SUCCESSIVE DETERMINATION OF BLOOD VOLUME OF NORMAL HUMAN SUBJECTS USING LOW DOSES OF I^{131}-HSA AND P^{32}-RBC

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INTRODUCTION

The determination of blood volume of a group of normal adult subjects, including both men and women, has been carried out using low doses of I^{131}-labelled human serum albumin, about 0.02 μc per kg of body weight. Further, a successive test was carried out using small doses of P^{32}-tagged red blood cells, about 0.01 μc per kg of body weight. These doses are at least 10 times less than the normally used tracer doses for such purpose. Some of the cases were again subjected for the estimation of blood volume by a conventional total dose of 20 μc of I^{131}-HSA and also T-1824 dye. In the present study an anticoincidence gas-flow counter has been utilised for the assay of the radioactivity which has helped to carry out the experiments with the reduced doses of isotopes.

The estimation of the blood volume depends on the dilution analysis where a known amount of a suitable substance is introduced into the blood stream. After allowing a suitable time for complete mixing, generally about 5-10 minutes, samples of blood are drawn out and the concentration of the injected substance is determined and the degree of dilution is calculated. Attempts have been made by laboratory and clinical investigators to utilise foreign proteins, vital dyes, gum accacia, haemoglobin, carbon monoxide, and more recently radioactive isotopes. Basically all the methods are simple which depend on the estimation of the dilution of the injected substance in the blood. However, the difficulty arises in an accurate estimation because of the physiological condition, the nature of the substance used and the errors involved during the procedure. The substance used should not undergo any change during the circulation at least for some time and should not alter the blood volume and must not be toxic. Further, it must not easily pass into the tissue spaces or be excreted rapidly and must not be taken up by the phagocytic cells of the blood. Amongst a number of materials attempted, the Evans blue dye (T-1824) and radioisotopes, specially P^{32}-RBC, I^{131}-HSA and Cr^{51}, are the materials of preference for the determination of blood volume.

Evans blue dye was accepted for the routine use for the estimation of plasma or blood volume (Gregerson, 1944). Hevesy showed that various isotopes may be incorporated into the red cells *in vitro*, and he made studies with P^{32}-phosphate (Hevesy and Zerahn, 1942; Hevesy *et al.*, 1944) and later with K^{42} (Hevesy and Nylin, 1951), and also thorium-B (Hevesy and Nylin, 1952). Further, Cr^{51} was introduced as red-cell tag (Gray and Starling, 1950) and also Rb^{86} (Tudhope and Wilson, 1956). Another way of tagging the RBC is the use of isotopes that can be built into the haemoglobin molecule metabolically, such as Fe^{59} (Gibson *et al.*, 1946), C^{14} (Bale *et al.*, 1949) and stable N^{15} (Shemin and Rittenberg, 1946). But difficulty arises in clinical work in maintaining a pool of donors of different blood groups who would also be subjected to greater radiation exposure. Frank and Gray (1953) used Cr^{51} as chromic chloride in normal saline for the plasma volume measurement, the solution being injected directly and the chromium gets bound by the plasma proteins *in vivo*. Storaasli *et al.* (1950), Crispell *et al.* (1950), Aust *et al.* (1951), Zipf *et al.* (1955), and Freeman (1957) have used radioiodinated human serum albumin for the same purpose, the isotopic label with I^{131} being incorporated in the protein molecules *in vitro* as described by Francis *et al.* (1951). The I^{131}-labelled human serum albumin is now available commercially. For repeated measurements at frequent intervals the protein-bound form of I^{132} has been preferred (Veall *et al.*, 1955).

METERIALS AND METHODS

Scheme :—

Normal adult subjects comprising of men and women were selected between the age group of 25-35 years for the repeated estimation of the blood volume, first with low doses of labelled albumin, then with tagged cells, and some of them were finally tested with conventional doses of labelled albumin and Evans blue dye. The gap between the successive tests was 12-20 days. Most of the cases received doses between 0.8–1.6 μc of I^{131}–HSA and 0.4-0.8 μc of P^{32} as tagged RBC, some of them however received an extra 15-30 μc dose of I^{131}–HSA.

