prodigiosus; while 3 others were put in the incubator without inoculation. After 24 hours the plates were removed. No growth at all had occurred on the 4.4 plate; on the other two, selective action had occurred as usual (see Fig. 2). The agars from each half of the plates were then carefully removed and their pH's taken. The results are recorded in Fig. 2. The pH of the dye containing agar was in every plate almost identical with that of the plain agar.

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The Use of Equations of the n-th Order to Describe the Action of Simple Haemolysins.

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In all recent work concerned with the fitting of formulae to curves obtained for the action of the simple haemolysins it has been assumed that the "fundamental reaction" between the cells and the lysin is one in which the latter combines with some component (probably protein) in the membrane of the former, thus forming a new compound as the result of the formation of which the integrity of the cell is destroyed. Thus, the quantity of the cell component, S, destroyed, is proportional to the quantity of lysin, x, used up in the system, and the velocity of the reaction is given by

$$(1) \quad dx/dt = k\,(c - x)$$

whence

$$(2) \quad t = \frac{1}{k} \log \frac{c}{c - x}$$

where c is the initial quantity of lysin (in milligrams), where t is the time required to produce lysis of an arbitrary number of red cells, and where S is large compared to c. Since it is assumed that the complete lysis of n cells corresponds to the utilization of a constant quantity of lysin, we obtain, by putting $x = \text{const.}$, and varying c in (2), a relation between the time for complete lysis of n cells and c, the initial concentration of lysin; when plotted, this relation gives the "time-dilution curve" for any particular lysin. If we are concerned with the number of cells, N, haemolysed from moment to moment by a particular concentration of lysin, from the beginning

of the reaction until its completion, we solve (2) simultaneously with

$$(3) \quad N = N_0 \int_0^x e^{-b^2x^2} dx$$

and obtain the S-shaped "percentage haemolysis curves".

For certain haemolysins under certain conditions, these expressions describe the experimental results excellently. Recently, however, we have examined the action of several simple lysins over very much longer periods than previously, observing the time-dilution and percentage haemolysis curves over periods as long as 300 minutes and as short as 6 seconds. When observations are extended in this way, it is plain that the above expressions require some modification, especially in 2 respects: (i) In the time-dilution curves the high concentrations of lysin produce haemolysis more rapidly than indicated by (2), and the low concentrations of lysin produce complete lysis after long times (100 to 300 minutes) when, according to expression (2), they should never produce complete haemolysis at all. Both these discrepancies were pointed out when the expressions given above were proposed as first approximations. (ii) The percentage haemolysis curves obtained in experiments with which low concentrations of lysin are used show far greater skewness (expressed by the ratio of the time required for 50% haemolysis to that required for 100% haemolysis) than the simultaneous solution of (2) and (3) provides for. This discrepancy is not easily detected, for technical reasons, and has hitherto escaped observation.

Suppose, however that the lysins concerned (saponin, the soaps, the bile salts, etc.) do not exist in a perfectly dispersed state, but that they exist in the form of aggregates of molecules of varying sizes, as may be expected from their semi-colloidal nature. Then an aggregate of 1, 2, 3 . . . molecules may react with each molecule of the cell component, S, and if each such molecule of S requires the interaction of a number of lysin molecules (say 6) it may obtain them by 6 additions of aggregates of 1, 3 additions of aggregates of 2, 2 additions of aggregates of 3, one addition of an aggregate of 6, or any one of a number of combinations of these possibilities. If, for example, the lysin existed in aggregates of 6, then the expression

$$dx/dt = k\,(c—x)$$

would describe the velocity of the reaction as in (1), but if it existed in aggregates of 3 only, then

$$dx/dt = k\,(c—x)^2$$

would give the velocity.

Thus, in general, if the lysin were to exist in aggregates of varying numbers we should have the velocity given by

$$(4) \quad dx/dt = k\,(c-x)^n$$

whence, if $1/p = n$,

$$(5) \quad kt = \frac{p}{p-1}\left\{c^{\frac{p-1}{n}} - (c-x)^{\frac{p-1}{n}}\right\}$$

in which n would be a measure of the mean state of aggregation of the lysin together with the mean number of combining molecules, and would have a meaning somewhat similar to that of the index n in Hill's equation for the dissociation of oxyhaemoglobin. The value of n in (4), moreover, will in general be greater than unity, and will not, as a rule, be a simple integer.

An expression such as (5) has been found experimentally to describe the action of all of the simple haemolysins examined. The fit of the calculated curves to the theoretical points is almost perfect and greatly superior to the fit obtained with the formulae used hitherto. As might be expected from the nature of the hypothesis on which the formulae are based, different values for n are found for different lysins under the same conditions, *e. g.*, for saponin $n = 2$; for sodium taurocholate $n = 1.8$; and for sodium oleate $n = 1.1$ (approximately, at 25°, with human red cells). Further, these values are altered by changes in temperature, electrolyte content, etc., which may be easily imagined to change the state of aggregation of the lysins, and although we do not at present consider the expressions given to have much more than empirical significance, such variations in n suggest that the hypothesis above is sound. It must be observed, however, that a similar formula could be arrived at by assuming that the molecules of the cell component react in various numbers with lysin molecules, and that a combination of both assumptions may ultimately be necessary.

It is of considerable interest that expression (5), together with (3), also describes the kinetics of certain complex haemolytic systems, *e. g.*, of complement-amboceptor systems. Thus, if the quantity of amboceptor is constant, (5) describes the relation between the time required for complete lysis and the concentration of complement c, provided that certain technical precautions are observed, while the combination of (5) and (3) gives the percentage haemolysis curves. In this system the value of n is usually about 1.3. The fact that these expressions apply to this form of lysis lends support to the views of Eagle and Brewer, *i. e.*, that the complement acts as a lysin and that the amboceptor merely "mobilizes" it at the cell surface.