THE DETERMINATION OF PLASMA VOLUME IN MAN WITH RADIOACTIVE CHROMIC CHLORIDE[1]

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Radioactive chromium has been found to tag red blood cells and plasma proteins (1). The anionic form, $Na_2Cr^{51}O_4$, labels the red blood cells, while the cationic hexavalent chromic chloride is firmly bound by the plasma proteins and is not taken up by the red cells. The affinity of the erythrocytes for sodium chromate has been used to measure the circulating red cell mass in man (2, 3).

Radioactive chromic chloride is rapidly bound by the plasma proteins both *in vivo* and *in vitro,* and the binding appears to be relatively stable (1). The utilization of radioactive chromic chloride in the determination of plasma volume was suggested by the observation that 98 per cent or more of the chromic chloride is bound immediately by the plasma proteins when injected intravenously into humans. Once the chromic chloride is bound to the plasma proteins, it can leave the circulation only at the slow rate at which the proteins leave it. The dilution of this tracer substance in the circulation after intravenous administration was, therefore, used to measure the plasma volume in man.

The plasma volume determination using radioactive chromic chloride presents a simple, accurate, and rapid technique, by which a stable, non-toxic chemical solution can be injected directly, obviating the necessity of tagging albumin or other proteins *in vitro.* Determinations can safely be performed repeatedly and in rapid succession. Self-absorption corrections are not necessary. Liquid phase gamma ray counting permits the measurement of the plasma volume within a few hours.

The plasma volumes of twenty-six normal adults have been determined by this method. In some of the subjects, a second determination has been done days to weeks later. The method was further tested by measuring the plasma volume before and after transfusion or hemorrhage of a known volume of plasma in hospital patients and volunteer subjects.

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METHODS

I. Preparation of chromic chloride

Radioactive chromic chloride is prepared from $Na_2Cr^{51}O_4$. The preparation of $Na_2Cr^{51}O_4$ from $Cr^{51}O_3$ has been described previously (1).[2]

$Na_2Cr^{51}O_4$, containing 25 to 30 mg. of chromium, is dissolved in 50 ml. of water in a 200 ml. beaker. Glass beads are added. Six ml. of concentrated HCl are added, turning the solution orange. When 6 ml. of 37 per cent formaldehyde are added the solution becomes green. This green solution is evaporated to dryness on a hot plate at 700° C. The solution must be shaken constantly while on the hot plate to prevent bumping. When evaporation is near completion, green crystals[3] form, which become light violet when complete dryness is reached. The beaker is removed from the hot plate as soon as the green crystals begin to turn violet and the crystals then resume their green color. Thirty-five to 40 ml. of water are added to the beaker to dissolve the crystals. The resulting green solution is filtered through No. 3 Whatman filter paper into a 50 ml. volumetric flask. This filtration should be done even though the solution looks clear. The pH of the solution is adjusted to 4.1 by the addition of 0.5 normal NaOH. If the pH is too high, $Cr(OH)_3$ will precipitate out. This can be corrected by the addition of a very small amount of HCl. The green solution in the volumetric flask is brought up to volume with water. It is stable at room temperature for several months.

[2] $Na_2Cr^{51}O_4$ is now obtainable from Abbott Laboratories.

[3] The crystals so obtained are probably a mixture of the hexahydrated forms of chromic chloride $(CrCl_2(H_2O)_4)Cl \cdot 2H_2O$, $(CrCl(H_2O)_5)Cl_2 \cdot H_2O$, and $(Cr(H_2O)_6)Cl_3$. Anhydrous chromic chloride is purple and quite insoluble. From pilot experiments it seems that not all the hexahydrates are equally efficient in tagging plasma proteins. A blue solution containing a preponderance of $(Cr(H_2O)_6)Cl_3$ does not tag as well as a green solution. Work is in progress to determine quantitatively the protein binding capacity of the different forms. For practical purposes, the crude color index seems adequate and the green solutions prepared by the method described have invariably tagged the proteins well.

II. Dosage

Electromagnetically enriched Cr^{50}, irradiated in the Oak Ridge pile for two months, yields Cr^{51} with a specific activity of about 0.6 microcurie per microgram chromium. The chromium obtainable through Abbott Laboratories also has a specific activity of 0.5 to 0.7 microcurie per microgram chromium.