Tagged Materials :—

Several consignments of I^{131}-labelled human serum albumin were obtained from Duphar Isotope Laboratory, Philips Rexane, Amsterdam. Each consignment was stored in refrigerator and used for about a week. It was diluted with normal physiological saline under aseptic conditions to have strengths 40 μc per ml and 2 μc per ml initially.

P^{32} in the form of Na_2HPO_2 was obtained from the same source and used for auto red cell tagging of the subject under investigation. The method

adopted for the *in vitro* tagging of the cells was similar to that of Reeve and Veall (1949). About 5 ml freshly drawn heparinized blood was incubated at 37°C for about an hour with 0.1 – 0.2 ml of the stock solution containing about 5 μc of the radioactive phosphate. After washing thrice with ice cold isotonic saline, the cells were suspended in saline approximately to the original volume of the blood. Uptake of P^{32} by red cells was found to be within 23-32 percent. Later an improved method of Mollison *et al.* (1958) was used for the labelling of the cells. The plasma was removed and the cells were suspended in citrate solution and then the isotope was added, about half of the quantity previously used, and incubated for 15 minutes only. By this method about 90 percent of P^{32} was taken up by the red cells. It can, however, be mentioned here that instead of the auto cells 0-group Rh-negative blood may also be used to facilitate investigation of more cases in short period.

Procedure :—

An initial blood sample of about 2 ml was collected from one of the antecubital veins, and keeping the needle in position a measured volume of the isotopic material was injected by a calibrated syringe through the same needle and flushed twice by withdrawing blood in the syringe.

For 50 percent cases three post-injection blood samples were collected within 30 minutes, one being at the 10th minute. They were used to evaluate the Zero-time correction factor. For the rest of the cases only one sample of blood was collected after 10 minutes.

A standard solution was prepared with the isotopic material using the same volume as used for the injection. It was diluted to 1000 ml in isotonic saline in standard measuring flask. For I^{131}-HSA, 1 ml of non-radioactive plasma and 5 ml of 1 percent NaI solution were added as carriers while preparing the dilute solution and this could be used for 2-3 days so long the same batch and same quantity was used for the injection. But in case of P^{32}-tagged cells, the standard solution was prepared each time corresponding to the each individual and saponin was used for haemolyzing the cells while preparing the dilute solution.

The initial blood samples collected were first used for obtaining the haematocrit values. The observed values were corrected by multiplying with 0.95. The separated plasma or RBC was used subsequently for the reference samples and the background samples.

During the simultaneous study with the conventional doses of I^{131}-HSA and T-1824 dye, one ml of the stronger solution of the I^{131}-HSA and five ml of the dye were injected consecutively through the same needle after taking the initial blood sample of about six ml. The Evans blue dye used was a

0.7 percent sterile aqueous solution. Five ml of blood specimens were collected for the radioactive assay and the estimation of the colour density. The Standard dilution of the same dye dose was made to 2 litres in isotonic saline. A blank sample was prepared by mixing 1 ml of the initial plasma with 4 ml of isotonic saline, the reference sample was prepared by mixing 1 ml of the initial plasma with 1 ml of the diluted dye solution and 3 ml isotonic saline, and the specimen samples were made with 1 ml of the post-injected plasma sample and 4 ml isotonic saline. The optical density of all the samples were read by a photoelectric colorimeter.

