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Simultaneous Use of I^{131}-Albumin and Cr^{51}-Labelled Red Cells in Blood Volume Studies in the Goat. (21292)

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(Introduced by B. J. Jandorf.)
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Several methods have been used to determine red cell and plasma volumes in various animals simultaneously(1-8). T-1824 dye and I^{131}-albumin are generally used for plasma labels. Frank and Gray(9) have recently reported the use of Cr^{51}-labelled plasma. Radioisotopes of P, Cr, and Fe are generally used for red cell labels. In many experiments here it was desirable to obtain plasma and red cell volumes simultaneously using labels which were available for other volume studies and which readily permitted several successive determinations. Small samples of blood for assay were also desired to reduce the blood loss due to frequent sampling. Most procedures require a considerable sampling loss where several volume determinations are made. The method of Gamble *et al.*(1), using I^{131} and P^{32}, although successful in the dog, was not used in the goat since goat red cells did not retain the phosphate.

In the method described below I^{131}-albumin and Cr^{51}-labelled red cells were used simultaneously. Hematocrit tubes were used for counting containers. Plasma was separated by centrifugation of blood and counted separately. The red cells were partially separated and counted. By correcting the cell plus plasma counts for the plasma counts, the red cell counts were obtained. The use of the hematocrit tube facilitates the separation of plasma and cells and requires no more than 1 cc of blood per sample. Volume determinations were made on normal goats using this method. Two *in vitro* recovery experiments were also performed.

Methods. Samples of blood were drawn from goats and the red cells were labelled with $Na_2Cr^{51}O_4$ as described by Gray and Frank (3). I^{131}-human serum albumin (Abbott) was diluted in isotonic saline to provide 0.5-1.0 μc/cc solutions. Wintrobe "EXAX" (Kimble Glass) hematocrit tubes were calibrated and used as containers for samples assayed for ra-

dioactivity. Volumes of samples were determined by reading the sample levels in the tubes. The hematocrit tube unit was used as the unit of volume. In order to avoid errors in counting due to changes in geometry an attempt was made to keep cell, standard solutions, and plasma levels approximately equal throughout an experiment. The level selected depends on the cell level as will be seen from the description of the method which follows. Blood samples were placed in hematocrit tubes and centrifuged for 30 minutes at 3000 r.p.m. A no. 17 hematocrit needle and syringe were used to remove all the plasma over the cells in the tube within a few units of the top of the cells. A very thin glass pipette was used to remove nearly all of the remaining plasma without disturbing the cells, leaving a fraction of a unit of plasma over the cells. The plasma thus removed was placed in another hematocrit tube at a level approximately equal to that of the cells from which the plasma was removed. Samples of standard solutions were similarly prepared in other hematocrit tubes. Volumes of red cells, white cells and remaining plasma were determined by reading their respective levels in the cell tubes. Volumes of plasma and standard solution samples were similarly obtained. Samples were counted in a well-type scintillation counter. Counts per unit volume (counts/volume) were calculated for standard solutions and plasma samples. The plasma counts/volume, volume of plasma remaining over the cells and the volume of plasma "trapped" in the cells were used to obtain the counts due to plasma (I^{131}) in the cell tubes. These counts were subtracted from the cell tube counts to obtain net red cell (Cr^{51}) counts from which counts/volume of red cells were determined. Hematocrit "trapped plasma" correction values (ratio of packed cell volume to packed cell and "trapped plasma" volume) were determined for each experiment using I^{131}-albumin. Since these values are large for goat blood they are particularly important in the determinations described below. About 10 cc of blood was taken from each source to be studied and a small drop of I^{131}-albumin (100-500 μc/cc) was added and mixed thoroughly. Three samples were placed in hematocrit tubes and centrifuged for 30 minutes at 300 r.p.m. Plasma and cells were separated as described above. Volumes of plasma, red plus white cells and plasma remaining above the cells were determined without correcting cell volumes for "trapped plasma." Cell and plasma samples were counted and the counts/volume were calculated for each as described above. The average counts/volume of plasma and cells were used to calculate the trapped plasma correction values: trapped plasma correction $= 1 - \frac{\text{counts/vol cells}}{\text{counts/vol plasma}}$. In order to check this method other methods were used simultaneously in some cases. *In vitro* plasma volume determinations were made using the dilution method with I^{131}-albumin and T-1824 dye (2,10,11). For some of these studies volumes were determined with I^{131} by counting 1 cc samples of plasma and standard solutions in plastic test tubes as well as in hematocrit tubes.

