XIII. POTASSIUM CONTENT AND CELL VOLUME OF CENTRIFUGED ERYTHROCYTES

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DANIELLI [1937] remarks that centrifuging bloods of certain species causes a loss of up to 40% of cell potassium from the erythrocytes. He has not worked with human erythrocytes and as all analyses of human cells depend directly or indirectly on their separation from plasma by centrifuging, the present writers have endeavoured to find out if such treatment causes any change in the potassium content or volume of these cells.

Potassium content. Rapidly sedimenting bloods from cases of rheumatoid arthritis were heparinized and divided into three portions. One was allowed to settle and the other two were centrifuged for 10 min. at 4500 and 10,000 rev. per min. respectively. After centrifuging, any potassium expressed from the cells would appear in the supernatant plasma and lest any local accumulation might occur in the layer immediately above the packed cell columns, the whole of the plasma was carefully mixed with a glass rod and then recentrifuged. In the case of the sample allowed to settle naturally, the clear plasma was removed with a pipette and any traces of suspended cells were removed by centrifuging. The potassium contents of the three separated specimens were estimated by the method of Kramer & Tisdall [1921] (Table I).

Table I

Rev. per min.	Potassium contents (m.eq. per 1000 ml.)			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	4.2	4.45	3.91	3.01
	—	4.51	3.94	2.91
4,500	3.6	4.73	3.88	3.25
	—	4.73	3.94	3.27
10,000	4.0	5.22	3.70	3.73
	—	5.12	3.91	3.75

Increase in plasma K as a result of centrifuging varies between 0 and 25%. Since the erythrocyte contains 25 times as much potassium as does plasma, it follows that less than 1% of cell K is lost as a result of centrifuging—provided that one assumes that no loss of cell water occurs simultaneously. And in fact loss of cell water could not account for the constancy of plasma K, since in order to mask a loss of 1% of cell K a simultaneous outflow of 35% of cell water would have to occur and to compensate for the loss of 2%, the cell would have to be desiccated assuming that water occupies 70% of the cell volume. It may therefore be accepted that no loss of cell potassium occurs as a result of centrifuging. On the other hand an apparent change of cell potassium might occur owing to expression of cell water; it seems unlikely that centrifuging could increase the amount of water in cells. In the following section it will be shown that cell volume remains practically unaltered during centrifuging.

Method 1. A solution of pure haemoglobin was prepared [Adair & Adair, 1934] and rendered isotonic with sodium chloride. A small amount of the coloured solution was added to a rapidly sedimenting blood. The mixture was set up in four graduated tubes and allowed to settle for 6 hr., after which time two of the tubes only were centrifuged. The supernatant haemoglobin-plasma was removed and a measured portion set aside as a colour standard. All the residual coloured plasma were then carefully washed out of the containing tubes without disturbing the cells. The latter were suspended in saline solution and in this way haemoglobin-plasma was washed out from between the cells and on recentrifuging appeared in the supernatant saline. The volume of this saline was measured and its haemoglobin content compared in a colorimeter with the standard solution. In this way the volume of intercellular haemoglobin-plasma is obtained. If then one subtracts the respective volumes of intercellular fluids from the corresponding volumes of settled and centrifuged cells one obtains the absolute volume of cells in each case.

Method 2. Here, plain heparinized blood was used, one portion being allowed to settle and the other being centrifuged. In both cases supernatant plasma was removed and intercellular plasma washed out of the erythrocyte columns by suspension in saline and recentrifuging. The protein content of this supernatant plasma was then compared with that of the original supernatant plasma, the colour reaction of Wu & Ling [1927] being used. Figures in Table II are expressed in volumes % of original suspension.

Table II

	Settled cells vol. %	Inter- cellular fluid vol. %	Absolute cell vol. %	Centrifuged cells vol. %	Inter- cellular fluid vol. %	Absolute cell vol. %
Method 1: tube 7 mm. bore						
Exp. 1	42	10	32	35	2	33
Exp. 2	41.5	9	32.5	35	1.5	33.5
Exp. 3	43	15	28	28.5	1.5	27
Exp. 4	44.5	10	34.5	33.5	2	31.5
Exp. 5	46.5	12.5	34	38	2.5	35.5
Method 2: tube 3 mm. bore						
Exp. 1	47	11.5	35.5	37	3	34
Exp. 2	55	11.5	43.5	45	1.5	43.5
Exp. 3	42.5	10	32.5	32.5	2	30.5
Exp. 4	42.5	7.5	35	34	1.5	32.5

It will be seen that with one exception the absolute volume of cells after settling differs little from the absolute volume after centrifuging, the variations lying between -4.5 and $+7\%$ —differences which are within the range of experimental error.

SUMMARY

Centrifuging human erythrocytes, at least in rapidly sedimenting blood, causes little or no change in the base or water content.

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