Radioactive assay:—

Samples were prepared with 0.2 ml of plasma or packed cells for the determination of the plasma or the cell volume corresponding to the use of I^{131}-HSA and P^{32}-RBC. Reference samples were prepared using 0.1 ml of the corresponding standard solution and 0 2 ml non-radioactive plasma or cell to keep the self-absorption factor same as those of the specimen samples. Further, samples were also prepared with 0.2 ml plain plasma or RBC, initially drawn, for the accurate background measurement. These samples were prepared on 22 mm round microscope cover slips; round paper strips of 25 mm diameter were pasted at the back of the cover slips to facilitate easy handling. In radioiodine samples a drop of 1 percent NaI solution was added in the planchets to suppress any loss of radioactivity during drying. In cell samples saponin was used for haemolyzing purpose and also 2-3 drops of 10 percent NaOH solution were added on the planchets to obtain uniformly thick samples; and after drying they were stored in a desiccator, otherwise absorbed moisture would give spurious counts in the gas-flow counters. Care was taken during drying the samples to avoid over heating and cracking in samples. Samples were dried under a heat lamp or a hot air oven, temperature being between 40-50°C.

The background sample, the reference sample and the specimen samples were counted in the anticoincidence gas-flow counter (Hosain, 1958 & 1960a). During the work with low doses of the isotopes 20-40 minutes were devoted for counting each sample to keep the standard error about or less than 3 percent. While using the conventional doses of I^{131}-HSA only 10 minute counting was sufficient to have standard error in counting less than 1 percent, but when the same sample was counted under thin walled end window beta counter 30 minute time was necessary for the same accuracy but 10 minute was again sufficient if 3-5 ml plasma samples were used for counting in well-type scintillation counter.

Precautions:—

The blood was withdrawn without stasis specially for the samples to be used for the haematocrit determinations.

The syringe used for the injection was never used for the collection of blood samples, and the post-injected blood samples were not drawn from the injected hand to avoid any contamination.

For taking out the measured volume of the isotopic material for standard solution it was preferred to use the same syringe used for injection after washing and drying.

While working with T-1824 dye, the case was advised for fasting and care was taken to avoid any haemolysis in the blood samples. Special centrifuge tubes, 6 cm long and 1 cm wide at the open end and 0.5 cm at the tapering end, were used for separating packed cells which would be used for the sample preparation. After removing the plasma it was washed once with ice cold normal saline by centrifuging at 3000 rpm for half an hour. After removing the saline it was kept for a few minutes and finally thin blotting paper strips were dipped to remove the saline from the surface of the packed cells.

Whenever there was gap between the collection of the sample and the final counting, the count rate was corrected for the hourly decay.

Zero-time correction:—

The concentration of the radioisotope at zero-time in the blood circulation was estimated by taking blood at different intervals, mostly at 10, 20 and 30 minutes, and graphically extrapolating the measured activities to zero time. Then the ratio of the zero time to 10 minute activities was obtained. The mean value of these ratios found to be 1.022 for I^{131}-HSA and that for P^{32}-RBC was 1.014. So for the rest of the cases these factors were utilised for correcting the 10 minute activities.

Calculation:—

$$V = \frac{N_r \cdot D}{N_o}$$

Where, V = Volume to be determined (Plasma or Cell in ml),
D = Standard dilution of the dose (here 1000),
N_r = Net counts per minute per ml of the reference sample,
N_o = Net counts per minute per ml of the specimen sample at zero time,

Then,

$$V_b = \frac{100}{100 - H_c} \times V_p \quad \text{or} \quad \frac{100}{H_c} \times V_c$$

Where V_b, V_p V_c and H_c are the blood, plasma, cell volumes and the haematocrit respectively.