Except for the procedure described above the blood volume studies were essentially the same as those of others(1-10) using radioisotope dilution methods. Standard solutions were prepared by diluting 1 or 2 cc of Cr^{51}-labelled red cells and 0.5 or 1 cc of I^{131}-albumin solution in separate 100 ml volumetric flasks of isotonic saline containing 1 cc of carrier plasma and a small pinch of saponin. *In vitro* volume determinations were made by introducing 2 cc of Cr^{51}-labelled red cell suspension into 100 ml of blood, followed by 0.5 cc of I^{131}-albumin solution. Three samples each of blood and standard solutions were processed as described above. The average

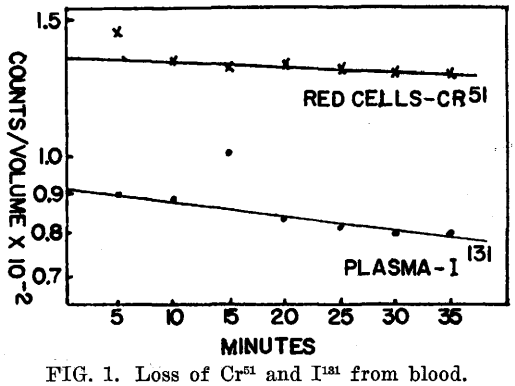

FIG. 1. Loss of Cr^{51} and I^{131} from blood.

TABLE I. Hematocrit "Trapped Plasma" Correction Factors Determined by Assay of I^{131}-Albumin Using the Direct Hematocrit Tube Method.

Observed hematocrit, %	Trapped plasma correction factor, %	Correct hematocrit, %
25.1	81	20.3
26.2	81	21.2
29.3	82	24.0
28.4	86	17.6
24.1	81	19.5
33.4	79	26.4
28.1	80	22.4
37.8	81	30.6
38.8	83	32.2
26.2	79	20.7
Mean	81	

counts/volume of red cells and plasma were compared to the corresponding average counts/volume of standard solution to determine the total plasma and red cell volumes in the flask. White cells were excluded from the determinations above but the volume of these in the cell tubes were averaged to obtain an estimate of the total white cells in the flask. Total blood volumes were obtained by adding the red cell and plasma volumes.

Four *in vivo* experiments were performed using the method described above. Seven to 35 cc of Cr^{51}-labelled red cell suspension and 2 to 10 cc of I^{131}-albumin solution were injected into goats through jugular catheters followed by a few cc of saline. Five 1 cc samples were taken in heparinized syringes between 10 and 30 minutes following the injection. A semilog plot of counts/volume of plasma and red cells was used to obtain values extrapolated to injection time (Fig. 1). The counts/volume at injection time were used to obtain plasma and cell volumes by comparison with counts/volume of standard solutions. Successive volume determinations were made at various times after the initial determination in 2 animals. In these cases 2 samples were taken just prior to injection. The counts/volume values were used to correct for activity already in the animal from previous determinations.

TABLE II. "Trapped Plasma" Correction Factors Using I^{131} and T-1824 Dye.

Hematocrit,* %	I^{131} hematocrit tube (direct)	I^{131} hematocrit tube (dilution)	I^{131} test tube (dilution)	T-1824 dye (dilution)
36.3	.84	—	.87	—
32.4	.81	.83	.87	.80
33.0	.81	.86	—	.82
31.8	.79	—	.81	.84
33.2	.83	—	.83	.93

* Uncorrected.

Results. "Trapped plasma" correction values (ratio of packed cell volume to packed cell and "trapped plasma" volume) determined by the methods described are given in Tables I and II. The mean value of ten determinations of different samples using the direct separation or hematocrit tube method was 81% and the range was 79-86%. There was good agreement among the direct and dilution methods (Table II). It is seen that the "trapped plasma" found in the goat is much greater than that reported for other animals(10-13). A "trapped plasma" correction value of 98% was found by the direct method described here for human blood which agrees with that found by others(10). "Trapped plasma" and corresponding hematocrit values are given in Table III for various centrifuging times. Changes in "trapped plasma" are seen to be related directly with changes in hematocrit.

TABLE III. "Trapped Plasma" Corrections and Hematocrits at Various Times of Centrifugation.

Centrifugation in min.	Uncorrected hematocrit, %	Trapped plasma corrections, %
30	27.8	82
60	25.1	90
90	24.6	90
120	24.2	92

The results of the *in vitro* volume experiments using Cr^{51} and I^{131} are given in Table IV. The differences in total blood volume and true volume in the two cases were 0 and 2%. White cell volumes (mean 1%) are not considered in total blood volume values so the deviations from the true values are augmented by a maximum of 1%, which increases the error in the first experiment and decreases it in the second. It is to be noted that not only accurate volumes but also accurate hematocrits were obtained in these experiments.

A summary of the *in vivo* experiments is given in Tables V and VI. The mean plasma volume was found to be 5.96% of body weight and that of red cell volume was 1.40% in 4 goats. The mean total blood volume of 7.37%

TABLE IV. *In Vitro* Volume Determinations Using I^{131} and Cr^{51} 100 cc Volumes.

