THE PERMEABILITY AND HAEMOLYTIC ACTION
OF THE FATTY ACIDS AND SOME OF THEIR
HALOGEN DERIVATIVES

BY M. BODANSKY.

(The John Sealy Memorial Research Laboratory and the University of Texas
School of Medicine, Galveston.)

(Received 28th June, 1932.)

(With Four Charts.)

A CRITICAL discussion of the methods of studying the permeability of erythrocytes
is contained in a recent review by Jacobs (1). In this review the procedures based
upon the osmotic volume changes are considered and the unique advantages of
the haemolysis method are pointed out in particular. By this method, the behaviour
of enormous numbers of cells is made easily visible to the naked eye, through mere
changes in the turbidity of the cell suspension. This procedure may therefore be
employed as a gross statistical method. Appropriate modifications and improve-
ments in the technique, depending on the nature of the problem, make possible
fairly quantitative results. A second advantage of the method is its great delicacy.
A third is that by means of certain refinements (2) it may be adapted to experiments
whose total duration is only a few seconds.

The possibilities of error are likewise carefully reviewed, the following being
emphasised by Jacobs. Haemolysis may be caused by factors other than osmotic
ones. Environmental factors, such as pH and temperature, produce secondary
osmotic effects and therefore must be properly controlled. Perhaps the most serious
type of error, according to Jacobs, arises from the attempt to draw conclusions as
to the relative rates of penetration of different substances or of the same substance
under different conditions, from the amounts of substances in question which are
observed to enter the cells in some single, arbitrarily selected time. This procedure
he considers objectionable "not merely because different results will, in general,
be obtained according to the time selected for the comparison, but even more
because such differences as are observed may be due rather to changes in what
might be called the 'capacity' of the cell than its permeability." Nor is the difficulty
avoided if the comparisons are made of the times required to reach some given
degree of penetration rather than of the degree of penetration attained in a given
time, owing to the possible simultaneous changes in the permeability of the cell
in the strict sense and in the position of final equilibrium of the system. Notwith-
standing these objections Jacobs expresses the opinion that, when employed with
discretion, the haemolysis method possesses certain unique advantages over all others.
On the basis of previous studies (3) in which the method was based on essentially Ponder's original technique (4), the advantages cited by Jacobs seem justified and may be taken for granted. On the other hand, in considering such problems as the effect of temperature on the permeability of the erythrocyte, the relative penetration of the fatty acids into the corpuscles of different species (dog, man), or the action of substitution products, such as the halogen derivatives, account must be taken of the objections outlined by Jacobs. Obviously the application of the haemolysis method to such problems will depend on whether the limitations preclude the possibility of drawing conclusions from the experimental results.

For example, as regards the effect of pH as an environmental factor, small differences are very significant, as indicated by the following illustrative data (Table I). On the one hand, these data show that, when the concentration of the acid is diminished, the rate of haemolysis is reduced. If the series of dilutions is sufficient, it becomes possible to establish for these and other acids the relation of haemolysis to concentration (and pH). That the results thus obtained would also define the relation as regards permeability in a strictly quantitative manner cannot be expected. Aside from other factors, it is clear that in the higher concentrations the surface effect of the acid will be significant, whereas in very low concentrations most, if not all, of the acid will be neutralised by the cell buffers. In the latter case there is superimposed upon the effect of the free acid as a simple lysin the effect of the neutralised acid. It should be noted, however, that even when the acid is wholly neutralised, the osmotic pressure within the cell is such that the cell volume increases, an already weakened membrane is stretched, with the resultant production of slow lysis (Ponder (5), Bodansky (3), 1931). Accordingly, the relations of concentration to permeability of the fatty acids, determined by the haemolysis method, are at best semi-quantitative, but as such are nevertheless of value in a general consideration of the problems of cell permeability.

Table I. Time required to produce complete haemolysis of a standard red blood cell suspension (human) at 25° C.

<table>
<thead>
<tr>
<th>Acid</th>
<th>n-Valeric</th>
<th>n-Caproic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial pH of system</td>
<td>2.94 3.93 3.22</td>
<td>3.04 3.23 3.38</td>
</tr>
<tr>
<td>Time (min.)</td>
<td>0.11 1.50 1.10</td>
<td>Inst. 1.45 1.65</td>
</tr>
</tbody>
</table>

To consider a second environmental factor—temperature—it is well known that haemolysis is accelerated with increasing temperature. Thus in an approximately 0.02 molar solution (made isotonic with saline) of valeric acid, a standard suspension of dog's corpuscles was completely haemolysed in 4.83 min. at 25° C., and in 1.9 min. at 37.5° C. A similar suspension of human cells required 11 min. at 25° C., and 2.16 min. at 37.5° C. Similar relations were observed for other fatty acids, including caproic. If the relative concentration gradients are determined for the latter fatty acid, according to a procedure outlined by Taylor (6), the following data are obtained (Table II).
The Permeability and Haemolytic Action of the Fatty Acids

Table II.