The radiation dosage is calculated by the formula of Marinelli, Quimby, and Hine (4), assuming that 10 per cent of the activity of Cr^{51} is due to gamma rays and that the half-life is 26.5 days (see Appendix A). The injection of the usual dose of 100 microcuries Cr^{51} into an individual weighing 70 Kg. represents a total radiation dose of 0.1 rep. Since the safety limits for human tracer studies ar 0.3 rep. per week, repeated administration is permissible.

The amount of chromium in the usual dose of 100 microcuries varies from 0.1 to 1.0 mg. or 1 to 14 gamma per kilogram of body weight, depending on the specific activity of the material. This is well below the toxic level.

III. Experimental procedure

A. Injecting and sampling procedure.

Approximately 100 microcuries of chromic chloride (0.1 to 1.0 mg. chromium) are suspended in 30 ml. of normal saline. This solution is autoclaved at 250° C. and 18 pounds pressure for ten minutes and then allowed to stand in the autoclave for another ten minutes. More intense heat and more prolonged heating seem to decrease the protein binding ability of the chromic chloride. Twenty-five ml. of the solution are drawn into a calibrated syringe, and the remainder is saved for counting.

A 10 ml. sample of blood is withdrawn from the subject into a heparinized syringe as the control sample. Syringes are changed and the chromic chloride solution is injected through the same needle. After allowing five minutes for mixing within the circulation, four samples of venous blood are drawn from the opposite arm at five minute intervals for twenty minutes. The time intervals are accurately established with a stop watch.

All samples are collected in heparinized syringes. The withdrawals are made from the opposite arm to that used for injection. Hemostasis is avoided as much as possible by releasing the tourniquet as soon as the needle is in the vein. Subjects need not be in the fasting state.

B. Counting procedure.

Radioactive chromium can be counted in the liquid or the dried state. For liquid sample counting two types of counters have been used in this laboratory: a well-type gamma ray counter (Texas Co., type H18-20 TR)[4]; and a liquid sample scintillation counter (W. S. McDonald Co., Inc., counter type 155, detector head type 152-H5). One microcurie of Cr^{51} records approximately 5900 counts per minute on the well-type counter and 65,170 counts per minute on the scintillation counter (Table I).

TABLE I

The counting efficiencies for Cr^{51} of several counters

Counter	Mean efficiency	Mean corrected counts *per minute μc.*	Background *counts per minute*
McDonald scintillation counter	2.96	65,170	450
Texas Company well counter	.3	5,900	390
Robinson flow counter	23.6	519,450	20
End window tracerlab tube	.8	17,120	18
End window amperex tube	.5	11,770	10

When radioactive chromium is to be counted in the dried state, an x-ray end window Geiger-Muller counter or a windowless flow type proportional counter can be used. The two end window tubes employed in this study were the North American Phillips, tube type 62017-Amperex, giving 11,770 counts per microcurie Cr^{51} per minute, and the Tracerlab. Inc., tube TCG 3, giving 17,120 counts per microcurie Cr^{51} per minute. The windowless flow type proportional counter (modification of the counter described by C. V. Robinson [5]) records approximately 519,450 counts per microcurie Cr^{51} per minute.

The plasma samples obtained after the injection of the usual dose of 100 microcuries of Cr^{51} will register approximately 150 to 200 counts per ml. in one minute on the end window Amperex tube. Our routine procedure is to count at least a total of 1300 counts, requiring in this case a counting time of 7 to 9 minutes per sample to keep the probable counting error within 2 per cent. Using the Tracerlab end window counter or the flow counter, the counting time is even shorter because of their higher efficiencies (see Appendix A). On both liquid sample counters, because of their higher backgrounds, a total of 10,000 counts is usually counted, requiring approximately five minutes counting time on the scintillation counter and fifteen minutes on the well counter.

C. Preparation of samples.

All blood samples are centrifuged in regular 15 ml. conical centrifuge tubes at 2700 rpm. at a radius of 14 cm. for one hour, and the plasma is separated. If dried samples are to be counted, one ml. portions of each plasma sample are pipetted into aluminum planchets. The planchets are prepared in duplicate.

