancy has been attributed to accumulation of CO in extravascular pigments, chiefly myoglobin, as proposed by Haldane and Smith(3), but the sites of accumulation of CO have never been demonstrated directly. We have analyzed homogenates of various tissues from rabbits and dogs after simultaneous administration of CO and autogenous red cells tagged with radiochromium (Cr^{51}), using the radioactivity of each homogenate as an index of its blood content, and found the principal sites of CO accumulation to be red skeletal muscle and myocardium.

Methods. Fifteen ml of blood from each animal was tagged with Cr^{51}, and the cells were stored overnight in saline or their own plasma at 5°C(2). Tracheotomy was done under narcosis with sodium pentobarbital (Nembutal), and the airway was connected to a small rebreathing system for administration of measured amounts (15 to 100 ml) of CO gas(4). Ten ml of the animal's own Cr^{51}-tagged cells was injected into a vein. Twenty minutes later, blood was drawn from the heart or a large vessel into tubes containing Wintrobe's oxalate mixture. The animals were killed by injection of Nembutal or by exsanguination. Pieces of heart, spleen, lung, liver, kidney, skeletal muscle, brain, bone and bone marrow were excised, wiped dry and homogenized. The blood was analyzed for CO by the palladium reduction method of Wennesland(5), and 3 g portions of homogenates were analyzed by a modification of a procedure used for blood clots(6). Five ml of a 1% solution of pepsin powder‡ in 1 N H_2SO_4 was added to each portion of homogenate to accelerate liberation of CO. Six to 8 hours were needed for complete liberation of the gas and reduction of an equivalent amount of $PdCl_2$. After the iodometric titration was completed, each sample was brought to a total volume of 12 ml by addition of distilled water. Its radioactivity in counts per second was measured in a flange-type scintillation counter. A total of 4,096 counts was made. The counting error was ± 2%. The error of the analysis of CO in homogenates was assumed to be the same as that in the analysis of blood clots(6). To obtain the desired accuracy in the tissue analyses the animals were given relatively larger doses of CO and of tagged cells than are used in measurements of blood volume. The CO content in volumes % was compared to the radioactivity in counts per second to obtain CO:Cr^{51} ratios for each tissue homogenate (R_t) and for the blood of each animal (R_b). For each tissue homogenate the ratio, R_t:R_b, was calculated. With no

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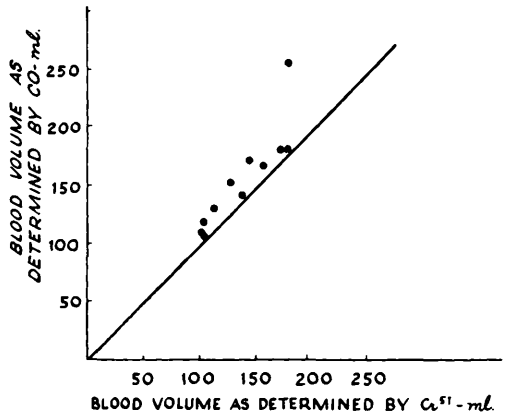

FIG. 1. Carbon monoxide-available space compared to Cr^{51} space in 11 rabbits. Mean difference is 13%.

loss of CO from the tagged circulating blood, the ratio $R_t:R_b$ would be one. Extravascular accumulation of CO would give a ratio larger than one. The more CO and the less blood contained in a tissue, the larger this ratio would tend to be. A ratio less than one might be accounted for either by accumulation of Cr^{51} in the tissue or by loss of CO during the analytical procedure. However, the strong affinity of CO for hemoglobin and related compounds makes it improbable that gas is lost during the few minutes it takes to prepare the homogenates. Blood can be left in open bottles for weeks without appreciable loss of CO (see Table I). The volumes of distribution of CO and Cr^{51} were determined in the usual way(2).

TABLE I. CO Content, at Various Time Intervals, of a Specimen of Human Blood Stored at 5°C, Half of It in a Stoppered Bottle and Half in an Open Erlenmeyer Flask.

	Carbon monoxide conc., vol %			
	Storage time, days			
	0	30	37	40
Stored in stoppered bottles	3.27*	3.10*	3.25†	3.24*
Stored in open flask	3.30†	3.20†	3.17*	3.25†

* 2 N H_2SO_4 used for release of CO.
† 1 N H_2SO_4 plus 1% pepsin used for release of CO.

