virus when subjected to a temperature of 28°C. for 28 days showed a diminution in titre for rabbit skin from 10^{-5} to 10^{-4}. Subsequent storage at 28°C. for a period of 69 days did not produce, however, a further fall in titre. The virus still produced lesions in a dilution of 10^{-4}. It has not been possible to keep the desiccated virus at undiminished titre for longer than 14 days at 37°C.

Although it was not our original purpose to test the potency of this cultivated strain of vaccinia virus in man, it is worthy of note that one previously vaccinated man inoculated intradermally with 0.2 cc. of the culture fluid from the 162d subculture developed a typical vaccinal lesion (accelerated reaction) with vesiculation, rupture of the epidermis and subcutaneous edema, induration, and ecchymosis. The lesion healed by cicatrization without secondary bacterial infection.

The possibility of the application to Jennerian prophylaxis of such a simple, practicable, bacteriologically sterile, and mammalian virus-free method as that perfected by Rivers and his coworkers is worthy of wide trial.

8289 C

Red Cell Size and Resistance to Osmotic Hemolysis.

ERIC PONDER.

From the Biological Laboratory, Cold Spring Harbor, L. I.

In explanation of the fact that the red cells of different mammals show different "fragilities", *i. e.*, different resistances to osmotic hemolysis, it has been pointed out that the extent dA to which the least resistant cell membrane can be stretched without there being a loss of pigment depends on the mean initial cell volume V, so that the ratio dA/V is substantially constant. This purely experimental result can be interpreted as meaning that molecules adjacent to the thin (perhaps bi-molecular) layer upon which semi-permeability depends can enter it when it is stretched, thereby allowing of a certain amount of stretching before lysis occurs (Ponder[1]).* Another well known fact is that different red cells of the *same* animal show different "fragilities", so that a resistance distribution of roughly

[1] Ponder, E., *J. Physiol.*, 1935, **83**, 352.

* It is to be borne in mind that in these experiments the cells were in their spherical form. We have no information as to how the area of the red cell in its discoidal form behaves during swelling.

symmetrical form arises with respect to tonicity. One would expect that here too the resistance might be related to the initial cell volume, and it is with this point that this note is concerned.

The volume which any cell of initial volume V_o reaches in a hypotonic solution of tonicity T is

$$V = V_o[RW(1/T - 1) + 100] \quad (1)$$

V and V_o being both measured in μ^3. In this expression W is the amount of water contained in the cell, expressed as a percentage by volume, and R is a constant which measures either the fraction of the total cell water which is "solvent water" (the view of Parpart and Shull[2]), or the extent to which the cell behaves as a "perfect osmometer" (the view of Ponder and Saslow[3]). The expression supposes that the volume of the hypotonic solution surrounding the cells is very great as compared with the volume of the cell water, as it usually is in fragility experiments. Supposing that the cell hemolyses when V reaches the critical volume V_c; then, if the stretching of the cell membrane is proportional to the initial volume V_o, we have hemolysis when

$$V_c = (0.207\ KV_o + V_o^{2/3})^{3/2} \quad (2)$$

Here the value 0.207 is that of $1/(0.75\pi)^{2/3}$. 4π, and the constant K is the constant in the expression $dA = KV_o$ which expresses the experimental result that the amount of stretching which a given cell can undergo is proportional to the initial volume V_o.

For values of K and of V_o such as are found in experiment, the right hand side of expression (2) is very nearly the same, numerically, as CV_o, where C is another constant which may assume a variety of restricted values. It therefore follows that if the cells of the same animal are distributed (roughly symmetrically) with respect to their initial volumes, both large and small cells will hemolyze in any given tonicity which produces lysis in about the same proportion as those in which they exist in the initial distribution. Thus if frequency distributions of cell volume are obtained in a number of different tonicities in which there are various degrees of hemolysis, the frequency distributions will all be of substantially the same form. This conclusion can be tested in the following way.