Trapped plasma, %	Red cell vol, cc	Plasma vol, cc	Total vol, cc	White cell vol,* cc	Red cell vol / Total vol, %	Corrected hematocrit, %
18	24	76	100	1	24.0	24.0
19	21	77	98	1	21.4	21.2

* White cell vol estimate.

of body weight agrees well with values found previously(2) using Cr^{51} and T-1824 dye, which in 20 animals ranged from 5.68% to 8.94% of body weight. The rate of loss of I^{131} and Cr^{51} from the blood of the animals here is well within the range of values found by the authors in previous studies using these labels separately. The graph in Fig. 1 illustrates the curves used for calculation of volumes *in vivo*.

The results obtained from successive volume determinations in two animals at various times are given in Table VI. Animal A in which an increase in hematocrit occurred showed an increase in red cell volume also, while the plasma volumes were little changed. Animal B shows the reproducibility of results with only minor hematocrit changes.

The method of simultaneous volume determinations described here has obvious limitations. Hematocrit tubes must be accurately calibrated and must permit accurate reading of volumes. The maximum level of suspension or solution which can be used is dependent on the depth of the well of the crystal counter. In a given volume determination it is necessary that all tube levels be nearly the same in order to reduce variation in counting geometry. This becomes more important with red cell levels above the top of the well of the counter. To reduce this problem in animals which have much higher hematocrits it may be necessary to use smaller volumes of blood in hematocrit tubes or to use a counter with a deeper well. The relative amounts of I^{131} and Cr^{51} which are used are also important. Since the largest error may be in obtaining net Cr^{51} counts in packed cells it is desirable to use more detectable Cr^{51} than I^{131}. The amounts used will be determined by the counter used. In cases where large changes in hematocrit occur during the sampling period of a given determination some error may be encountered due to changing counting geometry.

Summary. A method is described for determinations of cell and plasma volumes using Cr^{51} and I^{131} simultaneously in which hematocrit tubes are used for counting and separation of cells and plasma. *In vitro* determinations of volumes of blood were within 1% of actual

TABLE V. Plasma and Cell Volumes in Goats Using I^{131} and Cr^{51} Simultaneously.

Wt, kg	Trapped plasma, %	Corrected hematocrit, %	Red cell vol, % of body wt	Plasma vol, % of body wt	Total blood vol, % of body wt
*28.6	19	24.9	1.36	5.44	6.81
27.3	18	20.0	1.49	6.14	7.63
28.1	14	17.6	1.04	6.42	7.46
30.0	21	22.3	1.73	5.84	7.57
Mean			1.40	5.96	7.37

* Male.

TABLE VI. Successive Volumes Using I^{131} and Cr^{51} Simultaneously in Goats.

Animal	Wt, kg	Date, time	Trapped plasma, %	Corrected hematocrit, %	Red cell vol, cc	Plasma vol, cc	Total vol, cc
A	30.0	3/31 0830	21	22.3	518	1750	2268
A	30.0	3/31 1130	20	26.5	588	1730	2318
B	28.1	4/15 1025	18	18.4	316	1405	1721
B	28.1	4/15 1445	18	19.0	304	1423	1727

volumes. Volumes and distributions found by this method in 4 goats are within the range of values found in the goat by other methods. Limitations of the method are also discussed.

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Effect of Various Methionine Analogues on Bacteriostatic Action of Methionine Sulfoximine.* (21293).

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In recent years 3 groups(1-3) working with agene (NCl_3) treated proteins have isolated and characterized the causative agent of running fits in dogs. This compound, methionine sulfoximine, is of particular interest not only as a convulsant but because it acts as a methionine antagonist. Reiner *et al.*(4) and Gershoff and Elvehjem(5) have prevented the symptoms of methionine sulfoximine toxicity in animals by the administration of adequate quantities of methionine. Gershoff and Elvehjem(6) have also concluded that the anti-methionine activity of methionine sulfoximine is not specific for the enzyme systems directly involved in running fits but is general in nature. Methionine sulfoximine has also been shown to inhibit the growth of *Leuconostoc mesenteroides*(7), and this inhibition has been reversed by either methionine or glutamine (8,9).

The experiments to be reported here are the first of a series planned to investigate the biochemical and physiological mechanisms involved in the action of methionine sulfoximine. These investigations were made to determine the effect of various methionine analogs on the bacteriostatic action of methionine sulfoximine.

Experimental. These studies were carried out in 18 x 150 mm pyrex culture tubes using *Leuconostoc mesenteroides* P-60 as the test organism. All tubes contained 2 ml of modified Henderson-Snell media(10), containing 3.5 γ of DL-methionine per ml which is a suboptimal amount and allows approximately 50% of the maximum acid production. Acid production on this media with and without supplements of methionine sulfoximine and various methionine analogs was determined after incubation at 37°C for 68 hours. All determinations were made in sextuplicate.

Results. The results of these experiments are shown in Table I. These data, which were selected as being representative of a series of similar experiments, indicate that the ability of analogs of methionine to reverse methionine sulfoximine toxicity may or may not be related to their capacity to substitute for methionine in meeting the growth requirements of *Leuconostoc mesenteroides*.

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