one-half of the increase in total fatty acids, which at the end of 4 hours amounted to 1.27 millimols per 100 cc. of whole blood, or 2.3 g. of fatty acid (in terms of oleic acid). Considering the red blood corpuscles alone, the average maximum increase of the unsaturated fatty acids was .68 millimols, which is only about one-third of the total fatty acid increase (average of 1.92 millimols).

It is difficult to explain why after the administration of olive oil the increase in total and saturated fatty acids, particularly in the corpuscles, should be greater than the increase in oleic acid. This observation, first made by the author in 1924, is in agreement with a similar finding of McClure and Huntsinger[3] that the increased concentration of lipids in the blood which follows the absorption of oleic acid is not solely the result of accumulation of this substance. Either the oleic acid undergoes saturation, or what is more likely, saturated fat is mobilized from the fat depots during fat absorption.

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Relation of Hemolysis to the Primary Penetration of Fatty Acids Through the Red Cell Membrane.

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The hemolytic action of fatty acids may be attributed to the combined effects of injury of the erythrocyte at its surface and secondary penetration. It is obvious, however, that in high concentrations of the hemolytic agent, the damage to the membrane may in itself be sufficient to cause the dissolution of the cell, in which case the factor of permeability is of negligible importance. On the other hand, if the concentration is sufficiently low, the change in the red blood cell membrane may be so slight that a condition is approached in which primary penetration of the fatty acid may be regarded as the sole factor involved in the process.

That under certain conditions measurement of the rate of hemolysis also determines the rate of permeability of the hemolytic agent has been considered elsewhere[1] and is supported by the observation that the hemolytic action of fatty acids parallels their behavior in a

[3] McClure, C. W., and Huntsinger, M. E., *J. Biol. Chem.*, 1928, **76**, 1.

[1] Bodansky, M., *J. Biol. Chem.*, 1928, **79**, 241.

wide variety of biological phenomena in which primary penetration of these acids into tissues is involved.

Another test of the osmotic nature of the process may be made by varying the tonicity of the hemolytic system. It has been shown previously[2] that increasing the osmotic concentration of the fluid outside the corpuscles has little effect in altering their resistance to the hemolytic action of inorganic acids, which makes it seem probable that injury to the membrane is the primary factor involved. On the contrary, alterations in the concentration of the outside fluid exert a marked effect in hemolysis by fatty acids, which indicates that here penetration into the corpuscle is the predominant factor. This is illustrated in the accompanying chart, which represents the relation of tonicity (expressed in per cent NaCl) to the resistance of dog's corpuscles to the hemolytic action of fatty acids.

For a given tonicity, the ratio $\frac{\text{time required for complete hemolysis}}{\text{time required for complete hemolysis in a system isotonic with 0.85\% NaCl}}$ has been taken to represent the resistance. The circles represent data obtained in systems containing 0.04 N butyric acid; the squares, data obtained with 0.02 N valeric acid; the triangles, the data with 0.008 N caproic acid.

As is evident from the chart, the hemolytic effect is profoundly modified by changes in the osmotic concentration of the outside fluid. Thus, in a system equivalent to 0.68% NaCl (0.8 isotonic), the resistance of the erythrocytes to fatty acids is only about half the resistance in an isotonic solution. In a system equivalent to 1.2% NaCl, the resistance is increased approximately 50%.

Because of the independent effect of sodium chloride on erythrocytes, the data obtained in strongly hypertonic systems are not included in the present series of experiments. To illustrate the effect of exposure to hypertonic saline, the following experiments may be described.

Exp. 1. Cells were suspended in 2 x isotonic saline for 5 minutes, then washed and suspended in isotonic saline. The value of R (resistance) of the resulting suspension was 0.95. The control suspension was prepared by treating cells similarly for the same period of time, but with isotonic saline only.

Exp. 2. Cells were suspended for 2-3 minutes in 5 x isotonic saline, then washed and resuspended in isotonic saline. The resistance of these cells was found to be 0.756 to caproic acid (0.008 N) and 0.744 to valeric acid (0.02 N).

It should be noted that the technique employed in the present

[2] Bodansky, M., *J. Biol. Chem.*, 1928, **79**, 229.

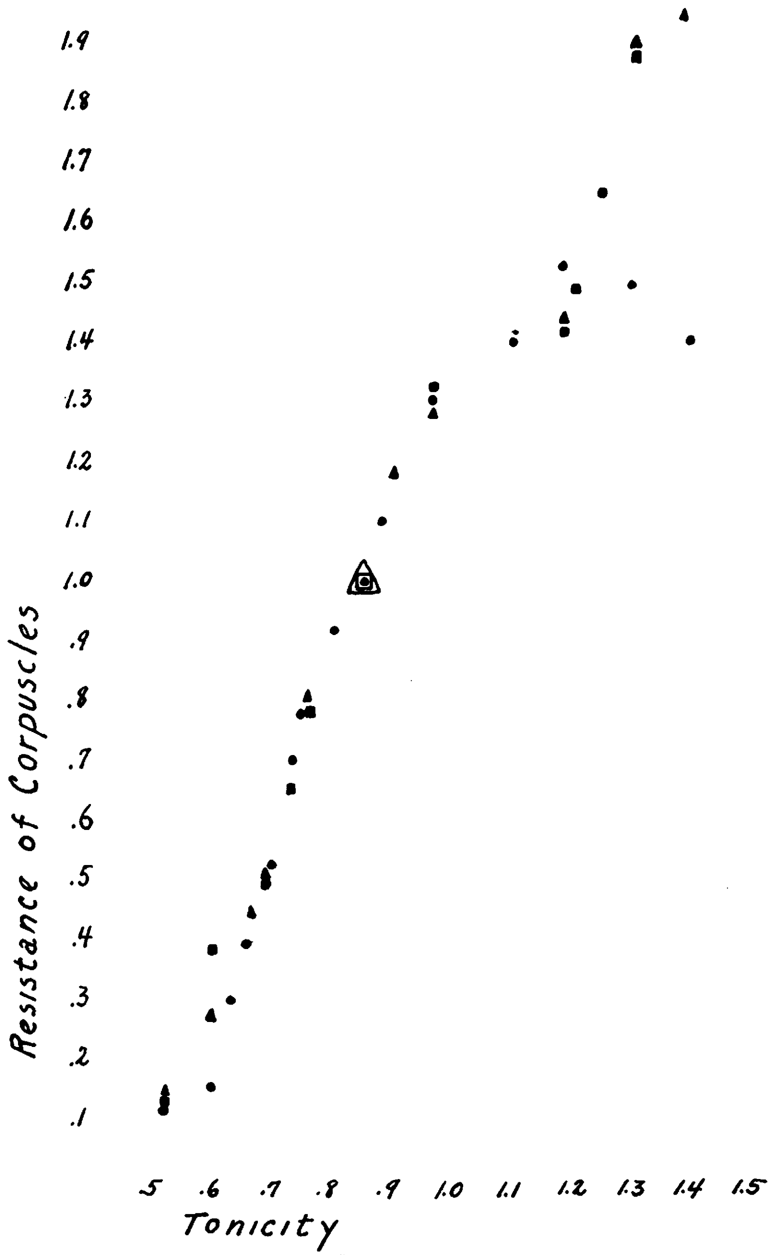

FIG. 1.

study is similar to that used in earlier work[1, 2, 3] and is based on methods originally devised by Ponder.[4]

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[3] Bodansky, M., *J. Biol. Chem.*, 1929, **82**, 567.

[4] Ponder, E., *Proc. Roy. Soc. London, Series B*, 1921, **92**, 285.