SIMULTANEOUS DETERMINATION OF EXTRACELLULAR VOLUME AND BLOOD VOLUME WITH THE VOLEMETRON

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SUMMARY

A new instrument, the "Volemetron"***, constructed to measure blood volume with radioactive isotopes, was adapted to determine ^{82}Br distribution volume. Details of the technique are given. Mean values of both RISA and ^{82}Br distribution volume in normal men and women were determined. They were in accordance with those given by other authors, and showed a better correlation with body surface than with body weight. Both determinations can be completed together within $2\frac{1}{2}$ h. The usefulness of a relatively simple combined determination is illustrated, and the desirability of a rapid and repeatable technique is stressed.

Many investigations have been conducted in the past years to evaluate the blood volume and extracellular volume in man. Despite the obvious value of quantitative information about the body fluid compartments in pathological conditions, the techniques elaborated, though reasonably accurate, have found as yet little application in clinical practice. Methods applying the principle of dilution of radio-isotopes have greatly simplified these problems, but still require some time for counting and calculation. The introduction of a semi-automatic counting and computing device, the "Volemetron", based on the "classic" dilution principles made it possible to determine blood volume within 15 min using ^{131}I-tagged serum albumin (RISA). We considered the need for a rapid and rapidly repeatable method for determination of ECV of equal importance. We have found it possible to use the same apparatus with some modification for the determination of the distribution volume of ^{82}Br.

TECHNIQUE

Determination of the blood volume

We used the standard technique, using ^{131}I-tagged albumin. This procedure has been amply discussed elsewhere[3]. The calibration of the apparatus is such that when ^{131}I is used, the dilution volume can be read directly from the scale in litres.

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*** "The Volemetron" manufactured by Ames Atomium Inc., Billerica, Mass., U.S.A. Ames Atomium (Holland), N.V. Burg. Patijnlaan 65, The Hague, The Netherlands.

Determination of the ^{82}Br volume

When distribution volumes of other radioactive substances are to be determined in the same way, two difficulties have to be overcome. First, the apparatus has not been calibrated for substances other than ^{131}I and ^{51}Cr. Second, with greater volumes, the scale is not sufficient. We solved these problems in the following way.

A lead screen was introduced into the hole where the dose is measured in order to increase the dose that can be counted within the "memory". Because of the very hard quality of the γ rays emitted by ^{82}Br, this measure merely reduced the counts by 30%. The slight increase of the injected dose thus made possible would still give such weak activity in the serum after equilibration that the method would be less sensitive and more time-consuming. We therefore, in addition, applied a stronger dose to fill the memory, and thus caused the arrow on the scale to stop long before the "pre-set time" was over.

The activity of the dose was then calculated from the time needed to fill the memory, as indicated by the fraction of the scale covered by the arrow. An arbitrary amount of counts was "fed" into the instrument later. To know this amount, one requires a visible counter, which was provided by the company "Atomium". The activity of the samples after dilution was then measured in the usual way. The procedure is as follows:

(1) Dose measurement is done using the lead screen. With an activity of $\pm 6\mu C$ in 2 ml, the memory is filled and counting stops when the arrow indicates $\pm \frac{1}{3}$ of the scale. As the capacity of the memory is known to be 960, the amount of counts that would be counted in the standard pre-set time (where the arrow stops at "10 l") is

$$\frac{10}{\text{scale reading in litre}} \times 960 = N \qquad (1)$$

(2) The dose is then injected, and the empty syringe is measured in the same way after the instrument has been re-set. The amount of counts is read and noted: (B) (residue). The memory of the machine is then "fed" with some radioactive sample—for instance from the sample kit—until its near maximum, because further steps are blocked when the capacity has been exceeded. The sample has to be removed quickly just before maximum capacity is reached. With some practice, it is possible to fill the memory repeatedly to exactly the same number by changing the switch from "measure" to "subtract" and vice versa. The counts are noted: (A). After a suitable equilibration time—usually $1\frac{1}{2}$ h—the "post-mix" sample is taken, and measured together with the "pre-mix" sample in the usual way. The volume is noted: (M). If the dilution is great, there are still counts left in the memory after the maximum volume (10 l) has been reached, this number is again noted: (E).

