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DETERMINATION OF PLASMA TRAPPING VOLUME OF THALASSEMIC ERYTHROCYTES BY RADIOHEMATOCRIT TECHNIQUE

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Packed red cell volume as determined by microhematocrit technique is being considered the most simple and reliable hematologic screening test since it has an inherent error of only 2% while the hemoglobin determination and red blood cell count have the error as high as 5 and 16% respeetively (1). Unfortunately when whole blood sample is centrifuged a certain amount of plasma remains adherent between cell surfaces and becomes trapped among the cells, thus resulting in an overestimate of the true packed cell volume (PCV). Although the uncorrected PCV value is adequate for most of clinical purposes it is recognized as the major source of error in calculations of blood volume, plasma volume, red cell mass, ferrokinetic studies and erythrocyte electrolyte concentrations. (2,3) Knowledge of plasma trapping value will permit the appropriate correction required for these calculations. Several plasma markers have been employed in determination of the amount of trapped plasma volume, but wide discrepancies exist with different markers used (3-15). Values of trapped volume reported in the literature range from 0.5 to 10% due to differences in experimental systems, separation techniques and methods of measurement (3-25).

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The volume of plasma trapped between the red blood cells of a centrifuged blood sample depends on the amount [6,8,16,26,27] and the intrinsic physical properties of the red cells including size [16, 27, 28], shapes [29,30], cell density [17,24,28,30] and flexibility of the red cell membrane [31]. Although Chaplin and Mollison [16] observed that neither anisocytosis nor spherocytosis significantly altered plasma trapping volume, it was subsequently shown that the presence of spherocytes and sickled red cells greatly increased trapped volume [25,29,30,32] while microcytic and hypochromic red cells [16, 25] raised this value only slightly.

Thalassemic blood picture is characterized by varying degrees of anisocytosis, poikilocytosis, target cells. hypochromic and fragmented red cells, It is reasonable to expect that plasma trapping volume of thalassemic red cells should be higher than normal red cells. The erroneously high PCV value will resulting in a falsely higher MCV value of thalassemic red cells. The only other study of trapped plasma volume of thalassemic red cells has been carried out by the old Winthrobe technique with low centrifugal force utilizing Evans blue dye as a marker [8]. The degree of increased plasma trapping volume observed by these authors was questioned since Evans blue dye not only can form a complex with serum albumin but certain amount of this dye can also be adsorbed onto the red cells [33].

We are reporting the results of our study by the radiohematocrit technique which clearly indicated that plasma trapping volume of thalassemic red cells is markedly increased to 4-5 times of normal red cells.

MATERIALS AND METHOD.

Freshly drawn heparinized blood samples (20 units heparin/ml. blood) were obtained from 9 healthy normal adults, 11 children (2-8 year of age) with severe beta thalassemia and 4 healthy children of the same age group. After they were mixed with ^{51}Cr. and / or ^{131}I-human serum albumin (HSA), radiohematocrit and microhematocrit determinations were carried out in 10 duplicates on each blood sample.

RADIOHEMATOCRIT DETERMINATION: I ^{51}Cr. TECHNIQUE.

To prevent the surface adsorption and penetration of Na_2 $^{51}CrO_4$ into red cells, this hexavalent chromium salt

was reduced to trivalent chromate salt by prior incubation of 0.05 ml (50 uCi) of stock $Na_2\ ^{51}CrO_4$ with 200 mg of ascorbic acid for 60 minutes. There is no detectable amount of ^{51}Cr adsorbed or diffused into the red cells observed in this that system. After mixing, 10 aliquots (1 ml volume) of blood sample were transferred into a set of 10 clean counting vials (ste A). They were then centrifuged at 1,500 rpm. (International Centrifuge Model K, I.E.C., Needham Hts., Mass., USA.) for 10 minutes. Four tenth ml. aliquot of clear supernatant fluid was then carefully transferred from each vial of set A into another 10 clean counting vials (set B). The radioactivity of each vial of both sets were counted in the Tri-Carb Liquid Scintillation Spectrophotometer, Packard Model 3320. The radiohematocrit value of each duplicate was calculated by the formula:

$$\% \text{ radiohematocrit} = \left(0.6 - \frac{0.4 \times \text{cpm. of vial A.}}{\text{cpm. of vial B}}\right) \times 100$$

The final radiohematocrit value of each blood sample was the average value of 10 duplicates.

II. ^{131}I-HSA TECHNIQUE. Whole blood sample was mixed well with ^{131}I-HSA (5 uCi / ml blood) before being aliquoted and proceeded as above.

MICROHEMATOCRIT DETERMINATION. Ten microhematocrit tubes were 3/4 filled with well-mixed whole blood sample and sealed with clay. They were centrifuged in the Microcapillary Centrifuge Model MB (I.E.C., Needham Hts., Mass., USA.) for 6 minutes at 11,000 rpm. (12,000 x G). The microhematocrit (PCV) value determined by the hematocrit reader (scale), and in certain sample, with microscope vernier. The microhematocrit value was the average value obtained from 10 duplicates.

PLASMA TRAPPING VOLUME. The plasma trapping volume was calculated from the formula:

$$\% \text{ PTV} = \% \text{ Microhematocrit} - \frac{\% \text{ Radiohematocrit}}{\% \text{ Microhematocrit}} \times 100$$

RESULTS.

The variation of radiohematocrit and microhematocrit value obtained from 10 duplicates of a single blood sample are shown in Table. I. These value are lower (better) than those observed by others (1, 32).