The counts contained in the chromic chloride solution, which is injected into the subject, are determined by diluting an aliquot of the saline solution 1 : 50 with distilled water. Five-tenths ml. aliquots of this diluted solution are pipetted into duplicate planchets. Self-absorption corrections can be eliminated by adding one ml. of the subject's control plasma to the 0.5 ml. aliquot. The plasma and the chromic chloride solution are mixed with a clean wire in the planchets.

[4] We should like to acknowledge our indebtedness to the Texas Company for supplying this counter.

All samples are dried over-night in air, or for about four hours in an oven at 60° C. At higher temperatures, chromium may be lost by sublimation. The samples are then stored in a desiccator until they are counted.

For liquid sample counting in the well type gamma ray counter, volumes of 6 to 7 ml. can be counted in ordinary thin-walled 15 ml. conical centrifuge tubes. In the scintillation counter 2 ml. samples are counted in small rimless culture tubes. No absorption corrections are necessary in these counters.

IV. Calculations

In the plasma volume determination with radioactive chromium, as with all other methods for plasma volume now available, the dilution curve is not "flat," and extrapolation to zero time is necessary (6). Mixing of the injected material with the blood is complete within five to ten minutes after injection (7). By obtaining four plasma samples at five minute intervals after the injection of chromic chloride and plotting the counts per ml. of plasma against time on semi-logarithmic paper, a straight line is obtained and the theoretical counts per ml. of plasma at zero time can be extrapolated. With this value, the plasma volume is calculated by the formula:

$$\text{Plasma Volume} = \frac{\text{Total counts injected} - 2 \text{ per cent}}{\text{Counts/ml. plasma at zero time}}$$

The total counts injected are calculated as the product of the number of counts per ml. of the diluted chromic chloride solution, the dilution factor, and the volume of chromic chloride injected (Appendix B). The 2 per cent subtracted from the total counts injected represent the average maximum counts lost to the erythrocytes (Table II).

Whenever the plasma volume determination is repeated, such as following hemorrhage or transfusion, a second dose of chromic chloride is administered. A significant number of counts may still be present in the plasma at the time of the second injection of chromic chloride.

TABLE II

Loss of radioactive chromic chloride to the red cells

Subject	Counts injected ×10⁶	Counts per ml. RBC's	Counts on RBC's ×10⁴	Per cent counts on RBC's
P. D.	24.8	193	42.4	1.7
J. McV.	22.2	365	80.5	3.6
W. T.	20.6	159	35.0	1.7
R. R.	24.3	379	83.4	3.4
L. G.	25.4	287	63.1	2.5
J. P.	25.8	172	37.8	1.5
J. G.	26.2	177	38.9	1.5
D. G.	21.6	291	64.0	2.9
C. H.	21.8	160	35.2	1.6
R. P.	105.1	449	98.8	0.9
B. B.	91.7	326	71.7	0.8
M. R.	100.1	839	184.6	1.8
P. C.	95.3	342	75.2	0.8
M. P.	38.0	562	123.6	3.2
L. S.	18.7	57	12.5	0.7
			Average	1.9
			Standard Deviation	±0.96
			Standard Error of Mean	0.25

In the calculation of the final plasma volume, these retained counts are subtracted from the counts in the plasma after extrapolation to zero time to obtain the corrected counts (Appendix C).

$$\text{Second Plasma Volume} = \frac{\text{Total counts in 2nd injection} - 2 \text{ per cent}}{\text{Corrected counts/ml. plasma at zero time}}$$

RESULTS

Plasma volume determinations with radioactive chromic chloride were performed on 26 normal adults (Table III). The mean plasma volume for the 21 males was 2894 ± 366 ml. by this method. When calculated on the basis of body weight, the mean plasma volume was 39.3 ± 4.9 ml./Kg. by the radioactive chromium technique, as compared to the value of 43.08 ± 5.9 ml./Kg. with Evans blue dye obtained by Gibson and Evans (8), and the Holden value of 40.6 ± 4.4 ml./Kg. with radioactive iodinated plasma obtained by Storaasli,

