Effect of Internal Emitters on Red Cell and Plasma Volumes of Beagle Dogs.* (23857)

JOHN E. PARKINSON AND JEAN H. DOUGHERTY†
Radiobiology Laboratory, Departments of Anatomy and Pathology, University of Utah, Salt Lake City

The hematological alterations produced by internally deposited radioisotopes have been studied in this laboratory for several years. The particular isotopes under investigation are plutonium, radium, radiothorium, mesothorium and strontium (Sr^{90}). Among blood changes that have been induced by these bone seeking isotopes, anemia is one of the most frequent. Relatively few studies have been made on anemias induced by internal irradiation as compared to numerous investigations on external irradiation. Our primary object is to ascertain the amount of retained isotope required to induce anemia, and to determine the time of onset, duration and type of anemia. Hematological alterations observed during the first year following administration of plutonium and radium have been reported(1). Absolute changes in red cell and plasma volume determined by Cr^{51} tagged red cells and I^{131} tagged human albumin are presented here.

Methods. The 21 control and 29 isotope treated animals are part of a colony of about 350 pure-bred Beagle dogs. They are housed in modern kennels and constantly under the care of full time veterinarians and their staff who maintain the animals on most optimum dietary conditions. Adult dogs, approximately 16 months of age, are injected intravenously with a single dose of isotope. Dogs bearing retained amounts of 0.27, 0.81 and 2.5 μc/kg of radiothorium and mesothorium and 0.81 and 2.5 μc/kg of plutonium and radium were used. Anemia occurs at these dose levels at one to 6 months post injection with recoveries and relapses at irregular intervals thereafter. Injected animals were studied from one to 45 months post injection and many were re-evaluated at multiple time intervals for total of 50 determinations on 29 dogs. Red cell volumes were measured by Cr^{51} tagging by modification of method by Read(2). Plasma volumes were obtained simultaneously by I^{131} tagged human albumin. About 50 ml of blood were collected in a plastic Fenwall bag containing 10 ml ACD solution. 0.5 to 12 μc Cr^{51} (Rachromate‡) and 0.5 to 1.5 μc I^{131} (RISA‡). After 45 minutes Cr^{51} uptake was stopped with 50 mg sodium ascorbate. Total counts were determined by obtaining an aliquot sample for counting and by weighing the bags before and after transfusion. Specific gravities of blood and plasma were determined with graded solutions of copper sulfate concentrations(3). Three 5 ml samples at 15-minute intervals $\pm$ 10 seconds served as dilution samples. Hematocrits were determined in Wintrobe tubes centrifuged at 3,000 rpm for 30 minutes and were not corrected for trapped plasma. Counting in sealed glass vials in shielded well-type scintillation counter with pulse height analyzer yielded about 90,000 cpm/μc Cr^{51}; 380,000 cpm/μc I^{131}; and 150 cpm background. Blood was placed in weighed vials (nearest 0.1 mg), centrifuged; plasma was withdrawn into weighed vials to be counted separately. Counts from packed cells were corrected for small amount of remaining plasma. From 15% to 50% of counts in the vial containing red cells represented remaining plasma. From 5% to 20% of counts in plasma were from Cr^{51} which did not tag red cells. Three points at 15-minute intervals were plotted on semi-logarithm graphs and the best fit line extrapolated to 0 time for plasma volume. The majority of determinations showed little scatter and constant slope. Red cell volume was calculated from the 30-minute dilution sample.

* This research was supported by Division of Biology and Medicine, U. S. Atomic Energy Comm.

† The authors wish to express appreciation to Dr. T. F. Dougherty for advice and assistance; to Dr. M. A. Van Dilla for advice on counting technics; to Kathryn Seymour and Warren Fisher for technical assistance; and to Betty Chamberlin for help with statistical analysis.

‡ Abbott Laboratories.

TABLE I. Comparison of Blood Volume Values in Control and Isotope Treated Beagle Dogs.

Group	No. of dogs	RBC vol (ml/kg)	Plasma vol (ml/kg)	Blood vol (ml/kg)	Venous hematocrit	Total body hematocrit	Hematocrit ratio
Control	21	43.0 ± 9.5*	51.4 ± 12.4	94.4 ± 16.6	46.4 ± 4.4	45.7 ± 6.8	.99 ± .09
Isotope treated (non-anemic)	11	43.3 ± 12.6	61.9 ± 8.7	105.2 ± 17.2	40.6 ± 5.1	40.7 ± 6.1	1.02 ± .21
Isotope treated (anemic)	22	24.4 ± 5.7	53.3 ± 11.1	78.7 ± 14.5	36.5 ± 5.3	32.1 ± 5.2	.88 ± .10

* Means are expressed ± stand. dev.

Results. The precision of this method was evaluated by making 2 determinations at one-week intervals in 10 dogs. Mean variation in red cell volume was 10% (0.5% to 19.2%) and in plasma volume 7.7% (0.2% to 16.3%). Red cells and plasma were counted immediately prior to second determination to correct for residual Cr^{51} and I^{131}. In 4 animals plasma volume was determined alone with I^{131} and then repeated 2 days later in combination with red cell volume. Mean variation was 6% (2.0% to 11.9%).

