Mg./K.) antipyresis of both rapid onset and long duration is obtained.

3. The presence of tolysin in no way augments the toxicity of phenacetin in rats.

8476 C

Permeability of Red Cell Membrane after Hypotonic Hemolysis.

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The generally accepted explanation for hypotonic hemolysis is that the red cells take up water, swell, and finally become permeable because their membranes are stretched or under tension. Conductivity measurements, however, show that the resistance of a hemolysed suspension is such that the cell membranes must have a very low conductance (Fricke and Curtis[1]), an observation which forces one to consider the possibility that the increased permeability associated with lysis is temporary only, and followed by a more or less complete repair of the membrane.

1. The simplest evidence is to be obtained from systems in which "reversible hemolysis" occurs. This phenomenon is observed when several volumes of water are added to blood or washed cells so as to produce more or less complete lysis, and enough hypertonic NaCl to restore the original tonicity added subsequently. The opacity which appears on the restoration of the tonicity was thought by Brinkman and Szent-Gyorgi[2] to be due to the taking up of liberated hemoglobin on or in the cells, but Bayliss[3] showed that it is the result of two occurrences: (a) if the amount of added water is small, swollen but unhemolysed cells shrink to their original size on the re-establishment of the tonicity, and (b), if the amount of added water is great, hemolysed ghosts shrink under the same conditions, and, trapping hemoglobin, bring about optical heterogeneity.

If to 10 cc. of a washed rabbit red cell suspension with a volume concentration of about 0.7 is added 40 cc. of water, a tonicity in the neighborhood of 0.2 is established, and this is low enough to hemolyse virtually all the cells, as can be shown by the hemoglobin con-

[1] Fricke, H., and Curtis, H. J., *J. Gen. Physiol.*, 1935, **18**, 821.

[2] Brinkman, R., and Szent-Gyorgi, A., *J. Physiol.*, 1923, **58**, 204.

[3] Bayliss, L. E., *J. Physiol.*, 1924, **59**, 48.

centration in the supernatant fluid after "reversal" has been produced and the ghosts centrifuged off. One can then calculate the volume which the ghosts ought finally to occupy if they shrink, on the addition of the NaCl which brings about the "reversal", in the same way as the original cells swelled on the addition of the water, *i. e.*, as osmometers of the same degree of perfection. In Table I ρ_1 is the volume concentration of the cells before the addition of n volumes of water, ρ_2 the volume concentration expected if the hemolysed ghosts were to shrink to the volume of the original cells, and ρ_3 the volume concentration of the ghosts as found by spinning the system showing "reversal" in a high speed hematocrite (12,000 r.p.m. to constant volume, which is usually attained within an hour).

TABLE I.

ρ_1	n	ρ_2	ρ_3
0.68	4	0.113	0.105
0.43	4	0.072	0.070
0.80	5	0.114	0.120
0.84	5	0.120	0.115

Allowing for some uncertainty both as regards the calculated tonicities and the hematocrite determinations, the calculated and observed results agree closely. The simplest way of accounting for the return of the ghosts to the original volume occupied by the cells from which they were derived is that the permeability to NaCl of the membrane surrounding the ghosts is very small compared to the permeability to water. Yet at the moment of lysis the state of affairs must have been very different, for a red cell loses pigment into a hypotonic solution which hemolyses it as if the permeability of the membrane to hemoglobin were about $4(10^{-4})$ cm./sec., or as if the membrane had no less than 6 holes of 1μ diameter punched in it (Ponder and Marsland[4]); a membrane so permeable to hemoglobin must be allowed to have had a still greater permeability to NaCl, unless our ideas about red cell permeability and hemolysis are entirely wrong.