The trapped plasma volume of approximately 4% is agreeable to most of the earlier studies in the literature. The value of plasma trapping volume (^{51}Cr. radiohematocrit technique) ob-

Table I. Variations of Results Obtained From 10 Duplicates

TECHNIQUE	% PCV	% VARIATION	TRAPPED VOLUME %
Radiohematocrit (^{51}Cr)	37.01 ± 0 49	1.18 ± 0.46	
Microhematocrit:			
- Vernier reading	37.81 ± 0.20	0 44 ± 0.27	2.12
- Scale reading	37.72 ± 0.28	0.64 ± 0.33	1.87

Table II- Plasma Trapping Volume of Thalassemic and Normal Erythrocytes (^{51}Cr. Technique).

SUBJECTS	No.	TRAPPED VOLUME (%)
Normal Adults	6	3.77 ± 2.42
Normal Children	4	3.95 ± 1.78
Thalassemic Children	,11	18.30 ± 9.58

tained from thalassemic blood is approximately 4-5 times of normal red cells.

The average value of plasma trapping volume obtained from ^{131}I-HSA technique (7.78%) is more than 100% higher than those from ^{51}Cr. technique (3.77%) due to the adsorption of ^{131}I-HSA onto the red cell surface.

COMMENTS.

Our results of plasma trapping volume (by ^{51}Cr technique and expressed as a percentage of PVC) of 3.77% in healthy adults and 3.95% in healthy children are comparable to those observed by other investigators (8,10,15-17, 19,24,25,30,34). The higher trapping volume of 7.78% when ^{131} I-HSA is used compared to 3.77% with ^{51}Cr technique indicated that the error is resulting from adsorption of ^{131} I-HSA onto the surface of red cells and possible transport of ^{131} I through the red cell membrane (14,35). In our laboratory, when the true hematocrit value is required the observed microhematocrit value is multiplied by a factor 0.96 to correct for 4% plasma trapping

volume. Although the feasibility and superiority of the microhematocrit technique are universally accepted, it should be kept in mind that in practice the theoretical advantage of microhematocrit over the conventional Winthrobe technique is overweighed by the slighty less well reproducibility of the former. The chief difficulty lies in getting a flat horizontal seal at the bottom of the microhematocrit tube in addition to accurate reading of the column. Even when these conditions are satisfied the S.D. of replicated estimate is about 0.5 [32]. In our experimental system, the S.D. of 10 duplicates are only 0 20 (with vernier reading) and 0.28 (with scale) which correspond to 0.44% and 0.64% of the red cell column respectively. The other source of minor error due to hemolysis and different degrees of oxygenation were eliminated by treating all samples alike and care was taken to minimize manipulation.

The finding of marked increased plasma trapping volume of thalassemic red cells indicated that packing of these red cells during centrifugation is less complete than normal red cells. It has been demonstrated earlier that the amount of trapped plasma volume in the lower layer of red cell column is smaller than the upper layer[16-18] and that the heavier cells packing more tightly than the lighter cells [24, 28]. Reticulocytes and young red cells are lighter than older red cells [17,37-41], and their presence in increased numbers in addition to red cells with varying shapes and sizes [42] in thalassemic blood may explain the reduced effectiveness of red cells packing during centrifugation and the increased plasma volume observed.

SUMMARY :

The plasma trapping volume of red cells was determined with the aid of the radiohematocrit technique. The trapped volume of red cells from healthy adults and children is about 4% (3.77-3.95%) while the average value for thalassemic red cells is 18.3%. The explanation for this increased trapped volume is the reduced effectiveness of red cell packing during centrifugation due to the presence of red cells with varying shapes and sizes and those with light weight i.e.. reticulocytes and young red cells. The higher trapping values obtained when ^{131}I-HSA is used in place of Na_2 $^{51}CrO_4$ is due to the adsorption of ^{131}I-HSA onto the red cell surface and possible transport of ^{131}I through the red cell membrane.

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ย่อเรื่อง

คณะผู้วิจัยได้ทำการตรวจและศึกษาเกี่ยวกับ plasma trapping volume ของเม็ดเลือดแดงโดยใช้วิธี Radiomicrohematocrit พบว่า trapped volume ของเม็ดเลือดแดงในผู้ใหญ่และเด็กปกติได้ค่า 4 %(3.77–3.95%) ในขณะที่ค่าเฉลี่ยจากเม็ดเลือดแดงของผู้ป่วยด้วย Thalassemia ได้ค่า 18.3%

การที่ค่า trapped volume เพิ่มสูงขึ้นในผู้ป่วย Thalassemia นี้ เนื่องมาจากความสามารถในการอัดแน่นของเม็ดเลือดแดงระหว่างการ centrifuge ลดลง ทั้งนี้ เนื่องจากมีเม็ดแดงที่แตกต่างกันทั้งรูปร่างและขนาด ตลอดจนเม็ดเลือดแดงที่มีน้ำหนักเบา อันได้แก่ Reticulocytes และเม็ดเลือดแดงอ่อน

ค่า trapping จะสูงขึ้นเมื่อใช้ ^{131}I-HSA แทน $Na_2{}^{51}CrO_4$ ทั้งนี้เป็นเพราะ adsorption ของ ^{131}I-HSA ต่อผนังเม็ดเลือดแดง และ ^{131}I ซึมผ่านผนังเม็ดเลือดแดงได้.