<table>
<thead>
<tr>
<th></th>
<th>Initial pH at which complete haemolysis was produced in</th>
<th>Relative gradients of caproic acid penetrating the red blood corpuscle and producing haemolysis of a standard suspension in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min. at 2 min. at</td>
<td>1 min. at 2 min. at</td>
</tr>
<tr>
<td>Dogs cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3°29</td>
<td>3°21</td>
<td>1°0 0°628</td>
</tr>
<tr>
<td>3°39</td>
<td>3°31</td>
<td>1°0 0°628</td>
</tr>
<tr>
<td>3°35</td>
<td>3°25</td>
<td>1°0 0°628</td>
</tr>
<tr>
<td>3°38</td>
<td>3°38</td>
<td>1°0 0°628</td>
</tr>
</tbody>
</table>

The calculations were based on the value 1°4 x 10^-4 for the dissociation constant of caproic acid, as given in the International Critical Tables. The relative gradients at different temperatures have also been determined for the other members of the saturated fatty acid series, but caproic acid has been selected for this illustration because it comes nearer than its lower homologues in producing relatively little injury to the cell membrane, largely by virtue of the small concentrations required. However, the acid is present in sufficient concentration, so that only a small part is neutralised by the cell buffers. If the assumptions, on which the determination of the relative gradients are based, are correct, it would appear that the permeability of the erythrocyte (dog, man) for caproic acid is nearly twice as great at 37° as it is at 25° C.

Although care was exercised in making the determinations as quantitative as possible, a more precise formulation of the results does not seem to be justified, partly in view of the previously stated contention of Jacobs that it is inaccurate to draw conclusions as to the relative rates of penetration of different substances, or of the same substance under different conditions, from the amount of material observed to enter the cells in some single arbitrarily selected time. In the case of the fatty acids, the assumption is that where the effect of injury to the surface may be largely excluded, the haemolysis depends upon the entrance into the cell of a certain amount of acid. This is essentially the basis upon which the relative concentration gradients of the various fatty acids were determined in an earlier study. If the results, given previously, are recalculated, with 1°0 as the gradient for butyric acid, the following values are obtained (Table III).

Table III. Relative concentration gradients of acids penetrating the red blood corpuscle at 25° C, and producing haemolysis of a standard cell suspension.

<table>
<thead>
<tr>
<th>Acid</th>
<th>In 1 min.</th>
<th>In 5 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric</td>
<td>1°00</td>
<td>1°00</td>
</tr>
<tr>
<td>Isobutyric</td>
<td>0°995</td>
<td>1°00</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0°477</td>
<td>0°60</td>
</tr>
<tr>
<td>Valeric</td>
<td>0°294</td>
<td>0°43</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0°154</td>
<td>0°25</td>
</tr>
<tr>
<td>Caproic</td>
<td>0°110</td>
<td>0°22</td>
</tr>
<tr>
<td>Heptolic</td>
<td>0°037</td>
<td>0°053</td>
</tr>
<tr>
<td>Caprylic</td>
<td>0°00116</td>
<td>0°0012</td>
</tr>
<tr>
<td>Pelargonic</td>
<td>0°000244</td>
<td>0°0005</td>
</tr>
</tbody>
</table>
These data illustrate the validity of Jacobs' contention that determinations based on the amount of a substance observed to enter the cell will differ according to the time selected for the comparison. Though this is admitted, it does not follow that results obtained in this way are without significance. Thus, a comparison of these data with the solubility of the fatty acids in lipoids brings out a definite relationship. Moreover, it may be judged from these data, that Traube's rule, which seems to hold for several of the lower fatty acids, does not apply to the higher members of the series. According to Traube's rule, it might be expected that the permeability of heptonic acid, as compared with butyric, should be approximately $3^3 = 27$ times as great. According to the data in the table, it was found to be 19 times as great for the 5 min. observation and 27 times for the 1 min. observation. The attraction intensity of caprylic acid, according to Traube's rule, should be about 81 times that of butyric acid, and for pelargonic acid it should be 243 times as great. For the 5 min. observations, the data based on the haemolysis measurements showed caprylic acid to have 833 times, and pelargonic acid approximately 4100 times the attraction intensity of butyric acid. Even without considering
The Permeability and Haemolytic Action of the Fatty Acids

that a certain proportion of the fatty acid was neutralised by the buffers within the erythrocyte, these values indicate the enormous attraction which exists between the red blood corpuscle and the more non-polar fatty acids in solution. It is doubtless because of this property that the erythrocyte plays such an important part in the transportation of fat in the organism. Moreover, the behaviour of the fatty acids, even in very low concentrations, suggests that physiologically these substances may be concerned with the process of red cell disintegration—an idea which is not new.

