Summary. An approximate curve representing the distribution of susceptibility to hemolysis among the erythrocytes of a given population is obtained by differentiation of the von Krogh equation. The von Krogh constant 1/n is shown to be an inverse measure of the uniformity of distribution. Variations in the value of 1/n are concluded to be indicative of variations in the uniformity of the cell suspension with respect to its susceptibility to hemolysis.

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Bacteria and Cellular Activities. I. Effect of *Streptococcus beta-hemolyticus* on Permeability of Chicken Erythrocytes.*

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In order to obtain a better understanding of some of the changes which may take place in cells under the influence of bacteria and their products, a series of experiments is being undertaken. The initial studies involved changes in the permeability of erythrocytes in the presence of a hemolytic bacterium.

Ponder[1] has reviewed the older literature on this general subject. Many similar experiments have been performed since (Maizels[2]). In all of the work cited interest was centered on the actual hemolysis of the erythrocytes. In the present investigation the permeability of the cells prior to hemolysis was considered.

The following data then are of interest not only with reference to the general problems mentioned, but also emphasize the care which must be exercised to eliminate the possible effect of bacterial contamination in permeability studies of erythrocytes.

Procedure. The test organism, *Streptococcus beta-hemolyticus* was secured from the American Type Culture Collection. It was seeded on proteose No. 3 agar and incubated for 24 hours at 37.5°C. An aliquot of the inoculum removed from the medium by washing with sterile Ringer-Locke solution was added to an equal volume of heparinized chicken erythrocytes obtained by cardiac puncture. This suspension of erythrocytes and bacteria was incubated at 37.5°C. A control suspension was made up in a similar manner except that the organisms were omitted. Aliquots were removed at varying intervals of time, and the permeability of the erythrocytes to glycerol was measured by the photronic cell technique employed by one of the authors (Hunter[3]). Change in volume of the erythrocytes alters the amount of light transmitted to the photocell, hence the current induced and the "scale reading" of the galvanometer. The volumes of the erythrocytes may be routinely varied (1) by placing them in an isotonic solution of a penetrating substance (0.3 M glycerol) which ultimately results in hemolysis or (2) by placing them in a hypertonic solution of a penetrating substance in Ringer-Locke solution (0.3 M glycerol in Ringer-Locke solution), which produces a rapid shrinkage and a subsequent swelling. The light transmission is increased both by swelling and by hemolysis. In the hypertonic solution swelling alone is meas-

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1 Ponder, E., *The Mammalian Red Cell and the Properties of Haemolytic Systems*, Berlin, 1934.