Results. Fig. 1 shows the volumes of distribution of CO and Cr^{51} determined simultaneously in 11 rabbits. The CO space exceeded the Cr^{51} space by an average of 13%. The difference is about the same as we found in human subjects, using the same technics(2), and as Root, Allen and Gregersen, using CO and P^{32}, found in splenectomized dogs(1).

Fig. 2 shows the results of analyses of blood and various tissue homogenates from 25 rabbits and 8 dogs, expressed as $R_t:R_b$ ratios. Ratios greater than one were found consistently in skeletal and heart muscle. The scatter of the data for skeletal muscle can be accounted for largely by differences in the blood content of the various specimens. Generally, higher ratios were obtained in muscle tissue from dogs killed by bleeding.

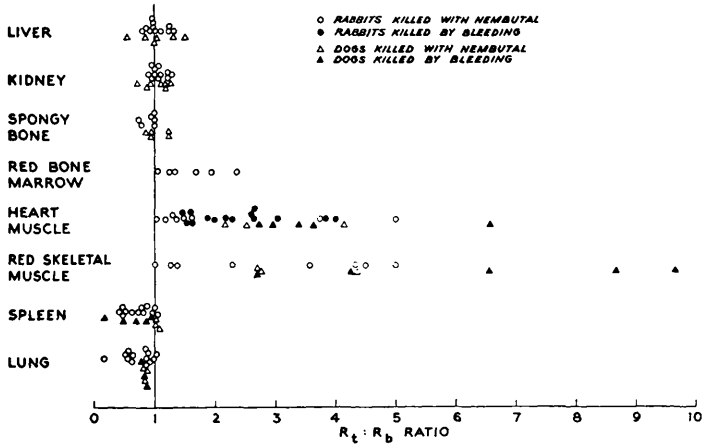

FIG. 2. $R_t : R_b$ ratios of tissue homogenates from rabbits and dogs killed 20 min. after simultaneous administration of CO and Cr^{51}. R_t = ratio in tissue homogenate of CO content (vol %) to Cr^{51} content (counts/sec.). R_b = ratio of CO to Cr^{51} content of corresponding blood sample.

than from those killed with Nembutal. The influence of blood content on the quantitative result was emphasized by data from 2 experiments not illustrated in the figure, in which dogs were killed by viviperfusion with saline and exsanguination. Here, the $R_t:R_b$ ratios were 19.0 and 24.0 for skeletal muscle and 19.0 and 7.1 for myocardium. A modest excess of CO appeared in the red marrow of the rabbits; good samples of red marrow could not be obtained from the dogs. $R_t:R_b$ ratios of about one were found for liver, kidney and spongy bone. In both species, $R_t:R_b$ ratios below one were found in homogenates of spleen and lung, suggesting retention of the Cr^{51}-tagged cells in these tissues. The problem of Cr^{51} retention is discussed in part II of this study(8).

Discussion. The high $R_t:R_b$ ratios found in skeletal muscle and myocardium, and particularly in nearly bloodless muscle, support the suggestion of Haldane and Smith(3) that most of the CO leaving the blood combines with myoglobin. Data from 10 dogs permitted an estimation of the total amount of CO accumulated in the muscular system. The total muscle mass was taken to be a third of body weight(7). The quantity of CO in each sample of muscle homogenate was corrected for blood content on the basis of its Cr^{51} content. Assuming that the samples were representative of the entire muscle mass, it could be calculated that between 5.6 and 20.0% (average 13.5%) of the administered CO had accumulated extravascularly in muscle. Thus, making no allowance for the small amounts of CO in the myocardium and bone marrow, most or all of the discrepancy between the blood volumes measured with CO and with radioactively tagged cells can be accounted for by loss of CO to muscle. Data from 6 rabbits gave similar results.

Summary. 1. Carbon monoxide gas and autogenous red cells tagged with Cr^{51} were administered simultaneously to rabbits and to dogs. Blood and homogenized tissues from various organs were analyzed for radioactivity and for CO content. 2. The volume of distribution of CO was 13% larger than the volume of distribution of Cr^{51} in the rabbit. The discrepancy is the same as that found in man and the splenectomized dog. 3. CO accumulated in red skeletal muscle and myocardium in amounts sufficient to account for the discrepancy between distribution volumes. 4. Evidence was found pointing to slight accumulation of Cr^{51} in spleen and lung. Further studies of this phenomenon are described in part II of this report.

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