Trapped Plasma in the Microhematocrit

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The amount of trapped plasma in the microhematocrit red blood cell column of samples from 25 normal individuals and 102 patients was investigated. The mean value for the normal individuals was 1.53%, and the mean values for the samples from the patient groups ranged from 1.41% to 1.82%. These groups included patients with sickle cell disease, iron deficiency, and hereditary spherocytosis. There was an inverse correlation between trapped plasma and the MCH in the samples from patients with iron-deficiency (MCH ≤ 25.0 pg). These findings have relevance to the determination of the PCV and derived red blood cell indices. (Key words: PCV; Microhematocrit; Trapped plasma) Am J Clin Pathol 1982; 78: 770–772

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MEASUREMENT OF THE VOLUME of a whole blood sample occupied by the red blood cell component, the packed cell volume (PCV), is an accepted routine technic in hematology. In addition to inclusion in complete blood count investigations, PCV values also are required in other laboratory measurements, *e.g.*, red blood cell folate and derived red blood cell indices.

Although the macrohematocrit method has been used[2] and advocated[5] to measure the PCV, the microhematocrit still appears to be the most widely performed manual method; in particular, the microhematocrit is used in establishing values for the reference material distributed by Coulter Electronics for the routine monitoring of their instruments' performance. In the determination of these values, it is assumed that there is plasma trapping of 3% in the red blood cell column of the microhematocrit in normal samples. This value for trapped plasma is derived from the work of England and colleagues,[1] but although this value has become widely accepted, there is evidence to suggest that this accepted value is too high.[4] The present study was undertaken to reexamine the amount of trapped plasma in the microhematocrit red blood cell column of samples from normal individuals and from patients with a variety of hematologic disorders.

Materials and Methods

Venous blood samples (3 mL) were collected from 25 normal control individuals and from 102 patients. The patients were grouped according to their principal diagnosis (Table 1). The patients with iron-deficient red blood cell changes (MCH ≤ 25.0 pg) were divided into two groups depending upon the etiology of their iron deficiency: (1) iron deficiency anemia *per se*, or (2) iron deficiency in polycythemia, where regular venesection was part of the treatment, and where PCV values were less than 0.500. Patients who did not primarily have a hematologic disorder (*e.g.*, obstructive jaundice, pre- and postsurgical cases, diabetes) were grouped together and included in the "other" category in Table 1; also included in this category was one patient with α-thalassaemia (Hemoglobin H disease).

The venous blood was anti-coagulated with K_2EDTA (1.5 mg/mL) and then mixed with iodinated ^{125}I-labeled human serum albumin injection B.P. (specific gravity 50 μCi/mL; 20 mg albumin/mL obtained from the Radiochemical Centre, Amersham, Buckinghamshire, UK). The radioisotope label was added to the blood from a 23-gauge needle, held vertically, in the ratio of 1 drop per mL of whole blood.

Trapped Plasma Measurement in the Microhematocrit PCV

The microhematocrit PCV was measured using glass capillary tubes (Bilbate 75 mm × 1 mm; BS 4316) which were filled with blood to give a blood volume of approximately 55 mm. The tubes were heat-sealed and the external surfaces cleaned with damp tissues, followed by centrifugation in a Hawksley microhematocrit centrifuge (Gelman Hawksley Limited, Lancing, Sussex, UK) for 10 minutes. The speed of rotation of the microhematocrit centrifuge was measured using a stroboscope to calculate the relative centrifugal force. The uncorrected PCV was assessed by measuring the height of both the red blood cell column and the total sample column, using metric graph paper.

The amount of trapped plasma was measured using a modification of the technic described by Garby and Vuille,[4] by separating the red blood cell column from the section of capillary containing the plasma and buffy-coat, and counting the radioactivity in each section. The

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Table 1. Trapped Plasma Values in the Microhematocrit of Samples from Normal Individuals and from Various Patient Groups

Subjects		Trapped Plasma (%)	
Group	Number	Mean	SD
Lymphoproliferative disorders	9	1.41	0.220
Macrocytic anemias	3	1.52	—
Normal	25	1.53	0.166
Iron deficiency (polycythemic)	13	1.56	0.173
Myeloproliferative disorders	8	1.68	0.195
Heterozygous Hb S	10	1.68	0.175
Homozygous Hb S	14	1.70	0.209
Hereditary spherocytosis	2	1.71	—
β-Thalassaemia minor	4	1.76	0.090
Chronic renal failure	5	1.78	0.066
Iron deficiency anemia	11	1.82	0.202
Others	23	1.58	0.224
All Samples	127	1.61	0.213

percentage trapped plasma was calculated using equation 1:

Trapped Plasma (TP)

$$= \frac{r(1 - PCV)\,100}{p(PCV)}\,\% \quad \text{(equation 1)}$$

where r = radioactivity from the red blood cell column, and p = radioactivity from the plasma column. This procedure was repeated in duplicate for each blood specimen.

Complete Blood Count (CBC) Investigations

A CBC was generated for each patient sample, using a Coulter Model S-Plus (Coulter Electronics Limited, Northwell Drive, Luton, Bedfordshire, UK), which was calibrated using Coulter 4C-Plus as the calibrant reference material for red blood cell count, hemoglobin, and mean cell volume.

Statistical Analysis of Data

The Student's *t*-test for sample groups with equal variance was used to determine the significance levels of the trapped plasma values for selected patient groups.

Results

Relative Centrifugal Force of the Microhematocrit Centrifuge

The relative centrifugal force (RCF) of the microhematocrit centrifuge as calculated from the stroboscope measurements was 18,700 *g* at the rim of the centrifuge. Therefore, for the mean PCV (0.381) in this study, where the capillary tubes contained a 55-mm column of blood, the RCF at the buffy coat was approximately 14,400 *g*. For the normal samples (mean PCV 0.423), the RCF was approximately 13,000 *g* at the same site.

