TECHNIQUES OF BLOOD VOLUME DETERMINATION EMPLOYING I^{131} AND Cr^{51}

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Several methods are used for the determination of blood volume but none can be regarded accurate and speedy. Recently, radioactive chromium has been used to determine the red blood cell volume by labelling erythrocytes with it, and radioactive iodinated human serum albumin is used for estimation of plasma volume. So far, no report is available in which a simultaneous use of Cr^{51} and I^{131} has been made to estimate the blood volumes in human beings, although the simultaneous use of the above isotopes was made by Klement *et al.* (1954) in blood volume studies in the goat.

The present work was undertaken to estimate the blood volumes (whole blood volume, RBC and plasma volume) by simultaneous use of chromium Cr^{51} and iodine I^{131} to evolve a speedy and fairly accurate method for this purpose. There can be three possible principles on which this simultaneous determination could be based.

(i) *Method of biological separation.*—Advantage is taken of the fact that Cr^{51} (in the form of sodium chromate) does not go into the plasma and I^{131} tagged human serum albumin does not enter the RBC. RBC are therefore labelled with radioactive chromium in the form of sodium chromate and injected. The volume of the cells of the host is then determined by dilution technique. Radioactive iodinated human serum albumin is similarly used to estimate the plasma volume.

(ii) *Method based on beta and gamma counts.*—In this method, advantage is taken of the physical property that radiochromium is a pure gamma emitter whereas radioiodine emits betas as well. The ratio of the number of gammas to number of betas from iodine alone is determined earlier. From the beta activity of the mixture the gamma counts of the iodine component of the mixture is calculated. Subtracting this out of the total number of

gammas in the mixture, the activity of chromium in the mixture, may be known thus,

Sample	Gamma/min	Beta/min	Ratio
I^{131}	A	B	A/B
I^{131} & Cr^{51}	C	D	

Number of gammas from I^{131} alone in mixture = Dx A/B = DA/B

Number of gammas from Cr^{51} alone in mixture = C – (DA/B).

(iii) *Single channel gamma ray spectrometer.*—With this apparatus it is possible to count gamma rays within a narrow band of energy, that is, characteristic of radiochromium or of radioiodine.

Emissions of I^{131} are counted in the presence of Cr^{51} by means of selecting a base line voltage (BLV) that is sufficiently high to reject all the pulses produced by the gamma rays of radiochromium. Under the conditions with the instrument that was used, this occurred at a BLV of 62.5 with a window width of 5 volt. To count Cr^{51} in the presence of I^{131} it is necessary to use a BLV and a window width that correspond to gamma rays from Cr^{51} alone. This may be accomplished by selecting a BLV of 32 5 and a window width of 1 volt. But as one gamma energy (0.364 MEV) of I^{131} is very close to gamma energy (0.32 MEV) of Cr^{51}, some of gamma rays of I^{131} will be present at the point at which Cr^{51} is counted. The activity of I^{131} present at the photoelectric peak for Cr^{51} may he corrected by means of subtracting an experimentally determined factor that relates the counts resulting from radioiodine at BLV 32.5 to the counts from this isotope at BLV 62.5. For example I^{131} alone and a mixture are counted as follows:

Sample	Corrected counts/min at BLV 32.5 & 1 volt window width	Corrected eounts/min at BLV 62.5 & 5 volt window width	Ratio
I^{131}	A	B	A/B
I^{131} & Cr^{51}	C	D	

Number of gamma counts from I^{131} alone in the mixture = DA/B

Number of gamma counts from Cr^{51} alone in the mixture = C – DA/B

All three methods have been followed for simultaneous study of I^{131} and Cr^{51}.

METHODS

Radioactive chromium labelled sodium chromate and I^{131} tagged human serum albumin were obtained fron Abbott Laboratories as sterile isotonic

nonpyrogenic solution. One hundred and twenty five microcuries of Cr^{51} were used as sodium chromate for estimating the red cell mass and 30 microcuries of I^{131} human serum albumin for plasma volume.

The scintillation counter, which was used for counting gamma photons, was a well type counter with thulium activated sodium iodide crystal. Volumes of 2 ml or less were ordinarily counted, thus obviating the need of correcting that for absorption (geometry).