2 Maizels, M., *Quart. J. Exp. Physiol.*, 1946, **33**, 183.

3 Hunter, F. R., *J. Cell. and Comp. Physiol.*, 1936, **9**, 15.

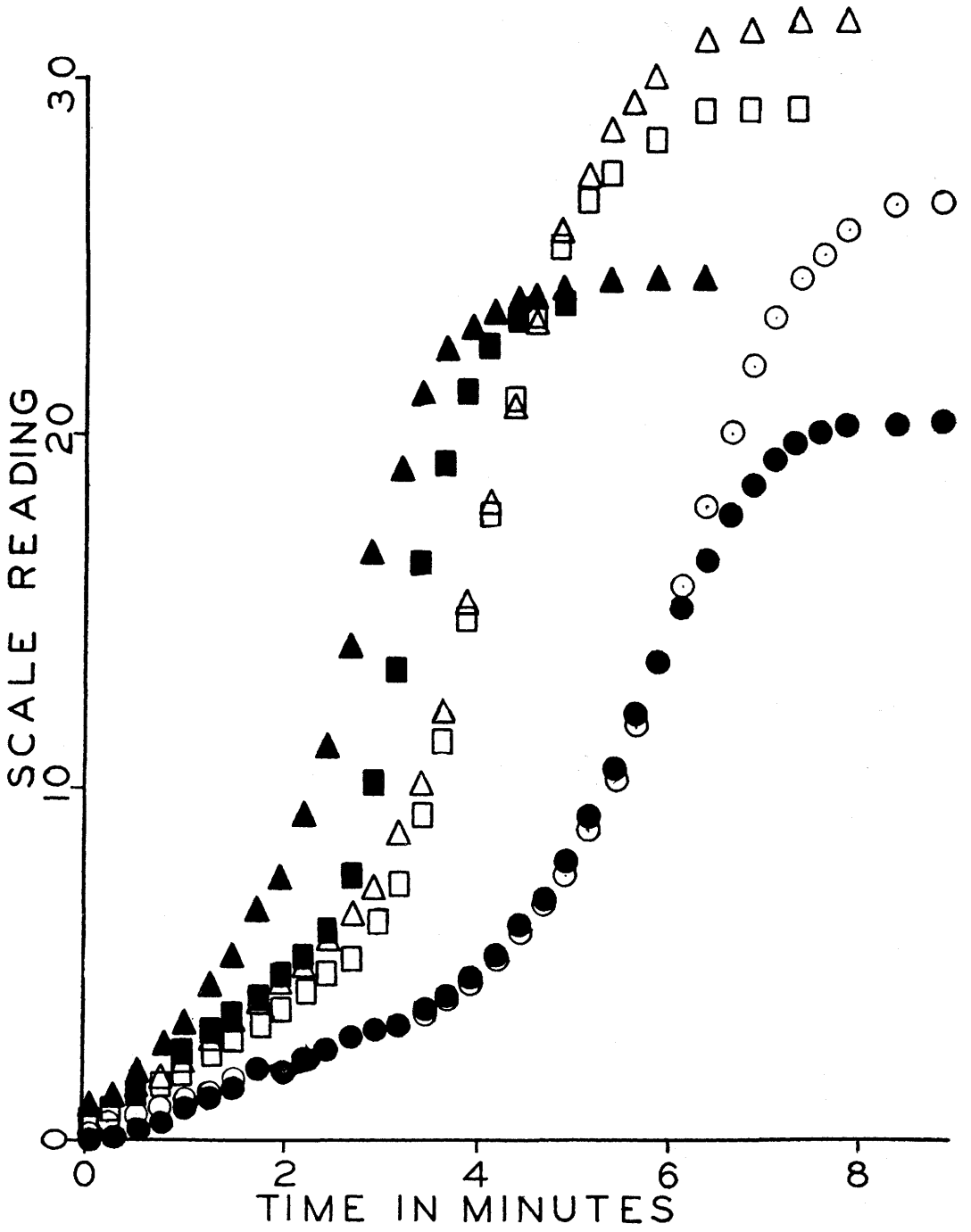

FIG. 1.

Effect of time of exposure to test organism on the hemolysis of chicken erythrocytes in glycerol. ○—control, 0 hr; ●—experimental, 0 hr; □—control, 12 hr; ■—experimental, 12 hr; △—control, 24 hr; ▲—experimental, 24 hr.

