A QUICK METHOD OF MEASURING BLOOD VOLUME WITH PHOSPHORUS^{32} LABELLED ERYTHROCYTES [1]

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IN a previous communication (Mukherjee and Rowlands, 1951) reference was made to the blotting-paper method for estimating quickly the circulating blood volume. This technique has been used in measuring blood volume in six children. The details of the technique and a preliminary report of the clinical work are presented.

The principles and the procedure adopted throughout this investigation are the same as described before (Mukherjee and Rowlands, 1951) except for the substitution of blotting-paper planchettes for ordinary nickel planchettes. Group O Rh-negative blood was used as the source for the preparation of labelled erythrocytes. Precautions were taken to maintain asepsis throughout the whole procedure.

METHOD

Preparation of Blotting-paper Planchettes.—A 12 in. sheet of standard blotting paper of 11 μ thickness was placed in a photographer's dry-mounting press with a similar sheet of photographer's mounting tissue, between a cardboard sheet and a metal sheet, in the following order from above downwards: (1) Metal sheet, (2) mounting tissue, (3) blotting paper, (4) cardboard. The press had already been brought to a temperature of 65° to 70° C. The blotting paper was kept in the press for one minute and then removed. The shellac of the tissue melted easily with the heat and became adherent to the blotting paper. The use of the metal sheet next to the other side of the mounting tissue was to avoid any adherence of the inner side which was used later to stick the preparation to the planchettes. Sheets of blotting paper and mounting tissue preparation, 12 in. square, can be rapidly prepared in this way.

A metal punch of 12 mm. diameter was then used with a screwing motion over a cork board to punch out circles of the size required to fit into the standard nickel counting trays described by Mukherjee and Rowlands (1951).

A small drop of absolute alcohol was placed on a clean, dry and warm planchette, spread over the surface and the excess shaken off. A circle of blotting paper was placed with the shellac side on the metal and firmly pressed to it with the thumb for twenty seconds. It is of advantage to slide the blotting paper to and fro over the planchette in the first few seconds to spread evenly the dissolving fluid and to obtain the maximum of adhesion. If the blotting paper becomes wet, too much alcohol has been used and the adhesion is imperfect.

[1] The details of the method were demonstrated at the Annual Meeting of the British Association of Plastic Surgeons, 8th September 1951.

Sources of Error

In addition to the usual sources of error in such technique (see previous paper), the error from absorption of radiations in the specimen itself has to be borne in mind. To estimate the extent of this error the following experiments were performed.

Labelled erythrocytes were prepared in the usual way and were suspended in inactive plasma so as to obtain blood with the same hæmatocrit as the original sample from which the erythrocytes were recorded. 0·1 ml. of the blood (with labelled cells) was pipetted into (*a*) each of three bare planchettes with a drop of water, and (*b*) each of three blotting-paper planchettes. The planchettes were dried overnight in an incubator at 35° C. The radioactivity of each planchette was measured in a Geiger-Müller counter for a period sufficient to allow reading of the average of four figures. The mean of three planchettes was taken. Ten such experiments were done, and the results in Table I show that this source of error is negligible. The overall error of the technique therefore remains within 3 per cent.

Table I

Variations in Amount of Radioactivity when the same volume of P^{32} labelled cells are pipetted into Blotting-paper Planchettes and Bare Planchettes respectively.
All counts are corrected for background error.

Number of Experiment.	Counts per 3 Min. with Blotting Paper (mean of three readings).	Counts per 3 Min. with Bare Planchettes (mean of three readings).	Ratio of Blotting Paper : Bare Planchettes.
1	3436	3531	0·97
2	5856	5927	0·99
3	5764	5851	0·98
4	7565	7074	1·07
5	4474	4915	0·9
6	3788	3796	1·0
7	5681	5690	1·0
8	5040	4776	1·06
9	3182	3275	0·97
10	3901	3974	0·98
		Total . . .	9·92
		Average . .	0·99
		Standard deviation ±	0·05

Results

In six convalescent children varying from 5 to 10 years of age and from 50 to 74 lb. in bodyweight, the circulating blood volume was measured by the use of this

technique. Within thirty minutes following injection the figures for blood volume data were available. These children were convalescing steadily. The results are shown in Table II.

Though the present series is a small one, the data are comparable with those of other workers (Table III).

TABLE II

Blood Volume Estimations with P^{32} (Six Cases)

Age.		Weight (Lb.).	Blood Volume.	Ml. per Lb.
Years.	Months.			
5	8	49¾	1446	28·9
6	6	42½	1638	38·1
7	6	56¾	1974	34·7
10	0	50¾	1987	37·9
10	4	73¾	2006	27·1
10	6	53	2067	38·1

TABLE III

Comparison of Blood Volumes estimated by means of P^{32} Labelled Erythrocytes

	Number of Cases.	Ml. per Kg. of Bodyweight.
Reeve and Veall (1949)	13 adults	78·03
Present series (1951) *	6 children (5 to 10 years)	81·80
Mollison *et al.* (1950)	9 infants	84·70

* Data from Table II have been converted to millilitres per kilogram of bodyweight.

SUMMARY

In six children, aged 5 to 10 years, a preliminary attempt has been made to estimate the suitability of measuring circulating blood volume by the blotting-paper planchette technique. Though the importance of measuring circulating blood volume in clinical work is being more and more realised, most of the available methods are complex, time-consuming and too specialised. The present technique is simple, reliable and quick.

REFERENCES

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