

Centrifugal Packing of Suspensions of Erythrocytes Hardened with Acetaldehyde* (32849)

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In rheological studies on blood or suspensions of erythrocytes (RBC) in various media, it is essential to have exact knowledge of the volume percent of RBC in the system. In calculating this cell percentage (H) from hematocrit readings (HCT) obtained by centrifugal packing, a correction must be made for the volume of suspending medium trapped in the packed cell column due to incompleteness of packing (1). The amount of medium trapped, and hence the trapping correction factor (F_t), varies with the force and duration of centrifugation as well as with the species origin of the RBC (2). Any rheological investigation on suspensions of RBC with altered physical properties should be preceded by an examination of the F_t of the suspensions under study. The experiments reported here were done on canine and human RBC fixed with acetaldehyde, a treatment which does not alter the shape, size, or electric charge of the RBC (3,4), but renders them much more rigid and far less deformable (5). The information obtained on the F_t of hardened RBC suspensions was essential for calculating the true cell percentage from hematocrit readings in our investigations on the viscometric behavior of these suspensions (6).

Materials and Methods. 1. *Preparation of hardened erythrocytes* (3). Blood was drawn from normal human subjects and mongrel dogs, and coagulation was prevented by the addition of heparin. After the removal of plasma, the RBC were washed three times with 0.15 M aqueous NaCl solution (hereafter

referred to as the physiological saline). The buffy coat, together with some RBC, was removed during the washing procedure. A 2% (w/v) acetaldehyde solution in physiological saline was prepared in the cold, and the pH was adjusted to 7.4 with 0.15 M aqueous NaOH solution. After the mixing of one volume of the washed RBC with approximately 10 volumes of the acetaldehyde solution, the suspension was kept for at least 1 month at room temperature to allow the completion of the hardening process. During this period, the pH of the suspension was checked and adjusted to 7.4 when necessary. Before use in experimental studies, the hardened RBC were washed three times with physiological saline or Eagle's solution (containing 0.25 gm/100 ml of albumin) and suspended in the same. The pH of the final suspension was adjusted to 7.4.

2. *Determination of trapping correlation factor* (2). The true volume of the suspending medium in samples of RBC suspension was determined from the dilution of known quantities of labeled macromolecules. The substances used were ¹³¹I-labeled human serum albumin (obtained from Abbott Laboratories, Teterboro, N. J.), ¹⁴C-labeled dextran (mol. wt. 60,000–90,000, obtained from New England Nuclear Corporation, Boston, Mass.) and ¹⁴C-labeled polyvinylpyrrolidone (PVP, mol. wt. approximately 40,000, obtained from Schenley Laboratories, Inc., New York City). One hundred μ l of a solution of one of the labeled macromolecules was mixed with a known volume (V , usually 3.0 or 5.0 ml) of RBC suspension. Microhematocrit (tubes with 1.2 mm i.d.) and macrohematocrit (4-ml Wintrobe tubes) samples were taken from the mixture. The macro- and microhematocrit tubes were centrifuged at 1500g and 15,000g, respectively. The duration of centrifugation was 30 min for the macrohematocrit tubes and 5 min or longer for the microhematocrit

* This investigation was supported by USPHS Research Grant HE-06139 from Nat. Heart Inst., U.S. Army Medical Research Development Command Contract DA-49-193-MD-2272, and a grant from Mrs. Alan M. Scaife for instrumentation.

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TABLE I. Trapping Correction Factors Determined with Albumin-¹³¹I.

Animal species	Erythrocytes	Suspending medium	Micro- F_t^a [mean \pm SEM (n)]	Macro- F_t^b [mean \pm SEM (n)]
Dog	Normal	0.9% NaCl	0.95 \pm 0.014 (8)	0.94 \pm 0.013 (8)
	Hardened	0.9% NaCl	0.60 \pm 0.019 (9) ^c	0.57 \pm 0.014 (9) ^c
		Water	0.62 \pm 0.012 (5) ^c	0.63 \pm 0.014 (5) ^c
Man	Normal	Eagle	0.97 \pm 0.007 (6)	0.94 \pm 0.007 (6)
	Hardened	Eagle	0.62 \pm 0.009 (5) ^c	0.61 \pm 0.009 (5) ^c
		Water	0.58 \pm 0.011 (19) ^c	0.57 \pm 0.019 (8) ^c

^a 15,000*g* for 5 min.

^b 1500*g* for 30 min.