RESULTS

A total number of 30 cases of age between 25 to 35 years were investigated for the repeated estimation of the blood volume of which case numbers 55 to 62 were exempted for the use of conventional dose of I^{131}-HSA and T-1824 dye. The body weight and the corrected haematocrit for the 16 men ranged between 46.4 to 65.9 kg and 38.0 to 45.6% respectively and those for the 14 women were 38.6 to 53.2 kg and 32.3 to 41.8%. The body weight and the haematocrit were same at the times of successive test except slight variations in few cases. The plasma volume and subsequently the blood volume obtained by the low dose I^{131}-HSA ranged between 36.6 to 50.6 and 60.8 to 88.6 ml/kg respectively for men and 37.4 to 50.7 & 60.8 to 80.8 ml/kg for women, and those by the low dose P^{32}-RBC were 24.5 to 34.7 & 65.0 to 81.1 ml/kg for men and 19.6 to 31.3 & 58.4 to 74.9 ml/kg for women. The blood volume as the mean of the two methods ranged between 63.5 to 84 9 and 59.1 to 77.9 ml/kg for men and women respectively. The grand mean value was 70.4 ml/kg.

The values of blood volume obtained by P^{32}-RBC method were in general lower than those obtained by I^{131}-HSA method. Further, women subjects showed comparatively lower values of blood volume per killogramme of body weight than the male subjects which were mainly due to comparatively low values of circulating red cell volume in the female subjects. The values of blood volume by the conventional dose of I^{131}-HSA were in good agreement (within ± 2 to 5%) with the previously obtained value by the low dose I^{131}-HSA method, but the values obtained by the dye method were comparatively higher (mostly 6 to 12% higher) than those obtained by the isotopic techniques.

Table 1 showes the values of the plasma and the cell volumes and the corresponding blood volumes as obtained by low dose I^{131}-HSA and P^{32}-RBC technique, the blood volume as ml/kg has been represented as the mean of the two values. The results, however, show a considerable variation in the values of blood volume of normal adult subjects when represented as ml/kg. A rough average value may be taken as 70 ml per kg of the body weight.

TABLE I

Successive estimation of blood volume of normal human subjects using low doses of I^{131}–HSA and P^{23}–RBC

Case	By low dose I^{131}–HSA		By low dose P^{32}–RBC		Mean
No. (BV)	Plasma Volume ml	Blood Volume ml	Cell Volume ml	Blood Volume ml	Blood Volume ml/kg.
Men :—					
1	1918	3324	1328	3141	69.7
2	1743	2900	1260	3159	63.5
3	2172	3797	1536	3589	76.0
4	2095	3570	1392	3370	70·6
5	2377	3896	1355	3474	73.7
6	2633	4455	1742	4259	82.7
7	2064	3794	1713	3757	69.3
8	2583	4671	1890	4324	80.8
9	2425	4070	1776	4395	73.4
10	2944	5146	2018	4718	84.9
11	2900	5151	1988	4550	79.2
12	2566	4140	1628	4282	63.9
55	2230	3927	1594	3691	74.1
56	2042	3321	1379	3583	66.0
57	2282	3923	1604	3837	71.2
58	2350	4137	1681	3891	68.5
Mean ± S. D.					72.8±6.2
Women :—					
13	1649	2545	812	2308	62.9
14	1595	2616	1022	2655	62.7
15	1922	2943	927	2672	64.4
16	2144	3355	1200	3235	74.8
17	1839	3084	1114	2758	63.7
18	1951	2881	919	2845	61.2
19	2225	3823	1482	3545	77.9
20	1907	2898	1012	3003	59.1
21	1938	3152	1164	3024	59.6
22	2441	3850	1387	3789	71.8
59	1899	2972	1017	2818	67.8
60	2144	3568	1309	3280	73.8
61	2464	3916	1309	3528	76.6
62	2232	3629	1497	3890	69.5
Mean ± S. D.					67.6±6.3

DISCUSSION

The present investigation is confined to a limited number of representative cases which shows that the anticoincidence windowless gas-flow counter (Hosain, 1958) can be used for the estimation of blood volume using 0.8–1.6 μc of I^{131}–HSA and 0.4-0.8 μc of P^{32}–RBC utilising only small quantity of blood for radioactive assay in place of the conventional use of 5-50 μc of I^{131}–HSA, 5-10 μc of P^{32}–RBC and about 5 ml blood samples. The reduction in dose specially helps to investigate babies and children, and expectant and nursing mothers (Hosain, 1960 b). The background count of the counter ranged between 6-12 cpm depending on the type of the 22 mm microscope cover slips used for the sample preparation (Hosain, 1959) and due to the effect of the residual radioactivity of an earlier test. The net count rates of the reference samples and the specimen samples ranged between 8-30 times the background count rate. A counting time of 10-40 minutes, depending on the activity of the sample, was used to keep the statistical error in counting less than 3 percent so that an overall error of about 3 percent can be expected in any result.