2. A concentrated suspension of ghosts can be made by hemolysing one volume of washed red cells with 4 volumes of water, adding one volume of NaCl hypertonic enough to restore the original tonicity (about 5% NaCl), spinning down the ghosts, and finally suspending them in a small amount of isotonic NaCl. The ghosts always contain a small amount of pigment, and it is impossible to remove this more than partially by washing. If a quan-

[4] Ponder, E., and Marsland, D., *J. Gen. Physiol.*, 1935, **19**, 35.

tity of such a ghost suspension, of known volume concentration, is added to hypotonic NaCl and the volume concentration again measured (high speed hematocrite), it will be found that the ghosts swell in hypotonic solutions in very much the same way as do intact red cells, *i. e.*, as "imperfect osmometers" with R-values between 0.5 and 0.8. Again, in fact, they behave as if their membranes were much more freely permeable to water than to NaCl, and as if the highly permeable state associated with hemolysis had been followed by a "repair" of the membrane and a restoration of much of the original impermeability to cations.

3. Fricke and Curtis[1] could not independently determine the volume concentration of the cells in their hemolysed systems, and so were unable to say how completely non-conducting the cells were after hemolysis. In the case of suspensions of ghosts obtained by producing "reversal" of hemolysis, one can measure the volume concentration (a) by hematocrite, and (b) by conductivity, and from the correspondence between the 2 figures can form an estimate of how completely non-conducting the cells are. The volume concentration found by conductivity is obtained from the expression

$$\rho = (r_1/r_2 - 1)/(r_1/r_2 + 1/X)$$

in which r_1 is the resistance of the suspension of ghosts, r_2 that of the fluid in which the ghosts are suspended, and X a "form factor" which can vary between about 1.0 for wholly non-conducting rabbit red cells in their normal discoidal form, and 2.00 for wholly non-conducting spheres. Since the ghosts are crenated objects, there is doubt as to the real value of X, but Table II shows ρ_1, the volume concentration found by hematocrite, ρ_2, that found by conductivity with a value of 1.20 for X, and ρ_3, that found by conductivity with a value of 2.00 for X.

TABLE II.

ρ_1	ρ_2	ρ_3
0.470	0.423	0.472
0.390	0.354	0.400
0.370	0.320	0.365
0.310	0.282	0.324
0.320	0.274	0.315

These figures show that excellent agreement as regards volume concentration would be reached if the ghosts could be considered as non-conducting spheres. Examination of the ghosts, however, shows them to be crenated, but essentially discoidal and certainly not spheres; there is, accordingly, a discrepancy between the results

for volume concentration obtained by hematocrite and the results found by conductivity on the assumption that the cells are altogether non-conducting. This discrepancy, however, is smaller than that which appears in the table between the values of ρ_1 and ρ_2, and probably much smaller, both because the hematocrite tends to give incomplete packing and too large values for ρ_1 and because the form factor for the crenated ghosts is certainly not as small as 1.20. Suppose that the discrepancy in volume concentration really amounts to about 0.02 as a maximum figure after all experimental errors have been allowed for; calculation then shows that it would be accounted for if the ghosts had a specific resistance about 25 times that of the surrounding medium, instead of being completely non-conducting. As the interior of the ghost, like the interior of the intact cell, has a resistance about the same as that of the surrounding medium, and as the surface membrane is certainly very thin, this would mean that the specific resistance of the membrane is of the order of thousands of times that of the suspension fluid, *at least.* Such a result is in keeping with those obtained for the osmotic behavior of the ghosts, and, as it is very difficult to think of the membrane having such a high resistance at the moment of hemolysis, we are again led to the idea of a more or less complete "repair".

Summary. Both the osmotic and the electrical behavior of ghosts obtained by producing the so-called "reversal of hemolysis" in suspensions of red cells hemolysed with water lead to the conclusion that the membranes surrounding the ghosts have a high degree of semi-permeability. Existing evidence makes it very difficult to think of this high degree of semi-permeability prevailing at the instant of hemolysis, and so the idea that the red cell membrane becomes more or less "repaired" after lysis needs to be considered.