Frequency distributions of red cell diameters are obtained for populations of rabbit erythrocytes in isotonic plasma and in plasma sufficiently hypotonic to produce anything from 10% to 60% hemolysis, the cells being converted into their spherical form by the

2 Parpart, A. K., and Shull, J. C., *J. Cell. and Comp. Physiol.*, 1935, **6**, 137.

3 Ponder, E., and Saslow, G., *J. Physiol.*, 1931, **73**, 267.

addition of small amounts of lecithin (see Ponder and Robinson[4]). From the distribution of diameters the distribution of volumes can easily be computed. The method used was that of Ponder and Millar,[5] the cells being photographed under the highest practicable resolution, and measurements being made from the plates. Table I shows the kind of result obtained; in this case the tonicity of the hypotonic plasma was 0.50, and the degree of hemolysis 62%. The number of cells measured for each distribution was 200, and the cells were distributed into groups differing by one division of the measuring scale, which, as the magnification was 580, was equal to 0.17μ. This is the finest grouping permissible, and relatively little is gained, in this particular case, by measuring a greater number of cells.

TABLE I.

Tonicity = 1.0		Tonicity = 0.50	
Diameter, μ	No.	Diameter, μ	No.
4.32	6	5.00	8
4.48	14	5.17	20
4.65	40	5.35	36
4.82	60	5.51	50
5.00	42	5.70	44
5.17	26	5.86	30
5.35	8	6.04	10
5.51	4	6.21	2

For the distribution in undiluted plasma the mean cell diameter is $4.8 \pm 0.018\mu$, with a standard deviation of $0.25 \pm 0.013\mu$. This corresponds to a mean cell volume of $58\mu^3$. For the distribution in hypotonic plasma the mean diameter is $5.56 \pm 0.019\mu$, with a standard deviation of $0.27 \pm 0.014\mu$.† This corresponds to a mean cell volume of $90\mu^3$. The 2 distributions become substantially the same if every cell in the first is increased to 1.155 times its initial radius, or to about 1.5 times its initial volume, which corresponds to an area increase of from 100 to 135%. It is true, theoretically (if the hypothesis that stretching depends on initial volume is correct), that the distribution in hypotonic plasma should show a small skewness as compared with the distribution in undiluted plasma, and this is because the right hand side of expression (2) is not exactly equal to CV_0; the methods used, however, are not sufficiently accurate to detect this small skewness, for the grouping of the cells is necessarily

4 Ponder, E., and Robinson, E. J., *J. Physiol.*, 1934, **83**, 34.

5 Ponder, E., and Millar, W. G., *Quart. J. Exp. Physiol.*, 1924, **14**, 67.

† The standard deviation of the cell diameters in hypotonic plasma ought, strictly speaking, to be 1.115 times that for the diameters in undiluted plasma. The apparent discrepancy is accounted for by the magnitude of the standard errors.

unusually coarse, a limitation being placed on the fineness of the grouping by the fact that measurements cannot be made with a precision greater than about 0.2μ, the limit of resolution. Thus the volume distribution which can be obtained in hypotonic plasma are not capable of deciding whether it is the increase in cell volume, or the stretching of the cell membrane, which is proportional to the initial cell volume.

In the course of the investigation it occurred to me that the final result might be influenced by the fact that all the small (or large) cells of the same animal do not necessarily contain the same amount of W, nor do they all necessarily have the same value of R. I accordingly computed what would happen if the values of RW were distributed with a coefficient of variation of 20%, and if the values were uncorrelated with cell size. The computations are exceedingly tedious, but the result is the same as that stated above: as the tonicity is reduced, there appears in the volume distribution of the intact cells a small skewness which would be experimentally undetectable.

Conclusion. The experimental frequency distributions for red cell volume obtained with spherical forms in hypotonic plasma in which there are varying degrees of hemolysis are compatible either with the idea that the volume at which the cell hemolyses is proportional to its initial volume, or with the idea that the stretching of the cell membrane at the moment of lysis is proportional to the initial cell volume.

8290 C

Nature and Permeability of Grasshopper Egg Membranes. II. Chemical Composition of Membranes.*

THEODORE LOUIS JAHN. (Introduced by J. H. Bodine.)

From the Zoological Laboratory, State University of Iowa.

The secreted membranes surrounding the diapause egg of the grasshopper, *Melanoplus differentialis,* consist of 4 distinct layers: the exochorion, a thin microscopically homogeneous layer; the endochorion, an underlying granular layer; a thin (about 1.5μ) yellow cuticle; and a thick white fibrous cuticular layer.[1] These can be fur-

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