The Geiger tube, used for beta counting, was window type coupled with an automatic sample changer and a scaler. Planchets of 1 ml sample or less were counted.

Single channel pulse height analyser, connected to well type detector and a scaler, was used to count samples containing radioactive iodine and radioactive chromium at different settings.

Preparation of Cr^{51} labelled RBC.—About 35 ml of blood was taken from the patient in ACD (anticoagulant) bottle. It was labelled with 150 microcuries of Cr^{51} (in chemical form of chromate) and then incubated at 37°C for half an hour after mixing it very gently. The blood was centrifuged at 20,000 r. p. m. for ten minutes, taken out, and plasma was removed with a sterilised syringe. The volume was restored with isotonic sodium chloride solution. It was centrifuged twice as before and after the last washing, the volume was made up to 35 ml.

Preparation of I^{131} labelled human serum albumin (RISA) and the standard.—A 100 ml of saline bottle was taken and labelled with 50 microcuries of I^{131} (RISA). It was gently shaken for twenty times and an aliquot of about 10 ml was taken in a beaker. Five ml out of this was pipetted and put into a volumetric flask of 50 ml and 45 ml of saline solution was added to make it up to 50 ml. This was standard No. 1 for I^{131} (RISA).

From the volumetric flask, 1 ml was pipetted into each of the plastic tubes for scintillation counter.

Two ml was pipetted in a 5 ml vial for the use in a pulse height analyser.

Two ml of whole blood from the patient was taken in a flask and 2 ml of water added to haemolyse it. One ml of RISA from standard No. 1 was added into it to make the total to 5 ml. It was well mixed. One ml of I^{131} labelled haemolysed blood from this 5 ml flask was pipetted twice and put into two planchets. Then 1 ml of dextrose solution was added into the planchet and a lens paper was put over all and then the planchets were put

on the planchets rotor under an infra red lamp to dry up. The planchets were made for beta counting in an endless window counter.

The bottle of saline labelled with RISA was weighed. Let its weight be W_1 g.

Technique.—Chromium labelled packed cells and RISA were taken to the bed side of the patient. First normal saline was given to the patient to find out that the flow is normal and then chromium packed red cells were taken from ACD bottle into a syringe. The syringe was weighed and the solution was injected into the tubing, fitted for saline flow. The syringe was weighed again after administering radioactive chromium to the patient. There was some solution left into the bottle to find the specific gravity and to make the standard.

After 15 min of the injection, a blood sample of 10 ml from the other arm was taken into a heparinised tube and marked as *blood sample No. 1.*

Immediately after this, the saline bottle was replaced with RISA labelled saline bottle and it was taken off when a small amount was left there to make the standard, etc.

After 20 min of iodine administration, another blood sample of about 20 ml was taken into another heparinised bottle and marked as *blood sample No. 2.*

The bottle with RISA was weighed. Let its weight be now W_2 and so the volume of RISA administered will be equal to $W_1 - W_2$ as the density was taken as 1.

Let the weight of the syringe filled with chromium labelled packed cells be W_3 and W_4 before and after injection. The volume of packed cells injected will be equal to $W_3 - W_4/d$ where d is the density of packed cells. To find d, a 5 ml vial was taken and weighed. Let its weight be W_5. Then it was filled with 5 ml of packed cells suspended into saline and weighed again. Let its weight be W_6 and so the density will be as such :

$$d = (W_6 - W_5)/5$$

and hence the volume of packed cells injected $= (W_3 - W_4)/\frac{(W_6 - W_5)}{5}$

An aliquot of 10 ml of RISA was again taken from the solution left and standard was made and the same procedure was followed as in preparation

of I^{131} labelled RISA and the standard. The standard was marked as standard No. 2.

From *blood sample No. 1*, 1 ml of the whole blood was pipetted into two plastic tubes and 2 ml was pipetted into a 5 ml vial for pulse height analyser. One ml of the whole blood was taken to find the haematocrit. Rest was centrifuged at 20,000 r. p. m. for 10 min and 1 ml of plasma was taken into two plastic tubes to find out whether there was any chromium activity in the plasma or not. It was found that there was no activity.