For the calculation of the apparent distribution volume of ^{82}Br (V Br) the empirically determined correction factor C must be known, as well as the decay factor G,—which is easily read from a graph when the time interval between dose measurement and volume computation is known. The formula is

$$V\ \text{Br} = \frac{N - B}{A - (E)} \times M \times C \times G \qquad (2)$$

RESULTS

(1) *Blood volume in normals*

The blood volumes in normal men and women of various size and age are shown in Table I. The values given are those indicated by the apparatus corrected by a factor

TABLE I

BLOOD VOLUME IN NORMAL PERSONS

♂	Age	Blood vol. ml/kg	Blood vol. l/m²	♀	Age	Blood vol. ml/kg	Blood vol. l/m²
1	37	88	3.13	1	50	65	2.42
2	46	79	2.50	2	58	66	2.39
3	64	71	2.87	3	37	77	2.68
4	66	53	2.26	4	47	56	2.37
5	55	70	2.35	5	37	77	2.64
6	24	63	2.47	6	43	62	2.44
7	25	62	2.48	7	61	49	1.87
8	33	74	2.45	8	29	57	2.20
9	24	63	2.67	9	28	63	2.48
10	48	66	2.56	10	62	44	1.85
Mean		69	2.57			61	2.33
S.D.		8(11.5%)	26(10%)			12(19.5%)	28(12%)

to compensate for the too high erythrocyte volume in the sample with resulting too low relative plasma content. This factor was calculated assuming a total body haematocrit (Ht) of 0.91 time the venous Ht[1]. In two subjects, determinations were repeated after seven days interval. The second determination showed differences of 2 and 3% from the first one. In one patient with congestive heart failure, blood volume was determined before and immediately after withdrawal of 0.45 l blood. A difference of 0.38 l was found.

(2) *Bromide distribution in vitro*

The factor C was determined by mixing the ^{82}Br with a known volume of water, and found to be 0.84. The constancy of this figure, that has since been determined regularly at 2-weekly intervals, proved the reliability of this method.

(3) *Bromide distribution volume in normal subjects*

$1\frac{1}{2}$ h after injection of the $[^{82}Br]NH_4$, blood was drawn and centrifuged, the serum sample being used for the measurement. The last step was done twice and the mean of the two readings taken. The results are given in Table II.

Determinations were repeated in two subjects, $1\frac{1}{2}$, 3 and 20 h after injection. With the longer periods, activity in the urine collected during this period was measured and appropriate correction was made. The same procedure was followed in three oedematous subjects. In one of them, activity in the ascites fluid was compared with that of the plasma. The results are given in Table III.

(4) *Simultaneous determination of the RISA and ^{82}Br distribution volumes and calculations of the extravascular volume*

In eight of our subjects, blood volume was determined after measurement of the ^{82}Br distribution volume, the post-mix sample of ^{82}Br determination being used

TABLE II

BROMIDE VOLUME IN NORMAL PERSONS

♂	*Age*	*Br vol. ml/kg*	*Br vol. l/m²*	♀	*Age*	*Br vol. ml/kg*	*Br vol. l/m²*
1	47	253	10.68	1	20	290	9.62
2	41	340	12.89	2	43	222	9.13
3	72	290	11.37	3	54	224	9.39
4	55	309	10.41	4	41	288	9.53
5	24	306	11.18	5	37	238	8.86
6	21	293	10.49	6	46	235	9.48
7	32	305	10.89	7	44	236	9.61
8	47	282	11.15	8	47	228	9.02
9	24	263	11.16	9	61	216	8.32
10	48	254	9.24	10	29	283	10.89
Mean		290	10.95			246	9.39
S.D.		27(9.4%)	0.7(6.4%)			29(11.8%)	0.42(4.6%)

TABLE III

^{82}Br DISTRIBUTION VOLUME

	Time after injection			
	1½ h	*3 h*	*6 h*	*20 h*
m ♂ normal	20.6	21.9		21.6*
f ♀ normal	16.6	17		17.7*
m ♂ with oedema	31.6	36.3	37.2	
f ♀ with oedema			28.4	29.8*
f ♀ with oedema	33	37.5	39.5	
Activity ratio ascites/serum	0.6	0.9	1.0	

* Correction has been made for ^{82}Br loss with urine.