In Table I the animals are divided into 3 groups: 1) controls, 2) isotope treated dogs in non-anemic phase, and 3) isotope treated anemic dogs. An animal was considered anemic when red cell volume was below the range of control values (<33.4 ml/kg). Blood volumes are expressed per kg of dog's average weight. In last column of Table the venous hematocrit is divided by total body hematocrit (red cell vol/total blood vol) and expressed as ratio (hematocrit ratio). This ratio expresses relative compartmentalization of red cells in large veins to body pool. There were 16 determinations on 11 non-anemic dogs and 34 determinations on 22 anemic animals.

Isotope treated dogs with decreased red cell volumes had normal plasma volumes. Only one severely anemic radiothorium dog (red cell vol 10.1 ml/kg) had a slightly decreased plasma volume of 35.2 ml/kg. Total blood volumes and hematocrit ratios in this anemic group were decreased from control values (t-test differences with $p = .001$). Blood volume values on isotope treated non-anemic dogs were generally similar to control animals.

Table II gives correlation coefficients between red cell volumes and hematocrit ratios. There is a good positive correlation in injected group (includes both anemic and non-anemic dogs) and a poor correlation in control group.

Red cell volumes and venous hematocrit values are correlated in Table III. There is good correlation in isotope treated anemic dogs in contrast to poor correlation in control and isotope treated non-anemic groups. In this Table the control group includes red cell volume and hematocrit values from 29 dogs previously measured without plasma volume determinations.

Discussion. A method is presented for simultaneous determination of red cell and plasma volume by isotope technics. A varia-

TABLE II. Correlation between Red Cell Volume and Hematocrit Ratio.

Group	RBC vol, mean	Hematocrit ratio, mean	No. determinations	Correl. coeff., r	P value
Control	43.0	.99	21	.429	.1–.05
Isotope treated	31.1	.93	50	.712	.001

TABLE III. Correlation between Red Cell Volume and Venous Hematocrit.

Group	RBC vol, mean	Ht, mean	No. determinations	Correl. coeff., r	P value
Control	42.8	48.6	52	.369	.01
Isotope treated (non-anemic)	40.5	45.8	32	.354	.05
Isotope treated (anemic)	25.8	38.9	46	.690	.001

tion of this method has been reported by others both as a simultaneous technic(4) and as a sequential technic employing Cr^{51} immediately following I^{131} plasma volume determination(5). Drawbacks to the method are errors introduced by inability to separate I^{131} and Cr^{51} in the counting device. In counting red cells correction must be made for contaminated I^{131}. A small amount of Cr^{51}, which cannot be corrected for, contaminates the plasma. Calculations from data published by Reeve *et al.*(6) demonstrate in 3 dogs, listed with repeat P^{32} red cell volume and T-1824 plasma volume, a variation of 9.1% to 18.3% and 0% to 8.8% respectively. These are very similar to variations seen here. Plasma volume determined alone differs only slightly from that determined simultaneously (average variation 6%). The red cell volume of control group (average $= 43.0 \pm 2.1$), is almost identical to a group of 29 dogs in which red cell volume was determined alone (average $= 42.6 \pm 1.7$). The method presented here offers advantages of gravimetric measurements, a single injection of isotope mixture, and concomitant plasma and red cell volumes. Experimentally the technic is reasonably reproducible and accurate.

From Table I it may be seen that in control dogs the mean venous hematocrit value is similar to total body or true hematocrit value with a hematocrit ratio approaching unity. However, in control dogs there is a poor correlation between red cell volume and venous hematocrit and hematocrit ratio (Tables II and III). Thus, the venous hematocrit does not reflect accurately changes in red cell volume. On the other hand, anemic isotope treated dogs have a higher venous hematocrit than total body hematocrit (different by T test of .002 level), and the venous hematocrit reflects fairly accurately changes in red cell volume (Table III). In these anemic dogs there is a striking contrast between reduction of venous hematocrit (80% of control) and decrease in red cell volume (60% of control). A decreased hematocrit ratio has been noted in people in various disease states, *i.e.*, chronic anemia, acute hemorrhage, heart failure, dehydration, hypoproteinemia, post operative shock(7); although some studies reveal a quite constant ratio over a wide hematocrit range(8).

These results suggest that when isotope treated dogs become anemic, the venous red cell compartment as represented by venous hematocrit increases with no accompanying change in plasma volume, and the red cell pool falls elsewhere. In other words, there is a relative shift of red cell mass from other areas into the venous pool. The red cells could come from the splenic pool or capillary bed.

