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The effect of washing on the resistance of erythrocytes to hypotonic hemolysis.

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Brinkman and van Dam¹ claim to have shown that the resistance of rabbit erythrocytes to hypotonic hemolysis is increased by washing them in a physiologically balanced solution, while Snapper² publishes data tending to show that washing in 0.9 percent NaCl decreases their resistance. This difference is not necessarily a general one due to the chemical composition of the solutions, as appears from the following experiments:

Blood was taken aseptically by heart puncture from a dog and from four guinea pigs whose blood was pooled. The blood was defibrinated by shaking with fragmented glass. 1.5 cc. of each kind of cell was suspended in 15 cc. of Ringer-Locke's solution lacking phosphate and bicarbonate, and a like amount in 15 cc. of 0.9 percent NaCl. After about ten minutes these, together with samples of undiluted defibrinated blood, were centrifuged, and the supernatant liquid drawn off so as to leave the total volume 15 to 20 percent in excess of that of the cell sediment, which was then stirred up in this volume.

Portions of 0.05 cc. of each of the six lots were added to 2.5 cc. of each of several appropriate dilutions of 0.9 percent NaCl, centrifuged, the supernatant liquid decanted, and the sediment of cells hemolysed in 2.5 cc. of distilled water. The color of both the decantate and the hemolysed sediment was then measured by comparison with a Madsen scale and the amount of hemolysis in percent calculated from these data. The method of calculation and the necessary corrections have been described.³

Curves were drawn showing degree of hemolysis in percent plotted against concentration of NaCl, and the relative concentrations required to produce 20, 40, 60 and 80 percent hemolysis

¹ Brinkman, R., and van Dam, E., *Biochem. Zeitschr.*, 1920, eviii, 35-51.

² Snapper, J., *Biochem. Zeitschr.*, 1912, xliii, 266-74.

³ Brooks, S. C., *J. Med. Res.*, 1920, xli, 399-409.

determined graphically. The resistance may be considered to be the inverse of these concentrations.

Ringer-Locke's solution, as used, lowered the resistance to 99 and 98 percent for dog and guinea pig erythrocytes respectively. The loss of resistance due to washing in Ringer-Locke's solution is so small that it may be considered to be without significance. Washing in a balanced solution must be repeated at least twice, as in the experiments of Brinkman and van Dam, if any considerable effect is to be observed.

NaCl 0.9 percent increased the resistance of dog and guinea pig red blood cells 2 and 20 percent respectively, but so altered the shape of the titration curve that the quantitative significance of these figures is dubious. Some increase in resistance is quite clearly shown.

It is apparent from this that NaCl may under appropriate conditions increase the resistance of erythrocytes, and that therefore the decrease in resistance found by Snapper, and considered by him to have been due to NaCl was quite probably due to mechanical injury from repeated centrifugation (he centrifuged his cells four times). Such injury can be prevented by the addition to the wash fluid of 0.125 percent gelatine.⁴ It is also possible that the effects of NaCl vary according to the duration of its action, subsequent injury reversing a primary increase in resistance such as Brinkman and van Dam produced by the washing out of lecithin in a balanced solution.

⁴ Rous, Peyton, and J. R. Turner, *PROC. SOC. EXP. BIOL. AND MED.*, 1915, xii, 107-8.