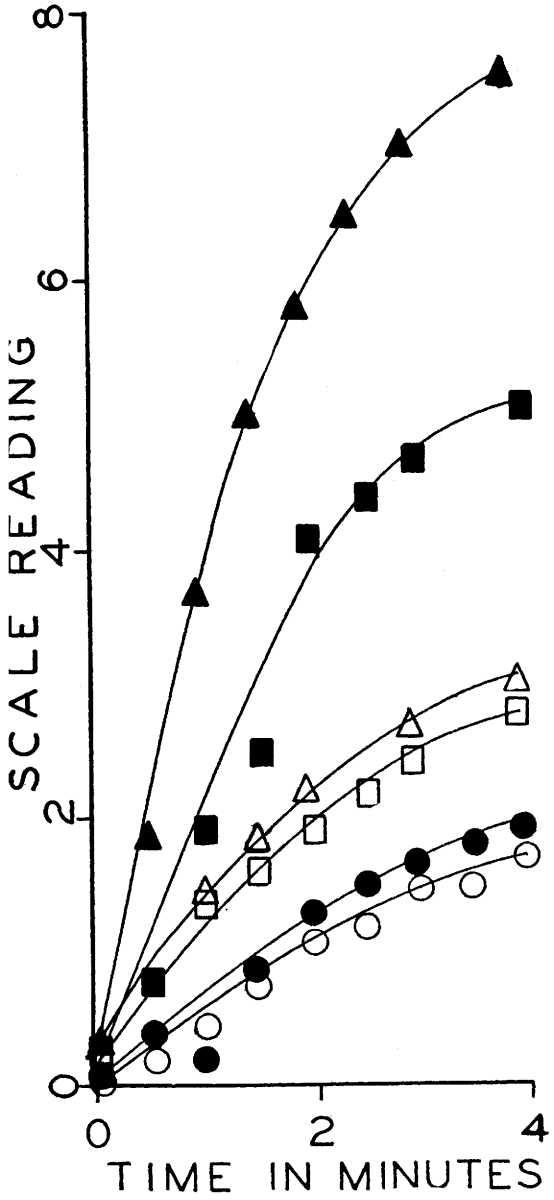

FIG. 2.
Rate of swelling of chicken erythrocytes exposed to test organism for varying times in 0.3 M glycerol in Ringer-Locke solution. ○—control, 0 hr; ●—experimental, 0 hr; □—control, 12 hr; ■—experimental, 12 hr; △—control, 24 hr; ▲—experimental, 24 hr.

ured. In the isotonic solution swelling precedes hemolysis, but since the light changes associated with hemolysis are so much greater than those due to swelling, the hemolysis curves are almost completely a consequence of the loss of hemoglobin from the cells.

Plates were poured to ascertain whether or not contamination had occurred: (1) when the blood was drawn, (2) after the erythrocytes had been centrifuged, (3) at the completion of each experiment, and (4) on the Ringer-Locke solution just before use. Only data from experiments which were free of contamination are considered. After the aliquot had been removed for the permeability studies further sterile precautions were not taken. Contamination introduced during the ten minutes or less required for the measurements would have had a negligible effect.

Results. In general exposure to bacteria for several hours increased the permeability of the cells to glycerol and decreased the time for hemolysis. (Fig. 1) To determine whether or not the change in hemolysis time was actually a consequence of a change in permeability, the rate of swelling was measured simultaneously in a hypertonic solution of 0.3 M glycerol in Ringer-Locke solution. The swelling curves are shown in Fig. 2. The decrease in the hemolysis times varies directly with the increase in the rate of swelling. As a final check fragility measurements were made. Fig. 3 shows that cells exposed to the test organism for 7 hours are not more fragile than control cells.

Discussion. The time for hemolysis and the rate of swelling of the control cells change over a period of many hours. Such a change has been noted in previous work (Hunter[4]), and might well be predicted on the basis of observations such as those of Jacobs and Parpart.[5] Preliminary experiments indicate that this change results at least in part, from volume changes of the cells. It is clear, however, that with erythrocytes the change is more marked in the presence of the test organisms.

Prior to hemolysis a gradual alteration of the cell membrane is induced by the test organism. This is indicated by the change in permeability to the non-electrolyte, glycerol. Hence, in permeability studies in which

[4] Hunter, F. R., *J. Cell. and Comp. Physiol.*, 1947, **29**, 301.

[5] Jacobs, M. H., and Parpart, A. K., *Biol. Bull.*, 1931, **60**, 95.

