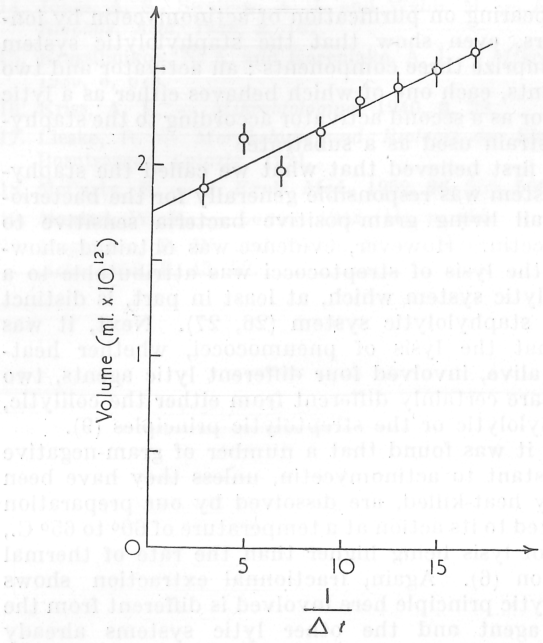

FIG. 3. — Plasmolysis of protoplasts in polyethylene glycol solutions as a function of the osmotic pressure of the medium. The volume of the plasmolysed protoplasts is plotted against the reciprocal value of the corresponding freezing point depression of the solution. The straight line is drawn by the method of least squares.

changed when the free protoplasts are formed. Hence the protoplasts cannot be regarded as strictly equivalent to the protoplasm in the cells, but some tentative conclusions concerning the structure of the living bacterium may nevertheless be justified. The validity of such conclusions is strengthened by the fact that the osmotic experiments with protoplasts and the permeability experiments with whole cells, as described above, indicate that the permeability of the two kinds of bodies is qualitatively the same in regard to sucrose, phosphate and urethan. Moreover, as has been shown earlier (1), the living cell and the protoplast have an identical endogenous respiration and oxidise glucose at the same rate.

The existence of a semipermeable membrane at the surface of the protoplasm in the living cell is strongly suggested by the fact that the volume changes of the protoplasts in heterotonic solutions follow the modified Boyle Van't Hoff law. The membrane may be very thin and soluble in lipid solvents; this would explain why a protoplasmic surface structure is not seen in electron micrographs of sectioned bacteria (6).

The volume of the osmoticly inert substances, *i.e.* the quantity b in the equation $P(V - b) = \text{constant}$ has been found to represent about 35 % of the volume of the protoplasm of the living *B. megaterium* cells. A comparison with the weight and volume of the desiccated protoplasmic constituents, obtained by simple gravimetric analyses and specific volume determinations, show that the osmoticly inert substances besides high-molecular weight compounds also consist of bound water.

The remaining part of the protoplasm should then represent the 'free space' inside the cells, containing osmoticly transferable, free water and dissolved low-molecular weight substances.

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The multiplicity of bacteriolytic agents in actinomycetin

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It is evident from Dr. Salton's report that enzymes from *Streptomyces* spp. are a useful tool for the study of bacterial structures. Therefore, it is not out of place to discuss briefly, in this symposium, the nature of the lytic systems involved.

The ability of many actinomycetes to dissolve bacteria was first incidentally observed in 1921 by Lieske (17), independently re-discovered, in 1924, by Gratia and Dath (11), and studied by Gratia and his collaborators between 1924 and 1934 (13). During that period, occasional observations of the same type were made by Rosenthal, in 1925 (21), and by Borodulina in 1935 (4). In 1936, one of us started a study of the bacteriolytic activities to be found in active culture-filtrates from actinomycetes (24), to which the name actinomycetin (23) was given : the study has been going on in his laboratory

ever since. At about the same time, contributions to the subject were published by Krassilnikov and Koreniako (15) and by Kriss (16).