TABLE III

Circulating plasma volumes in twenty-one adult males and five females

Subject: Male	Wt. *Kg.*	Ht. *cm.*	Surface area *sq. m.*	Total P.V. *ml.*	Plasma volume *ml./Kg.* of body weight	Plasma volume *ml./Sq. M.* Surface area
P. D.	70	166	1.76	2830	40.4	1610
J. McV.	64	190	1.88	2930	45.8	1560
W. T.	61	170	1.70	2960	48.5	1740
R. M.	80	189	2.05	3180	39.7	1550
D. P.	68	178	1.83	3010	44.3	1640
R. P.	71	174	1.84	2530	35.6	1375
B. B.	88	180	2.07	3030	34.4	1460
M. R.	77	174	1.90	2210	28.7	1165
P. C.	62	178	1.76	2740	44.2	1555
J. McK.	82	186	2.04	3330	40.6	1630
S. P.	66	186	1.86	2750	41.3	1480
J. T.	85	186	2.09	3000	35.3	1425
L. F.	80	186	2.03	3190	39.9	1570
L. G.	68	188	1.90	3010	44.3	1580
M. P.	87	179	2.04	3570	41.0	1750
R. C.	65	166	1.71	2140	32.9	1250
J. P.	77	187	2.00	2680	34.8	1340
F. H.	79	178	1.95	3400	43.0	1740
W. W.	77	180	1.90	2680	34.8	1410
T. D.	66	173	1.78	2470	37.4	1390
D. McD.	83	172	1.96	3130	37.7	1600
			Average	2894	39.3	1515
			Standard Deviation	±366	±4.9	±157
			Standard Error of Mean	80	1.1	34
Female						
E. P.	60	169	1.67	2380	39.6	1420
L. V.	57	168	1.65	1860	32.6	1130
E. C.	54	160	1.55	2190	40.5	1410
S. K.	55	158	1.55	1910	34.7	1230
M. C.	56	165	1.60	2120	37.8	1325
			Average	2092	37.0	1303
			Standard Deviation	±212	±3.3	±123
			Standard Error of Mean	95	1.4	55

TABLE IV

Plasma volume determination by radioactive chromic chloride before and after transfusion or hemorrhage

Patient	Sex	Weight Kg.	Initial P.V. ml.	Transfusion or hemorrhage ml.	Final P.V. Calculated ml.	Final P.V. Measured ml.	Final P.V. Difference ml.	Final P.V. Difference (%)
R. C.	M	65	2144	−239	1905	1930	−25	−1.3
F. H.	M	79	3414	−240	3174	3260	−86	−2.7
M. C.	F	56	2116	+257	2373	2380	− 7	−0.3
W. J. W.	M	77	2676	+507	3183	3200	−17	−0.5
S. P.	F	43	2356	+550	2906	2833	+73	+2.5
H. D.	M	60	2159	+259	2418	2425	− 7	−0.3

Krieger, Friedell, and Holden (9). The values obtained by all three methods agree to better than one standard deviation. The correlation between the total plasma volume and the plasma volume related to body weight and surface area was calculated. The correlation coefficient between the total plasma volume and the plasma volume per square meter of surface area was 0.86, between the plasma volume per kilogram of body weight and that per square meter of surface area was 0.84. Both represent definitely significant correlations. The total plasma volume was somewhat less significantly related to the plasma volume per kilogram of body weight, with a correlation coefficient of 0.54.

In the five women studied, the plasma volume was lower than in the males, with a mean plasma volume of 2092 ± 212 ml. or 37.0 ± 3.3 ml./Kg. The figures of Gibson and Evans for women are 2284 ml. as the mean plasma volume or 41.5 ml./Kg. (8).

The accuracy of the method was verified by a second plasma volume determination immediately after the transfusion or hemorrhage of a known volume of plasma. The volume of plasma transfused or removed varied between 250 and 500 ml. The results of the second plasma volume determinations agreed with the expected values within 3 per cent (Table IV).

When the percentage error is calculated in terms of the actual volume of plasma lost or gained by the subject, the error is of necessity larger, averaging 11.3 per cent. This is inherent in the calculation and depends on the volume of plasma lost or gained and its proportion to the total plasma volume.

When 100 microcuries of radioactive chromic chloride are injected intravenously, only a very small fraction is lost to the erythrocytes. The radioactivity of the red cells was measured on all four blood samples drawn after the injection of chromic chloride in 15 subjects. The number of counts per ml. of washed red cells was measured and the counts in the total circulating red cell mass were calculated. The average loss of counts to the erythrocytes was 1.9 ± 0.9 per cent of the total counts injected (Table II). To correct for this small loss, 2 per cent of the counts were routinely subtracted from the total injected counts.