Spleens of normal dogs can store from 2 to 17% of red cell volume depending on degree of contraction or relaxation(6). Splenectomy reduces this wide fluctuation(6). The splenic pool could become depleted in isotope treated dogs, and this has been borne out at autopsy as some dogs have had small atrophic spleens.§ About 17% of total blood volume is found in minute vessels of the dog(9). Preliminary study of post mortem sections suggests that there is a decreased capillary bed in many isotope treated dogs. Further study is necessary to determine the main source of additional red cells in larger veins.

Summary. Simultaneous red cell and plasma volumes have been measured with Cr^{51} and I^{131} in control beagle dogs and dogs with internally deposited radioisotopes. The reliability of the method is discussed. In isotope treated anemic dogs the venous hematocrit tended to be higher than the total body or true hematocrit but more accurately reflected red cell volume changes than in controls. Plasma volume did not change to account for this relative increase in venous hematocrit. It is postulated that this relatively high venous hematocrit probably represents a shift of red cells into large caliber veins.

1. Dougherty, J. H., Bowers, J. C., Bay, R. C., Keyanonda, P., *Rad.*, 1955, v65, 253.

2. Read, R. C., *New Eng. J. Med.*, 1954, v250, 1021.

3. Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., Dol, V. P., Emerson, K., Jr., Archibald, R. M., *J. Biol. Chem.*, 1950, v183, 305.

4. Klement, A. W., Jr., Ayer, D. E., McIntyre, D. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 81.

5. Gurney, C. W., Bolt, R. J., *Univ. of Mich. Med.*

§Unpublished observations.

Bull., 1956, v22, 319.

6. Reeve, E. B., Gregerson, M. I., Allen, T. H., Sear, H., *Am. J. Physiol.*, 1953, v175, 195.

7. Hope, A., Verel, D., *Clin. Sci.*, 1955, v14, 501.

8. Chaplin, H., Jr., Mollison, P. L., Vetter, H., *J. Clin. Invest.*, 1953, v32, 1309.

9. Gibson, J. C., 2nd, Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J., Evans, R. D., *ibid.*, 1946, v25, 848.

Received August 26, 1957. P.S.E.B.M., 1958, v97.

Production of Experimental Cardiac Hypertrophy in Rats by Reduced Atmospheric Pressure and Elevated Environmental Temperature.* (23858)

HARRY SOBEL,† AND STUART GRABOYES

Institute for Medical Research, Cedars of Lebanon Hospital and Department of Biochemistry and Nutrition, University of So. California, Los Angeles

In our investigations on proteins of the heart a need arose for production of graded cardiac hypertrophy in the rat. It was also necessary that the cardiac changes be related entirely to increase in work load. Use of an aorta or renal artery tie yielded erratic results. The possible occurrence of secondary cardiac pathology due to renal necrosis made these technics undesirable. Hypertrophy has been reported frequently following exposure to discontinuous hypoxia. Van Liere and Feder (1) observed 24% increase in relative heart weight following exposure of rats to atmospheric pressure of 303 mm Hg, 4 hours a day for 41 days. This technic seemed to offer many advantages and it was employed along with elevated environmental temperature. It was possible by this means to produce graded hypertrophy of any desired degree. For example a 50% increase in heart weight occurred after approximately 12 days of treatment.

Methods. Rats of the Addis-Slonaker strain maintained on stock diet were used. The weight range was 135-165 g. They were daily exposed (including weekends) for 4 hours to 37°C ± 1°C and an elevation of 25,000 ± 1,000 ft. in an evacuation apparatus.‡ When heart weights were to be obtained, the rat was killed on the morning following last exposure and the heart was freed of the great vessels and both atria. The chambers were cut and blood removed as completely as possible by tapping on filter paper. Growth did not occur during period of treatment; the animals either gained or lost a few grams over the starting weight. If an occasional rat lost more than 6 g, it was discarded.

Results. The findings were documented as regression of log of relative heart weight (R) on accumulated exposure time (T) (Fig. 1). R is the ratio of observed heart weight/predicted heart weight. For predicted heart weight the equation of Addis and Gray(2) was used. This had been derived for animals of this strain. R for untreated animals is approximately .96 rather than 1.00 since the Addis-Gray equation is based upon the intact heart. Since a sex difference was not observed, the data of males and females were pooled.

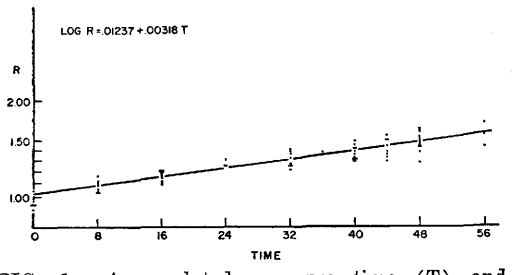

FIG. 1. Accumulated exposure time (T) and heart wt observed/heart wt expected (R). T = time in hr. n = 75. Correlation coef. = .83. z transformation yielded $\frac{z}{\sigma_z} = 10$. t = 12.7. V = .00144.

* Supported by grant from Am. Heart Assn. and Public Health Service.

† Investigator, Howard Hughes Fn.

‡ The authors are grateful to Dr. Benjamin H. Ershoff for loan of this equipment.