Trapped Plasma Values in the Microhematocrit

The trapped plasma (TP) results for all 127 samples ranged from 1.18% to 2.25% (mean 1.61%; SD = 0.213), with the samples from the normal individuals giving a mean TP of 1.53%. There were only six samples which had trapped plasma values equal to or greater than 2.0%. Of these, three were from patients with marked microcytic hypochromic changes due to iron deficiency anemia. The remaining three samples were from one patient who was homozygous for Hb S (TP = 2.25%), one patient who was heterozygous for Hb S (TP = 2.00%), and one patient with Hemoglobin H disease (TP = 2.12%). The TP results for the samples from each of the patient groups are shown in Table 1.

The Effect of Iron Deficiency on the Trapped Plasma Value

A comparison was made between the TP values for the patients with iron deficiency anemia (MCH ≤ 25.0 pg), the polycythemic group with iron-deficient red blood cell changes (MCH ≤ 25.0 pg), and the normal individuals. The results show that there was a significantly higher value for TP for the patients with iron deficiency anemia compared with the normal individuals ($P < 0.001$), and the polycythemic group with iron-deficient red blood cell changes compared with the patients with iron deficiency anemia ($0.01 > P > 0.002$). However, there was no significant difference between the TP values for the polycythemic group with iron-deficient red blood cell changes compared with the normal subject group ($P > 0.20$).

Correlation analysis demonstrated a significant inverse relationship between TP values and the MCH in the patients with iron deficiency anemia (r = −0.638; $0.05 > P > 0.02$), whereas the polycythemic group with iron-deficient red blood cell changes gave an inverse relationship which was not significant (r = −0.410; $P > 0.10$). However, when all patients with iron-deficient red blood cell changes were considered together, there was a highly significant inverse correlation between MCH and TP (r = −0.651; $P < 0.001$).

Trapped Plasma Values in Patients with Hb S

The difference in the TP values for the samples from the homozygous and the heterozygous Hb S patients was not significant ($P > 0.20$). However, there was a significant difference between the normal individuals and the homozygous Hb S patients ($0.01 > P > 0.002$), and to

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Table 2. Reported Value for Trapped Plasma in the Red Blood Cell Column of the Microhematocrit for Normal Samples Given by Various Authors

Author(s) and Year	Number of Samples Examined	Approximate Relative Centrifugal Force	Centrifugation Time (minutes)	Mean Trapped Plasma Value (% of Red Blood Cell Column)
Furth, 1956[3]	10	10,000 *g*	4	2.0
Garby and Vuille, 1961[4]	5	10,000 *g*	10	1.31
Rustad, 1964[6]	5	6,040 *g*	10	2.78
England, Walford, and Waters, 1972[1]	27	12,000 *g*	5	3.22
Present Study	25	13,000 *g*	10	1.53

a lesser extent between the normal individuals and the heterozygous Hb S patients ($0.05 > P > 0.02$).

Discussion

Apart from intrinsic differences in red blood cell behavior, the amount of trapped plasma (TP) in the red blood cell column of the microhematocrit depends on the centrifugal force and the time of centrifugation. A number of authors have examined the amount of trapped plasma in the microhematocrit, and their results are listed in Table 2. The mean TP result in the present study agrees fairly closely with that of Garby and Vuille,[4] who used a relative centrifugal force (RCF) of 10,000 *g* for 10 minutes, but only examined five individuals. The higher results obtained by Furth,[3] who used a shorter time (RCF 10,000 *g* for 4 minutes), and Rustad,[6] who used a lower centrifugal force (RCF 6,040 *g* for 10 minutes), are in the same order as the mean results of the present study if allowance is made for the differences in technic. The highest reported value in normal individuals is that of England and co-workers[1] who used a Hawksley microhematocrit centrifuge (RCF 12,000 *g* for 5 minutes).

It might be expected that hematological disorders in which the red blood cells are less deformable would produce higher values for plasma trapping since the packing of the red blood cells in the microhematocrit is determined by their deformability.[7] In the present study, marginally higher values for the TP have been observed in sickle cell disease and sickle cell trait, iron deficiency, and hereditary spherocytosis. In sickle cell trait and sickle cell disease, England and colleagues[1] gave values of approximately 5.3% and 4.5%, respectively, for TP. The result obtained for these patients in the present study is much lower than theirs, but they also found higher values in normal individuals. In the present study, a value of 1.71% was found for TP in samples from patients with hereditary spherocytosis, in contrast to the finding of 4.9% by Furth,[3] but the shorter centrifugation time (4 minutes) used by Furth possibly explains the observed difference. Both the present study and that of England and associates[1] found higher values for TP in patients with iron-deficient red blood cell changes. Similarly, the present study has confirmed their observation that there was an inverse correlation between the TP value and the MCH.

In conclusion, the present study has confirmed the previous reports that the amount of TP in the microhematocrit red blood cell column is small, and the currently accepted value of 3% for normal samples appears too high. If a value for TP of 1.5% is used instead of 3% when determining the values for the control material issued by Coulter Electronics, this will alter the PCV value, and hence, the derived red blood cell values, namely the MCV and MCHC. In addition, although statistically higher values are found in certain hematologic disorders if the centrifugation conditions of the present study are used, these values are not much higher than those for normal samples. Thus, if a value of 1.5% TP is applied to all samples, even in the sample with the highest TP (2.25%) in this present study, the error in the actual PCV would only be 0.003 at a PCV of 0.400.

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