TABLE IV

SIMULTANEOUS DETERMINATION OF ^{82}Br AND ^{131}I ALBUMIN SPACE

♂	*Age*	*Blood vol. (corr.)*	*^{82}Br vol.*	*Extravasc. vol.*	*Br V / Bl. V*	*E.V.V. / Bl. V.*
	55	4.02	17.8	14.3	4.4	3.6
	24	4.91	20.55	16.35	4.2	3.4
	48	4.63	17.73	14.1	3.9	3.1
	47	6.37	25.6	20	4.1	3.2
Mean					4.1	3.3
Mean ratios calculated from Table I and II					4.2	3.4
♀	47	4.09	16.5	13.3	4.0	3.3
	61	3.20	14.2	11.5	4.4	3.6
	29	3.89	19.3	16.6	5.0	4.3
	37	4.02	17.5	14.2	4.4	3.6
Mean					4.4	3.7
Mean calculated from Table I and II					4.0	3.2
Pat. P.	21					
With oedema		3.6	29.9	26.8	8.3	7.4
Without oedema		4.2	22.3	18.7	5.3	4.5

as pre-mix sample for the blood volume. As the apparatus automatically corrects for the activity from the ^{82}Br still present, there is only a slight increase in the statistical error, that can be minimised if necessary by more frequent repetition of the last step. The results are given in Table IV.

We also calculated that part of the bromide distribution volume that is localised outside the vascular system, by substracting from the total volume that part of the ^{82}Br that was present in the plasma and red blood cells. This last value we called "extravascular Br volume". The ratios of the "total" and "extravascular" Br volume to the blood volume are given in the last two columns.

Practical application. The value of these ratios can be illustrated by patient P with a nephrotic syndrome, in whom we found a small blood volume and an elevated Br volume, with consequently greatly altered ratios. After therapy, which resulted in 7 kg weight loss, these figures reached nearly normal values, but as can be seen from the last line in Table IV, the ratios were still well outside the normal range.

(5) *Penetration of Br into red blood cells*

It is known that Br (like Cl) penetrates into the red blood cells, where its concentration is about 60% of that in the plasma[2]. We found the activity ratio between red blood cells and plasma in 3 different subjects to be 56, 63 and 61%, respectively.

DISCUSSION

Blood volume determination

The advantages of the Volemetron for blood volume determination, consisting in simplifying and accelerating the procedure and in reducing the sources of error, have been discussed elsewhere[3]. Of particular interest seems to us the automatic safety check, by which direct measurement of the dose in the syringe, makes errors in the dosage virtually impossible. The error of the electronic part of the determination is well below the desirable minimum of a clinical test. Systematic and sometimes variable errors are introduced by the following facts:

(a) Freely diffusible ^{131}I in the preparation. We have found this to be not more than 2%.

(b) Disappearance of RISA out of the blood. This happens at a rate of + 10–20% after 60 min according to different authors[4]. If the sample is taken 10 min after injection, the error is so small that correction by extrapolation of the curve to zero time was shown to yield no better results[4].

(c) Errors in the haematocrit determination.

(d) A systematic error is introduced by any method labelling only one of the two blood constituents (plasma or cells) by the fact that the "over-all cell percentage" is only 91% of that of venous blood[1]. From this fact, methods labelling red cells can be expected to give 11% lower values than those labelling plasma constituents. We have done our determination with whole blood, using a correction for the factor sub (d) only, according to the formula:

$$\text{blood volume} = \frac{100 - \text{Ht}}{100 - 0.91\ \text{Ht}} \times \text{measured volume.}$$

All the errors mentioned, however, are probably less important than the large physiological variations between normal subjects; these variations are attributed to the lack of a suitable parameter[1]. The best parameter, the "lean body mass", is hard to determine. As shown in Table I, variations are somewhat less if the values are related to body surface than to body weight. Our results agree well with those of other authors who found values between 60 and 77 ml per kg for normal women and men[1,5-7]. Like other authors, we found lower values at comparable weight and length for women (Table V).

