ON THE FREEZING POINT OF THE UNHAEMOLYSED CORPUSCLES DURING HAEMOLYSIS OF BLOOD AND ON THE EFFECTS OF EVAPORATION ON THE RESISTANCE OF ERYTHROCYTES TO HAEMOLYSIS

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In the *Bio-Chemical Journal*, volume IV, page 284, I have pointed out that the resistant corpuscles during haemolysis of blood are either less permeable to water or can bear the tension of distension better than those that haemolyse.

In order to determine whether the unhaemolysed corpuscles are less permeable to water or not, I have estimated the freezing point of the unhaemolysed corpuscles as well as that of the supernatant fluid after the separation of the unhaemolysed corpuscles by centrifugalisation.

In all of the experiments the blood was allowed to clot, the clotted blood squeezed through thin muslin and mixed with two parts of distilled water, and the mixture thoroughly centrifuged after one hour.

The following results have been obtained:—

(1) HUMAN BLOOD. (Blood taken from the jugular veins four hours after death.)

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.195
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.195

(2) FOWL'S BLOOD

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.224
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.285

(3) FOWL'S BLOOD

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.210
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.210

(4) FOWL'S BLOOD

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.210
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.200

(5) FOWL'S BLOOD

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.235
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.208

(6) FOWL'S BLOOD

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.230
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.190

(7) FOWL'S BLOOD

1.	Δ for the haemolysed corpuscles	...	...	...	...	...	0.250
2.	Δ for the unhaemolysed corpuscles	...	...	...	...	...	0.250

It will be seen from the above that the freezing points of the haemolysed corpuscles were nearly the same as those of the unhaemolysed corpuscles, and therefore osmosis must have taken place to the same extent in the haemolysed as well as the unhaemolysed corpuscles. In other words, the unhaemolysed corpuscles are nearly as much permeable to water as the haemolysed ones. The fact that some of the corpuscles do not haemolyse must therefore be due to their being able to bear the tension of distension better than those that haemolyse. We may describe this resistance of the erythrocytes to rupture as their specific resistance. This specific resistance to rupture varies in different animals, and may be expressed by the relative haemoglobin value of their resistant erythrocytes.

In the following tables the erythrocytes were invariably suspended in 0.85 per cent. saline, and the amount of suspension taken was always half that of the dissolving fluid, which in the present case was distilled water.

Specific resistance of erythrocytes to rupture in different animals

1. Human blood	...	...	...	...	...	...	...	...	...	0.2934
2. Fowl's blood	...	...	...	...	...	...	...	...	...	0.1875
3. Dog's blood	...	...	...	...	...	...	...	...	...	0.3333
4. Frog's blood	...	...	...	...	...	...	...	...	...	0.7917
5. Sheep's blood	...	...	...	...	...	...	...	...	...	0
6. Rabbit's blood	...	...	...	...	...	...	...	...	...	0

Effect of evaporation on the resistance of erythrocytes to haemolysis

If we spread the erythrocytes, from a suspension of these in N/10 sodium chloride solution, over a slide and then allow them to dry gently, we find that these dried corpuscles when treated with N/10 sodium chloride solution completely dissolve. Let us consider what happens when the erythrocytes are drying on the slide. They tend to stick to the slide and, as evaporation goes on, they tend to contract. As a result of these two antagonistic processes there is, perhaps, rupture of their membraneous structure, and they dissolve when they come in contact with what would be an isotonic solution in the case of undried erythrocytes. Their complete solution indicates complete disruption of their cellular framework in the process of drying up.

This complete disruption is, as just now stated, either purely mechanical due to rupture of all the membraneous portions inside the erythrocytes that become adherent to the slide, or the removal of water during evaporation brings about a complete change in the chemical

constitution of the erythrocytes, converting them into particles soluble in a saline of any strength, just as a lump of sugar dissolves in water. This latter view best explains the complete solution of erythrocytes when treated with saturated solution of sodium chloride in water. In the process of desiccation of the erythrocytes by the saturated sodium chloride solution, water is removed from them. A portion of this water is perhaps in chemical combination with the erythrocytes, and this compound is broken up when they are allowed to dry up over a slide or are treated with saturated sodium chloride solution. Water, therefore, as it exists inside erythrocytes, is partly in a chemical combination with its structure, and

this compound may be expressed as $X \begin{matrix} \nearrow H \\ \searrow OH \end{matrix}$.