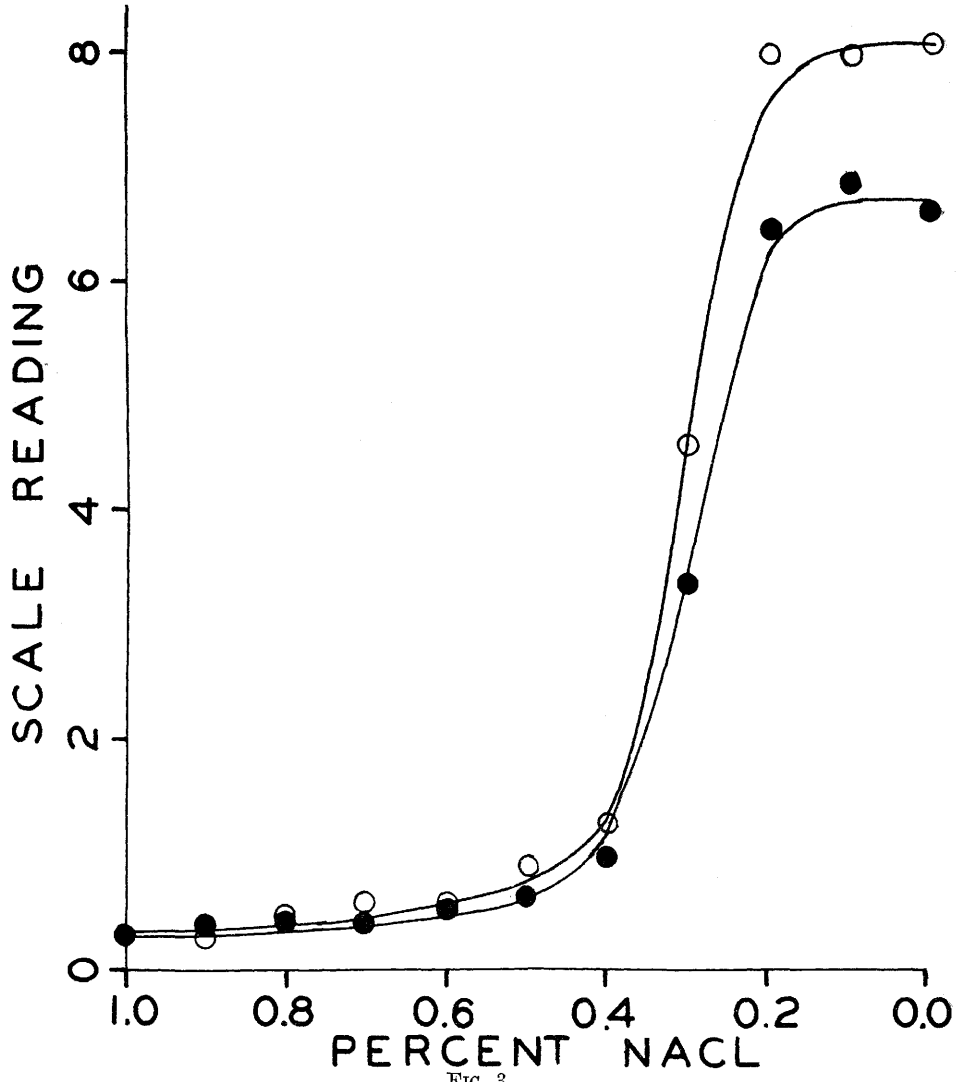

FIG. 3.

Fragility of chicken erythrocytes. ○—control; ●—cells exposed to the test organism for 7 hr.

erythrocytes are maintained for several days under conditions favoring sepsis, the possible effect of bacterial action on the cell membranes must be considered.

Investigations of this nature are being continued to determine the generality of change in the permeable properties of cells under the influence of bacterial toxins.

Conclusions. 1. The permeability to glycerol of chicken erythrocytes exposed to a suspension of *Streptococcus β-hemolyticus* was increased after several hours of exposure to the test organism.

2. Experimental cells were no more fragile than controls.

3. The possible theoretical implications of this work are indicated.