TABLE V

BROMIDE VOLUME

References	*Method*	*Equilibr. time*	*Mean vol. ml/kg* ♂	*Mean vol. ml/kg* ♀	*Mean vol. l/m²* ♂	*Mean vol. l/m²* ♀	*No. of subjects* ♂	*No. of subjects* ♀
7	10 μC i.v.	14 h	275 S.D. 24	265 S.D. 19	–	–	10	10
8	10–15 μC oral	24 h	265 S.D. 28	260 S.D. 36	–	–	12	9
9	24.4 μC i.v.	1 h	275 S.D. 68		9.03 S.D. 2.2		total	8*
9	24.4 μC i.v.	2½ h	291 S.D. 74		10.75 S.D. 2		total	8*
present investigation	6 μC i.v.	1½ h	290 S.D. 27	246 S.D. 29	10.95 S.D. 0.7	9.39 S.D. 0.42	10	10
BLOOD VOLUME								
1	T1824: 20 ml 0.075% i.v.	15–30–45 min	–	66.5 S.D. 6.8	–	2.34 S.D. 0.251	–	8
	^{32}P 2 μC i.v.	15–30–45 min	–	66.5 S.D. 6.8	–	2.34 S.D. 0.251	–	8
	^{131}I Alb 50 μC i.v.	10 min	78.1 S.D. 10.5		–	–	total	25**
5	^{32}P ½ mC i.v.	15 min	69	64.4	–	–	71	16
6	^{51}Cr 50 μC i.v.	20 min	–	62 S.D. 6	–	2.22 S.D. 16	–	200
7	^{51}Cr 50 μC i.v.	20 min	71 S.D. 12	64 S.D. 4	–	–	10	9
7	T1824 10 ml 0.1% i.v.	5–20–40 min	71 S.D. 12	64 S.D. 4	–	–	10	9
present investigation	^{131}I Alb. 4 μC i.v.	10 min	69 S.D. 8	61 S.D. 12	2.57 S.D. 26	2.33 S.D. 28	10	10

* Sex not indicated.
** The blood volume has been calculated according to the formula:

$$\text{total blood volume} = \frac{\text{Plasma volume}}{1 - \text{corrected haematocrit.}}$$

The bromide space

Several substances have been used to measure "extracellular volume". Though they are supposed not to enter into the cells, they still show different distribution volumes. The reason is, that "extracellular space" still comprises several compartments that are not penetrated at the same rate by different substances. Among the methods used, those employing inuline, mannitol, sucrose, sulfate and thiosulfate give lower values than Br, Cl, Na and thiocyanate[8]. Chloride and bromide penetrate partially into the red blood cells, and are excreted into the digestive juices, whereas CSF contains only 33% of the plasma contents[8].

Several investigators have confirmed that bromide space is identical with chloride space for all practical purposes[9]. It seems profitable for clinical problems to obtain rapid and repeatable information about the chloride space.

Chloride distribution volume may not be identical with extracellular volume, but the exact nature of the latter is debatable. It may be assumed that the volume in which ^{82}Br is distributed after a standard time is closely related to the extracellular fluid volume and that the value obtained, as well as changes in this value in a given patient reflect some clinically useful parameter.