However, the present interest in bacteriolytic enzymes from actinomycetes developped at a later date, when three new teams of workers, enterered the field : in 1948, Webb and his collaborators (14, 20) ; in 1951, van Heyningen and his collaborators (3, 22) ; and lastly, in 1952, McCarty (18). This renewal of interest in the question seems to have been stimulated by Maxted's paper (19) showing that lysis of streptococci by *Streptomyces* enzymes is a good means of releasing streptococcal group antigens, a particular example of a general principle reported earlier by Gratia and Dath (12) and later confirmed by one of us (5).

Different organisms and techniques being used by the different workers who, in addition, are stimulated by interests of various kinds, it is very difficult to validly compare their results. In the following, we shall endeavour only to show very briefly that our own actinomycetin, that is a suitable culture-filtrate from our *Streptomyces albus* strain G, contains a number of different principles acting specifically upon given bacterial species or even strains.

At first, only two types of activity were distinguished : lysis of heat-killed bacteria on the one-hand, lysis of living gram-positive bacteria on the other hand. The typical substrate used for the study of the former activity being heat-killed *Escherichia coli*, the responsible agent was called the colilytic principle. The properties of this agent clearly show that it is a protein and an enzyme (24). A number of facts suggest that it is not identical with the many proteases and other enzymes (1, 2, 10, 25) to be found in actinomycetin and direct evidence in favour of this point was later on obtained by Ghuysen. The typical substrate used for studying the lysis of living bacteria was *Micrococcus pyogenes* var. *aureus*, and the responsible agent was therefore called the staphylolytic principle.

A number of observations show that colilytic and staphylolytic principles are distinct. The kinetics of lysis by actinomycetin follows a different course whether heat-killed *E. coli* or living *M. pyogenes* are used as a substrate (24). The conditions for the production of the two types of activity by *Streptomyces albus* G are sharply different, and so are the respective degrees of stability in culture of the two principles (7). The most suitable conditions for lysis of heat killed *E. coli* are quite different from those required for the lysis of living staphylococci : in particular, with respect to activating and inhibiting agents (7). The two principles behave quite differently with respect to absorption and elution on a variety of substrates, especially on the bacterial cells themselves (8). It was our first belief that the staphylolytic system comprized the colilytic agent plus something else, as in fact it is quite easy to obtain colilytic actinomycetin without any staphylolytic activity. But, it has now been possible to obtain separate fractions possessing only either the colilytic or the staphylolytic activity.

The staphylolytic system however is a complex one : it comprizes a lytic agent and a non lytic activator, the separation of which can be achieved by fractionnal extraction of actinomycetin with ammonium sulfate solutions of various strengths. In fact, recent observations, bearing on purification of actinomycetin by ion-exchangers, even show that the staphylolytic system might comprize three components : an activator and two other agents, each one of which behaves either as a lytic principle or as a second activator according to the staphylococcal strain used as a substrate.

It was first believed that what we called the staphylolytic system was responsible generally for the bacteriolysis of all living gram-positive bacteria sensitive to actinomycetin. However, evidence was obtained showing that the lysis of streptococci was attributable to a complex lytic system which, at least in part, is distinct from the staphylolytic system (26, 27). Next, it was shown that the lysis of pneumococci, whether heat-killed or alive, involved four different lytic agents, two of which are certainly different from either the colilytic, the staphylolytic or the streptolytic principles (9).

Lastly, it was found that a number of gram-negative rods, resistant to actinomycetin, unless they have been previously heat-killed, are dissolved by our preparation if submitted to its action at a temperature of 60° to 65° C., the rate of lysis being higher than the rate of thermal sterilization (6). Again, fractionnal extraction shows that the lytic principle here involved is different from the colilytic agent and the other lytic systems already known.

In conclusion, the bacteriolytic properties of actinomycetin from *Streptomyces albus* G are attributable to a variety of enzymes, or rather enzymatic systems, displaying each a very high degree of specificity. Work is now in progress to try and separate the many active components of actinomycetin. When this will be achieved it will be fascinating work to test their respective potential activities upon the various bacterial components that are being isolated in other laboratories, since the result of such collaborative work might well enlighten our knowledge of bacterial structures.

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