The amount of chromic chloride bound by the plasma proteins was determined by means of a sulfonated cationic exchange resin, Dowex No. 50,[5] in the sodium cycle, with a 20 to 40 mesh and 8 per cent cross linkage. The materials to be tested were run at a speed of 1 to 2 ml. per minute

[5] National Aluminate Corporation, Chicago 38, Illinois.

TABLE V

The binding of radioactive chromic chloride by plasma proteins

	Radioactive chromic chloride solution in saline	Plasma tagged *in vitro* with radioactive chromic chloride	Plasma tagged *in vivo* with radioactive chromic chloride
Corrected counts before passing through resin	9276	10276	635
Corrected counts after passing through resin	375	10150	633
Counts removed by resin	8901	126	2
Per cent counts removed by resin	95.6%	1.2%	.3%
Per cent of radioactive chromium bound to protein	—	98.8%	99.7%

through resin columns with a length of 10 cm. and a diameter of 1 cm. Under these conditions the resin removed 90 to 100 per cent of free chromic ion, within the range of concentration of chromic chloride used. When plasma, tagged with chromic chloride *in vivo* or *in vitro,* was passed through the resin, 98 to 100 per cent of the chromium was recovered, indicating that practically all of it was bound by the plasma (Table V).

The proteins of several plasma samples which had been tagged with radioactive chromic chloride *in vivo* were separated by fractional extraction (10). All the fractions showed radioactivity indicating that all fractions are tagged by radioactive chromic chloride.

In several normal subjects a second plasma volume determination, weeks to several months after the first one, agreed with the initial determination within 5 per cent.

DISCUSSION

The Cr^{51} method of determining the plasma volume in humans is based on the rapid *in vivo* binding of chromic chloride by the plasma proteins. All methods for the determination of plasma volume now available depend on the binding of the tracer substance to plasma proteins *in vivo* (8) or *in vitro* (9, 11–14), and are, therefore, open to the criticism that the plasma proteins do not constitute a well-defined compartment. However, although proteins do leave the vascular bed, the amount that escapes during the twenty-minute test period is probably too small to influence significantly the measurement of plasma volume (15).

The mean values for plasma volume obtained by the Cr^{51} method agree with those of other investigators, using Evans blue and iodinated plasma albumin, (8, 9, 15, 16) within one standard deviation. This would seem to indicate that all three methods measure the same protein pool.

From a practical standpoint, Cr^{51} has several advantages. Lipemia and hemolysis do not interfere with the determination. *In vitro* tagging of proteins is unnecessary, since 98 per cent or more of an injected dose of chromic chloride is immediately bound by the plasma proteins *in vivo*. The simple chemical solution of chromic chloride is stable for long periods. The low dose of radioactivity (0.1 rep.) permits repeated use of the Cr^{51} method on the same individual, and the amount of chromium (0.1 to 1.0 mg.) used in a single experiment is also sufficiently low to permit repeated administration with complete safety. Results of the determination are available within six to seven hours if a Geiger or proportional flow counter are used, for which the planchets must be dried. When the samples are counted wet in a gamma ray counter, the final results are available within one to two hours. This makes the technique a rapid and useful clinical tool. Furthermore this method makes possible the simultaneous determination of plasma volume and red cell mass with two forms of the same isotope, radioactive chromic chloride and sodium chromate.

SUMMARY

1. Radioactive chromic chloride with a half-life of 26.5 days has been used for the determination of plasma volume in man.

2. A saline solution of radioactive chromic chloride was injected into the circulation where it is immediately bound by the plasma proteins.

3. After a short period to allow for mixing, samples of blood were drawn at five-minute intervals and the radioactivity of the plasma was determined.

4. The circulating plasma volume was calculated by the isotope dilution principle, correcting for the loss of protein-bound radioactive chromic chloride from the circulation by a curve extrapolating the radioactivity to zero time.

5. The accuracy of the method was within 3 per cent as verified by hemorrhage and transfusion experiments with measured volumes of plasma.