We therefore chose a rather short time of equilibration, $1\frac{1}{2}$ h, which has the advantage of rapidity and usually negligible urinary excretion. Investigations of others[9,10] as well as ourselves (Table III) show that by that time, more than 90% equilibration is reached in subjects without oedema. In case of great excess in extracellular fluid, 3 h equilibration is to be preferred. The values thus obtained, though still somewhat too low, reflect the extent of the abnormality sufficiently well, the error being always in the same direction and proportional to the magnitude of the abnormality present. With longer equilibration time, corrections are needed for loss with urine (and in some cases other excreta) introducing more potential errors. Moreover, the activity of the ^{82}Br decreases rapidly, which not only makes determination less accurate, but also increases the errors due to the presence of radioactive impurities with longer half-life time[2]. We have not corrected the bromide distribution volumes for plasma protein nor for the Donnan equilibrium, as the influence of these factors is not only small but also relatively constant.

As was the case with blood volume values, a better correlation was obtained if the volumes were related to body surface than to body weight. Our values are within the same range as those obtained by others who found mean volumes between 250 and 291 ml/kg using varying methods of administration and equilibration times[7-9] (Table V).

Relation of RISA and ^{82}Br distribution volumes

We have shown that with the present technique, both volumes can be determined within $2\frac{1}{2}$ h. Three venipunctures are required with a total blood loss of 50 ml, using a very small amount of short-lived radioactive material. This enables one to determine two important physiological parameters in acute situations for diagnostic and investigational purposes.

As both are closely related, it seemed of interest to determine the ratio of one to the other. Because the greater part of the blood volume is comprised within the bromide volume, we also calculated what might be called the "extravascular volume" by subtracting from the bromide volume that part of the ^{82}Br that was present in the vascular system. For this calculation we assumed a ^{82}Br content of the red blood cells of 60% of that of the plasma. We also related the fluid volumes outside and inside the vascular system to each other. While the variations of the two volumes are so large that small abnormalities cannot be recognised with certainty, the latter ratio in particular may be clearly abnormal, as illustrated in the second determination in the patient given in Table IV. It may be mentioned further that men and women seem to have approximately the same ratio.

Statistical error

The apparatus is so constructed that in the step "measure dose" each registered count represents 64 divisions, as is the case in the step "compute volume". During "compute volume", however, one in every 16 divisions is registered.

We calculated the error in the determination, on the basis of the statistical variations in the number of counts computed by the apparatus, making the following assumptions: the patient is free from pre-existing radioactivity; the injected dose is 4 μC ^{131}I and 6 μC ^{82}Br. The reading error on the scale was found to be 0.02 l.

Blood volume

The S.D. of the first step "measure dose" was 3.75 on $\pm$ 900 counts and in the step "substract residue" 0.5 on $\pm$ 16 counts. Together, this constitutes an S.D. of 3.8 on 884 impulses or 0.43%.

In the step "compute volume", the "pre-mix" and "post-mix" wells add and subtract from the memory $\pm$ 37 and $\pm$ 921 counts respectively, after which the memory is empty. The S.D. of these two computations is 1.5 and 7.6 respectively. The end result, as read on the scale, had a S.D. of 7.8 on (921 − 37) = 884 counts, or 0.88%. This represents a scale reading of 0.98% or 0.05 l. If all these errors are taken into consideration, the eventual result as read from the scale has a S.D. of 0.054 l or 1.1%.

The ^{82}Br distribution volume

In this procedure, according to the formula (1), the memory is filled completely (960 counts). This step has a S.D. of 4 or 0.42%, which is equivalent to a scale reading of 0.015 l (as the arrow stops at about 3.6 l with a dose of $\pm$ 6 μC). Together with the reading error, this results in a S.D. of 19 for factor N, formula (2). The factor B (residue) is about 20 counts, S.D. 0.5. Factor A is an arbitrary amount of counts (usually $\pm$ 930) and the factors C and G are constants.

During the "compute volume" step, $\pm$37 counts are added to the memory from the "pre-mix" well, so that 967 impulses from the "post-mix" well have to be subtracted from the memory. The S.D. of these two simultaneous procedures is 1.5 and 7.8 counts respectively. Taken together, the S.D. is 7.9 or 0.85% of 967 counts. The eventual scale reading of factor M has a S.D. of 0.07 l (reading error included). This makes the total S.D. for formula(2) 1.14%.

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