^c $p < 0.001$ when compared with the F_t of the normal RBC suspension.

tubes. After the reading of hematocrit values, 1.0 ml of the supernatant fluid in the macrohematocrit tube was pipetted for the counting of radioactivity (C) with a gamma-beta ray spectrometer (Packard Instrument Co., Downers Grove, Ill.). If Q is the amount of radioactivity contained in 100 μ l of the labeled solution, then the volume of suspending medium in $(V + 0.1)$ ml of the suspension is equal to Q/C . The volume of cells is then $(V + 0.1) - (Q/C)$, and the cell percentage (H) is $100(V + 0.1 - Q/C) / (V + 0.1)$. The ratio of the calculated cell percentage to the hematocrit value, i.e., H/HCT , is the trapping correction factor (F_t). This factor represents the volume fraction of the packed cell column occupied by RBC. The RBC counts and RBC volumes were determined in an electronic particle counter and size-distribution plotter (Coulter Electronics, Hialeah, Fla.).

Results and Discussion. The trapping correction factors (F_t) determined with albumin-¹³¹I on suspensions of normal and hardened RBC are shown in Table I. For suspensions of normal, deformable RBC of either canine or human origin, the HCT value (macro- or micro-) was only slightly higher than the true cell percentage (H) determined by isotope dilution, and the F_t values (H/HCT) were between 0.94 and 0.97. Thus only 3 to 6% of the packed column of normal RBC was occupied by trapped fluid. In contrast, the HCT values for the hardened RBC suspensions were much higher than H , giving F_t values for canine and human RBC suspensions ranging from 0.57 to 0.63. That is, only some

60% of the packed column was composed of hardened RBC and the trapped fluid occupied approximately 40% of the column. Essentially the same results were obtained regardless of the origin of the hardened RBC (canine or human) or the nature of the suspending medium (water or salt solution) tested. A comparison of the F_t of the hardened RBC suspensions with the corresponding values obtained on normal RBC suspensions by the Student t test shows that the difference was significant (p values < 0.001).

The validity of F_t determined by the dilution of albumin-¹³¹I depends on a uniform distribution of the labeled protein in the suspending medium without being adsorbed onto the RBC surface. If there should be a significant adsorption of albumin-¹³¹I onto the surface of the hardened RBC, the F_t would be falsely low for the hardened RBC suspensions. In order to test this possibility, the F_t values of normal and hardened RBC were also determined by the dilution of ¹⁴C-labeled dextran and PVP; and the results obtained with these two nonprotein macromolecules are similar to those with albumin-¹³¹I (Table II). Since it is unlikely that these different types of macromolecules would adsorb to the surface of the hardened RBC to similar extents, the findings indicate that the low F_t value for the hardened RBC suspension is not a result of surface adsorption of the labeled macromolecules. Table II also shows the slight increase in F_t by increasing the duration or the force of centrifugation.

The validity of the difference in F_t between the normal and the hardened RBC sus-

TABLE II. Trapping Correction Factors for Human Hardened RBC in Eagle Solution: Comparison of Results Obtained with Different Labeled Macromolecules.*

Labeled macromolecules used	Conditions of centrifugation		
	(A) 15,000g, 5 min.	(B) 15,000g, 30 min.	(C) 1,500g, 30 min.
Albumin- ¹²⁵ I	0.62 ± 0.009 (5)	0.64 ± 0.009 (5)	0.61 ± 0.009 (5)
Dextran- ¹⁴ C	0.57 ± 0.016 (5)	0.60 ± 0.015 (5)	0.57 ± 0.012 (5)
PVP- ¹⁴ C	0.65 ± 0.007 (5)	0.67 ± 0.007 (4)	0.62 ± 0.010 (4)

* Values given are mean ± SEM (number in parentheses indicates no. of expts.)

pensions is further strongly supported by the following experiments in which the microhematocrit values of RBC suspensions were followed during the period of hardening. Normal canine erythrocytes were washed and suspended in physiological saline to give hematocrits of 12–30%. After centrifugation the saline supernatant was replaced by exactly the same volume of 2% acetaldehyde solution. Immediate determination of hematocrits of these acetaldehyde–RBC suspensions gave the same readings as the saline–RBC suspensions (12 and 30% for the two samples shown in Fig. 1). Daily measurements of hematocrits from these two samples showed a sharp increase during the first day and a much slower rise during the subsequent days. At the end of 1 month, the hematocrit readings of the acetaldehyde–RBC suspensions apparently