6. The circulating plasma volumes of 21 normal adult males were determined:

Mean circulating plasma volume 2894 ml. ± 366
Mean plasma volume per Kg. body weight 39.3 ml. ± 4.9
Mean plasma volume per sq. m. surface area 1515 ml. ± 157

APPENDIX A

Assay of Cr^{51} and Dosage Determination

Cr^{51} is a soft x-ray emitter with a half life of 26.5 days. Recent determinations by A. W. Sunyar (17) have indicated that 10 per cent of the disintegrations are accompanied by gamma rays, in disagreement with a previous figure of 3 per cent given by Bradt and his co-workers (18). W. S. Lyon, at the Oak Ridge National Laboratory, has found 8.3 per cent gamma radiation in Cr^{51} (19).

We have standardized Cr^{51} against a Bureau of Standards radium standard, using a Lauritsen electroscope and a ½ inch aluminum absorber. In the calculations, we used the revised Cr^{51} disintegration scheme with 10 per cent gamma radiation and made due allowance for the difference of the energy of the gamma rays of Cr^{51} and the energies of the several gamma rays of the radium standards. By this assay method we obtained results higher by a factor of 2.55 than by our earlier method (1). The errors involved in the several measurements of the present method, including the fraction of gamma rays per disintegration, indicate that the results are probably not accurate to better than 20 per cent.

We have measured samples that have also been assayed by Abbott Laboratories and agree with them to better than 20 per cent.

Using the Lauritsen electroscope assay, the counting efficiencies for Cr^{51} were determined on several kinds of counters (Table I). These efficiencies can only be considered as qualitative estimates, and in view of the complicated disintegration scheme should not be used to give the gamma ray or x-ray efficiency of any of the counters.

The radiation dosage has been recalculated on the basis of 10 per cent gamma radiation. Using the formula of Marinelli, Quimby, and Hine (4) we have:

$D = D_\beta + D_\gamma$

where D is total dosage and D_β and D_γ are the soft x-ray and gamma contributions, respectively.

$D_\beta = 88\ ETC$ rep.

where E = average energy per disintegration in MeV.; assuming a fluorescence yield of 0.263, this

$= 4.92 \times 10^{-3} \times 0.263$

$= 1.294 \times 10^{-3}$ MeV.

T = half life of the isotope, 26.5 days

C = concentration in microcuries per gram of tissue

$= 3.0\ C$ rep.

$D_\gamma = K_\gamma C\ g\ 10^{-1}$ rep.

where $K_\gamma = 1.44\ t\ I_\gamma\ 10^{-3}$

where t = half life of the isotope, 636 hours and $I_\gamma = 1.8$ r/mc.-hr.

$= 1.65$

g = geometrical area of distribution

$= 314 - 4140\,\mu$

where μ for .320 MeV. = .033

$= 177$

$= 29.2\ C$ rep.

It is assumed that the x-radiation affects the blood stream only, that the gamma rays irradiate the whole body, and that there is no excretion of Cr^{51}.[6] With these conservative assumptions: $D = D_\beta + D_\gamma$

If 100 μc of Cr^{51} are administered to an individual weighing 70 Kg.

$$D_\beta = \frac{3.0 \times 100}{5000} = .06 \text{ rep.}$$

and

$$D_\gamma = \frac{29.2 \times 100}{70 \times 1000} = .04 \text{ rep.},$$

which represents the contribution arising from the gamma rays, neglected in the previous calculations.

$$D = .1 \text{ rep.}$$

Daily dosage = $D\lambda$ rep.

where λ = disintegration constant of Cr^{51} per day, 0.0261

= .003 rep. for 100 μc Cr^{51} in a 70 Kg. individual

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[6] The possibility of accumulation of Cr^{51} in the spleen as a result of red cell destruction at that site has also been considered. Data obtained on a series of dogs show a concentration of no more than 0.05 percent of the injected Cr^{51} dose per gram of spleen during the first three weeks after injection. The spleen in the dog therefore may receive twice the radiation that the blood stream does. However, since the disappearance curve of tagged red cells is steeper in the dog than in man, the human spleen may well receive a lower total dosage. Cr^{51} accumulation in the spleen following the injection of labeled erythrocytes does not appear to be significant in normal subjects; however, it may be considerably greater in patients with rapid red cell destruction from any cause.

APPENDIX B

Protocol and calculations of plasma volume determination

D. P., 21-year old male medical student; Wt., 68 Kg.; Ht., 178 cm.; Surface area, 1.83 sq.m.

1:30 p.m., 10 ml. of blood withdrawn from subject. Injected 32.5 ml. of $Cr^{51}Cl_3$ solution in saline through same needle in right antecubital vein.