The dye method using the T-1824 is still commonly used but this is likely to give higher percentage of error and there is objection of multiple doses. The radioisotope methods are more accurate and repeated estimations are possible. Further, simultaneously the plasma and the cell volumes can be determined independently (Berson and Yallow, 1952; Gray and Frank, 1953). The only objection that may arise in the administration of repeated doses is due to the possibility of a significant amount of body irradiation. However, in blood volume measurements the use of radioisotopes is not open to serious criticism on the grounds of radiation hazard. It is, however, always preferable to use the minimum possible quantities of r adioisotopes with sensitive counting techniques, and reasonable time may be spared for sufficient statistical accuracy necessary for the medical inference. Further, I^{132}–laballed serum albumin can be used for serial measurements over a comparatively short space of time and at the same time total whole body radiation due to the short lived I^{132} would be about 13 times less than the corresponding use of I^{131} (Cook *et al.*, 1956), but the labelling of the albumin should have to be done in the laboratory which might be done in an hour by suitable procedure (Veall *et al.*, 1955).

In practice solid sample preparation for radioactive assay need careful attention whereas liquid sample counting is much easier. In the earlier work with P^{32} Hevesy and his collaborators digested the packed red cells with added carrier phosphate and precipitated as magnesium ammonium phosphate for counting under end-window GM counter (Hevesy *et al.*, 1944). Zerahn designed a special cuvette to present wet packed

cells to an end-window beta counter (Zerahn, 1943). Later, Reeve and Veall (1949) used the haemolyzed blood for counting with liquid G.M counter. For the work with I^{131}-HSA the well-type scintillation counter is convenient for the assay of about 5 ml specimen. Crispell *et al.* (1950), however, used dry thick plasma samples for counting under the end-window beta counter and administered 12 μc dose. Zipf *et al.* (1955) preferred well-type scintillation counter and gravimetric method for their routine plasma volume estimations using 20 μc dose. Sen *et al.* (1957) used beta counter for assaying small quantities of plasma with 50 μc dose. Generally about 10 μc dose of I^{131}-HSA is commonly employed. Albert *et al.* (1956) have devised a plastic coil in an attemt to simplify radioactive counting in liquid phase using the well-type scintillation counter, the fluids were drawn into or passed through a thin plastic tubing coiled inside the hollow space of the crystal. They used chromium-51 for their investigation. At present, however, it appears that Cr^{51} is more suitable for red cell tag and can be used both for blood volume measurements and the determination of the survival of erythrocytes.

SUMMARY

The determination of blood volume of a group of normal adult subjects including men and women has been carried out with reduced doses of radio-isotopes. Each individual has been subjected to repeated estimations of the blood volume, with an interval of about a fortnight between the successive tests, using reduced doses of 0.8–1.6 μc of I^{131}–labelled human serum albumin and 0.4-0.8 μc of P^{32}–tagged red blood cells and also a conventional dose of about 20 μc of I^{131}–HSA along with T-1824 dye. The reduction in dose has been made possible by the use of an anticoincidence windowless gas-flow counter and only 0.2 ml fluid has been used to make thin dry sample for the assay of the radioactivity. The results have been represented in tabular form. Considering the variations in the values of the estimated blood volume with respect to the body weight a rough average value can be taken as 70 ml per kgm for normal adult cases.