Blood sample No. 2 was taken. Out of it 1 ml was taken into two plastic tubes and 2 ml was pipetted into a 5 ml vial. One ml was taken to find out the haematocrit.

Two ml was taken into a flask with 2 ml of plain water to haemolyse it. One ml out of this was taken into each of two planchets with 1 ml of dextrose and a lens paper in each planchet. The planchets were put into rotor under an infra red lamp to dry up.

Rest of the blood was taken and centrifuged. One ml of plama was pipetted into each of two plastic tubes, 2 ml was pipetted into a 5 ml vial.

RESULTS

Blood volume determination was done in two patients by all the three methods.

In case No. 1 about 35 microcuries of chromium labelled red blood cells were injected intravenously to a women aged 30 years. The sample of blood was collected after 15 min from another arm and just after this 12 microcuries of I^{131} tagged human serum albumin was given through the saline tubing in the intra-cubital vein of the patient. The results are given in Table I.

In case No. 2 about 125 microcuries of chromium labelled red blood cells and 30 microcuries of RISA were given to a women aged 35 years in the same way as in case No. 1. Samples of blood were also collected similarly. The results are given in Table I.

TABLE I

Blood volume determinations by three methods using radio isotopes

Methods	Case	Iodine		Chromium
		Plasma vol. ml	Whole blood vol. ml	Whole blood vol. ml
Biological Separation	1	1770	...	3060
	2	2770	...	3480
Counting of betas and gammas from whole blood sample	1	...	2910	2320
	2	...	4280	3212
Gamma ray spectrometry using pulse height analyser	1	..	2440	3075
	2	...	4180	3840

Case No. 1 Corrected haematocrit, 34%; Case No. 2 Corrected haematocrit 35.5%

DISCUSSION

It is evident that employing the method of counting betas and gammas on the whole blood sample, determination of blood volume was more with the use of iodine alone (2910 ml) than when only chromium was employed (2320 ml). The difference in the results was not due to planchets being made on the second day of study. In the second case, when the planchets were made on the same day, the pattern of result still remained the same. This indicates that the actual space of iodine was more than the plasma space. It might be said that in case of iodine some of the activities go to extracellular fluid besides going to plasma.

While employing the method of biological separation, the blood volume was higher by the chromium method (3060 ml) than by the iodine method. The high blood volume by the chromium method was due to high RBC volume. The reason of high RBC volume could be that all the red cells were not drained from the pipette and the sample counted lower than what it actually was. As the defect was overcome in the second case by pipetting whole blood instead of red blood cells, the results of Table I (case 2), showed that the blood volume was more by iodine method than by the chromium method (3480 ml), in contrast to the results of Table I (case 1). The same conclusion that the iodine space is more than the plasma space could again be drawn in this case.

The result of case No. 2 when compared showed that by the method of biological separation the blood volume using iodine and chromium was 4290 ml and 3480 ml respectively with mean average of 3885 ml, showing a variation of ± 11 per cent. By the method of counting betas and gammas the blood volume using iodine and chromium was 4280 ml and 3212 ml respectively with mean average of 3746 ml, showing a variation of ± 14 per cent. By the method of gamma ray spectrometry the blood volume using iodine and chromium was 4180 ml and 3840 ml respectively with mean average of 4010 ml, showing a variation of ± 4 per cent only which was least of all the three methods. It may be said that the method of gamma ray spectrometry was most accurate of all the three methods employed and as it was least time consuming. It may be used as a routine method in the determination of blood volumes with a double label.

SUMMARY

By the simultaneous use of Cr^{51} and I^{151} a comparative study was undertaken for the determination of blood volume by three different methods, viz., biological separation, counting of beta and gamma from the whole blood, and gamma ray spectrometry using pulse height analyser.

It was observed that the simultaneous use of Cr^{51} and I^{131} provided a means for determination of plasma, RBC and whole blood volumes at the same time.

The method of gamma ray spectrometry was more accurate than the other two methods. It was the least time consuming and may be used as a routine method for the determination of blood volumes.

Iodine space was more than the plasma space (i. e. iodine went into extracellular fluids besides going to plasma).

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REFERENCE

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