Ten ml. samples of blood withdrawn from left antecubital vein at 1:35, 1:40, 1:45, and 1:50 p.m.

Sample	Geiger counts/ml.
Injected sol'n diluted 1:50	300 304
5 min. sample	150 149
10 min. sample	134 138
15 min. sample	128 142
20 min. sample	116 120

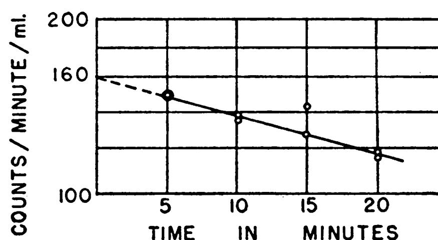

FIG. 1

Calculations: 32.5 ml. $Cr^{51}Cl_3$ solution injected × 302 counts/ml. × dilution of 50 =

	491,000	counts injected
−2%	9,820	counts lost to RBC's
	481,180	counts in plasma

$$\text{Plasma Volume} = \frac{481{,}180}{160 \text{ counts/ml. plasma at zero time}} = 3007 \text{ ml.}$$

APPENDIX C

Sample protocol and calculations of bleeding experiment

F. H., 32-year old normal male; Wt., 79 Kg.; Ht., 178 cm.; Surface area, 1.83 sq.m.

Initial Plasma Volume: 1:40 p.m., 10 ml. of blood withdrawn from subject.
Injected 28.8 ml. $Cr^{51}Cl_3$ solution in saline through same needle in right antecubital vein.
Ten ml. samples of blood withdrawn from left antecubital vein at 1:46, 1:51, 1:56, and 2:02 p.m.

Hemorrhage: 452 ml. blood removed. Hct. 47.0.

Final Plasma Volume: 2:22 p.m., 10 ml. control sample of blood withdrawn from subject.
Injected 28.8 ml. $Cr^{51}Cl_3$ solution in saline through same needle in right antecubital vein.
Ten ml. samples of blood withdrawn from left antecubital vein at 2:28, 2:37, and 2:47 p.m.

Sample	Geiger counts/ml.
Injected sol'n diluted 1:50	960 996
6 min. sample	393 379
11 min. sample	279 273
15 min. sample	224 223
22 min. sample	189 193
Control sample prior to 2nd injection	122 116
6 min. sample after 2nd injection	458 448
15 min. sample	311 317
25 min. sample	252 248

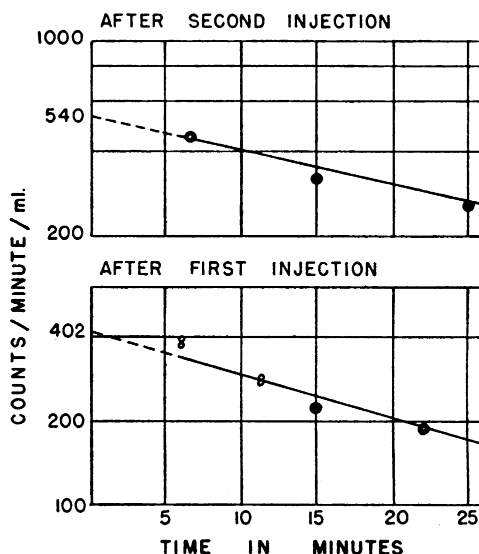

FIG. 2

Calculations: 28.8 ml. $Cr^{51}Cl_3$ solution × 978 counts/ml. × dilution of 50 =

	1,415,000	counts injected
−2%	28,300	lost to RBC's
	1,386,700	counts in plasma

$$\text{Initial Plasma Volume} = \frac{1{,}386{,}700}{402 \text{ counts/ml. plasma at zero time}} = 3449 \text{ ml.}$$

$$\text{Final Plasma Volume after Hemorrhage} = \frac{1{,}386{,}700 \text{ (2nd Injection)}}{540 \text{ counts/ml. plasma extrapolated to zero time} - 119 \text{ (counts remaining/ml. plasma prior to 2nd injection of chromic chloride)}} = 3294 \text{ ml.}$$

Initial Plasma Volume	3,449 ml.
−Hemorrhage	240 ml.
	3,209 ml.

Error = 2.5%

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