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REFERENCES

1. Albert, S.N., Spencer, W.A., Finkelstein, M., Shibuya, J., Alpert, S. and Coakley, C.S. (1956): *J. Lab. Clin. Med.*, **48**, 471.
2. Aust, J.B., Chou, S.N., Marvin, J.F., Brackney, E.L. and Moore, G.E. (1951): *Proc. Soc. exp. Biol.*, **77**, 514.
3. Bale, W.F., Yuile, C.F., DeLaVergne, L., Miller, L.L. and Whipple, G.E. (1949): *J. Exp. Med.*, **90**, 315.

4. Berson, S.A., and Yallow, R.S. (1952) : *J. Clin. Invest.*, **33**, 377.
5. Cook, G.B., Eakins, J. and Veall, N. (1956) : *Intern. J. Appl. Rad. Isotopes.*, **1**, 85.
6. Crispell, K.R., Porter, B. and Nieset, R.T. (1950) : *J. Clin. Invest.*, **29**, 513.
7. Francis, G.E., Mulligan, W. W. and Wormal, A. (1951) : *Nature*, **167**, 748:
8. Frank, H. and Gray, S. J. (1953) : *J. Clin. Invest.*, **32**, 991.
9. Freeman, W. (1957) : *Amer. J. Clin. Path.*, **27**, 393.
10. Gibson, J.G., Weiss, S., Evans, R.D., Peacock, W. C., Irvine, J.W., Good, W.M. and Kip, A. F. (1946) : *J. Clin. Invest.*, **25**, 616.
11. Gray, S.J. and Frank, H. (1953) : *J. Clin, Invest.*, **32**, 1000.
12. Gray, S.J. and Sterling, K. (1950) : *J. Clin. Invest.*, **29**, 1604.
13. Gregerson, N. I. (1944) : *J. Lab. Clin. Med.*, **29**, 1266.
14. Hevesy, G., Koster, K. H., Sorensen, G., Warburg, E. and Zerahn, K. (1944) : *Acta. Med. Scand.*, **116**, 561.
15. Hevesy, G. and Nylin, G. (1951) : *Acta Physiol. Scand.*, **24**, 285.
16. Hevesy, G. and Nylin, G. (1952) : *Circulation Res.*, **1**. 102.
17. Hevesy, G. and Zerahn, K. (1952) : *Acta Physiol. Scand.*, **4**, 376.
18. Hosain, F. (1958) : *Naturwissenschaften.*, **45**, 107.
19. Hosain, F. (1959) : *Naturwissenschaften.*, **46**, 139.
20. Hosain, F. (1960a) : *Ind. Jour. Med. Res.*, **48**, 250.
21. Hosain, F. (1960b) : *Ind. J. Radiol.* (in press).
22. Mollison, P.L., Robinson, M. and Hunter, D. (1958) : *Lancet*, **1**, 766.
23. Reeve, E.B. and Veall, N. (1949) : *J. Physiol.*, **108**, 12.
24. Sen, H.K., Mukherjee, S.R., Roy, B. and Banerjee, S.K. (1957) : *Ind. J. Physiol. Allied Sci.*, **11**, 115.
25. Shemin, D. and Rittenburg, D. (1946) : *J. biol. Chem.*, **166**, 627.
26. Storassli, J.P., Krieger, H., Friedell, H.L. and Holden, W. D. (1950) : *Surg. Gynec. Obst.*, **91**, 458.
27. Tudhope, G. R. and Wilson, G. M. (1956) : *Brit. J. Haematol.*, **2**, 75.
28. Veall, N., Pearson, J. D. and Hanley, T. (1955) : *Brit. J. Radiol.*, **28**, 633.
29. Zerahn, K. (1948) : *Acta. Physiol. Scand.*, **16**, 117.
30. Zipf, R. E., Webber, J. M. and Grove, G. R. (1955) : *J. Lab. Clin. Med.*, **45**, 500 & 648.