Chart 2. Relations are as in Chart 1. Isobutyric and α-bromoisobutyric acids.

Human red cells are haemolysed more slowly than dog's corpuscles by similar concentrations of fatty acids. Thus in an approximately 0.01 molar solution of caproic acid, complete haemolysis of a standard suspension of dog’s erythrocytes occurred in 2.92 min., whereas a similar suspension of human cells required 16.5 min. On the basis of a large number of observations, the concentration gradient for caproic acid was found to be approximately 50 per cent. greater for human than for dog's corpuscles. This difference in behaviour is probably not related to an actual difference in permeability, but to a difference in the buffer value of the two kinds of cells, as was determined in an earlier study by means of potentiometric measurements.
In summarising the behaviour of the fatty acids from the standpoint of both haemolytic action and permeability, certain features stand out. The results are reproducible from time to time. The relations existing between the fatty acid homologues are constant within very narrow limits. For a given fatty acid, the relation of concentration to haemolytic effect is fairly well defined. The halogen derivatives of these acids, on the other hand, do not show these predictable relationships.

Halogen derivatives of the fatty acids. From the standpoint of their permeability, the derivatives of acetic acid are of comparatively little interest. The concentration of acetic acid required to produce moderately rapid haemolysis is so high that a considerable share of the effect is due to the injury of the cell membrane. The order of lipid solubility of these acids is:

acetic < chloracetic < bromacetic < dichloracetic < trichloracetic < iodoacetic.

For most concentrations this is also the order for their haemolytic effect and probably for their permeability through the red cell membrane. A striking feature of the behaviour of the halogen compounds is that there is no constant relation of concentration to the rate of red cell destruction, a higher concentration often showing less effect than a lower concentration. This peculiarity has been observed.
The Permeability and Haemolytic Action of the Fatty Acids

repeatedly, both in human and dog’s corpuscles, at 25°C, as well as at 37°C. It can hardly be regarded as accidental and is probably associated with peculiarities in the surface activity of these compounds.

The order of lipoid solubility of propionic acid and some of its halogen derivatives is:

propionic < β-chlorpropionic < β-brompropionic < α-brompropionic.

Their haemolytic effect is in general in the same order, and shows peculiarities similar to those noted for the acetic acid derivatives.

Permeability is a dominant factor in haemolysis by butyric acid, but it is quite probable that this is not true of its α-halogen derivatives, owing to their high dissociation constants. Chart 1 shows the relations for butyric acid and its α-brom derivative. It will be noted that, for a given molar concentration (0.05), haemolysis is much more rapid with the halogen compound than with butyric acid. The ratio of their oil-water distribution coefficients (for a 0.1 molar concentration) is:

\[
\frac{\text{α-brombutyric}}{\text{butyric}} = \frac{1.29}{0.656} = 1.956,
\]

and the ratio of the true partition coefficients is:

\[
\frac{1.39}{0.596} = 2.32.
\]
The relations for isobutyric and α-bromisobutyric are represented in Chart 2. Chart 3 shows the relations determined for valeric and α-bromvaleric. The curves for n-caproic and the corresponding α-brom derivative are given in Chart 4.

A study of these and a much larger number of similar observations leads to the conclusion that even an approximately quantitative evaluation of the permeability of the halogen derivatives of the fatty acids cannot be made by the haemolysis method. Undetermined surface effects complicate the situation. That the cells take up the halogen derivatives more rapidly than the fatty acids is, however, quite obvious and is to be expected from the greater solubility of these compounds in lipoids.

SUMMARY.

The permeability of the red blood corpuscle for fatty acids has been studied by the haemolysis method. It has been found that in spite of certain limitations the method may be applied to the study of various factors, such as the effects of pH, concentration, temperature and buffer content of the corpuscles. If account is taken of the possibilities for error and the experiments are adequately controlled, the results have a quantitative significance.

For a given concentration the halogen derivatives are more effective than the fatty acids in producing haemolysis, but no definite relationship of concentration to haemolytic effect has been determined for these compounds. They are apparently taken up by the red cells more readily than the fatty acids, as might be expected from their greater solubility in lipoids. It has not been possible, however, quantitatively to evaluate these differences in permeability.

REFERENCES.