approached steady levels. Since there was no detectable increase in either RBC diameter (microscopic observation) or the RBC volume (electronic measurement of particle size), the increase in hematocrit of the acetaldehyde–RBC suspensions resulted from incomplete packing rather than RBC swelling. When the cell percentage before hardening (H_0) is compared with the hematocrit reading obtained after one month of hardening in acetaldehyde (HCT), the ratio H_0 /HCT is approximately 0.6. This again shows that the packing of hardened RBC is only about 60% complete, a result which is in agreement with the F_t values determined with isotope dilution. It is interesting to note that, on the basis of simple geometric consideration of human RBC, Burton (7) has calculated that nondeformable RBC can be packed to only 58% cells. The present results support his theoretical prediction. Hence it is obvious that some rheological studies on hardened RBC suspensions (5) should be reevaluated, taking into account this trapping correction factor.

A relatively low F_t value (0.8–0.9) has been reported for goat blood (with normal, untreated RBC) and has been attributed mainly to the smaller RBC size in this species (2,8). In view of the present findings and evidence from unpublished observations on filtering goat RBC through polycarbonate sieves (9), the less complete packing of goat RBC may actually reflect that the RBC are less deformable in this species.

Summary and Conclusions. The centrifugally packed column of human or canine RBC hardened with acetaldehyde consists of only 60% cells, in contrast to the near 100% packing for normal RBC. This incomplete packing of hardened RBC was demonstrated

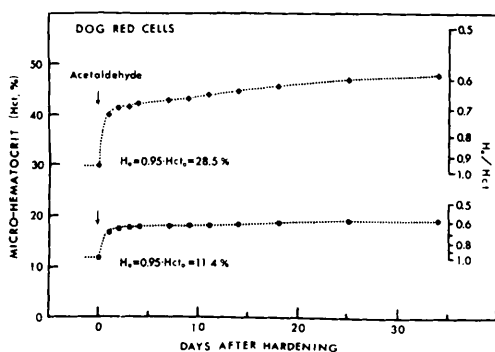


FIG. 1. Changes of microhematocrit (Hct) with time in two samples of dog RBC after being suspended in acetaldehyde solution. The values for the original cell percentage (H_0) of the two samples before acetaldehyde hardening are given. As hardening proceeds, note the gradual rise of Hct and hence a decrease in the ratio H_0 /Hct (scales shown on the right) from 0.95 to approximately 0.6.

with the isotope dilution method using three different macromolecules (albumin, dextran, and PVP) and by following the hematocrit values during the hardening process, and the results agree with theoretical calculations. The striking difference in the centrifugal packing of hardened vs normal RBC indicates that normal RBC are deformable during centrifugal packing and that the low degree of packing must be considered in any study on hardened RBC.

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Received Nov. 30, 1967. P.S.E.B.M., 1968, Vol. 127.

Thioacetamide Inhibition of Liver and Serum Amylase Production* (32850)

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Administration of thioacetamide will cause centrolobular liver necrosis (1,3) in acute experiments and cirrhosis (2,3) when fed at relatively lower levels for extended periods of time. It is also known that hepatoma (3) may be a result of long term administration. We have shown that liver damage caused by *N*-nitrosodimethylamine or carbon tetrachloride (4) results in lowered liver and serum amylase levels in the rat and would have predicted that damage due to thioacetamide would do likewise. However, Muramatsu and Busch (5) showed that administration of thioacetamide to rats caused an increase in the uptake of radioactive lysine into liver proteins. Also, Kizer *et al.* (6) showed that adenylic acid (AMP) deaminase activity in rat liver was significantly elevated by administration of thioacetamide, both by feeding and intra-abdominal injection.

The results presented here show that ad-

ministration of thioacetamide, as shown by others (1,2,3), causes liver damage in our rats and this damage is accompanied by lowered liver and serum amylase levels.

Methods. The white rats used in these experiments were males, weighing 200–300 gm, of the Sprague-Dawley strain obtained from Rolfsmeyer Laboratory Animals. Thioacetamide used was obtained from Nutritional Biochemicals Corporation. When injected intraperitoneally the drug was dissolved in sterile 0.9% NaCl in a concentration of 20 mg/ml; when administered by stomach tube the concentration was 10 mg/ml.

With each rat used, blood was drawn for serum amylase determination before administration of thioacetamide. After administration of the drug, which was given in varying amounts for different time periods (see Table I), all rats were sacrificed 1 day following the last injection or feeding; and blood, liver, and kidney samples were obtained. Appropriate control animals, to whom only sterile 0.9% NaCl was administered, were included

* This investigation was supported by Grant AM 02610, from National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.