BACTERIAL LYSIS AND ANTISEPTICS

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(With Plate 3)

Autolysis is shown by bacterial species to varying degrees; among pneumococci it is a diagnostic feature. This autolysis can be accelerated by processes which kill the cell rapidly without destroying the enzymes responsible (Dubos, 1945). It is known that, in the case of the pneumococcus, excess of formalin will prevent lysis, and removal of the inhibitor induces a change from a Gram positive to a Gram negative reaction.

Gratia & Dath (1925) noted a lytic action of mould filtrates on many organisms. In Thom's Monograph (1930) this is mentioned as due to the action of a bacteriophage. Fleming's original observation on penicillin showed lysis of staphylococci, but Todd (1945) first drew attention to the almost complete clearing of certain bacterial fluid cultures under its influence.

It is the purpose of this paper to indicate some of the conditions under which bacteriolysis takes place, and to show that not only penicillin but a large variety of antiseptics under suitable conditions induce bacteriolysis.

METHOD

The minimal bacteriostatic value of the antiseptics is found by serial dilution in broth.

The organism is grown in Hartley's broth previously warmed to 37° C., and inoculated with 1 c.c. of a 12 hr. broth culture. Experiments were done with 50 c.c. volumes in screw-capped flat bottles; but the results are identical using plugged containers. Lysis is not found if the growth is poor or slow, from whatever cause.

Antiseptics are added when the organism is in its logarithmic phase; e.g. in 2 hr. with *B. coli*, 3 hr. with streptococci, and 4 hr. with staphylococci. At hourly intervals for 5 hr. samples are withdrawn for examination, and final tests are done at 24 hr. and at 48 hr.

In each test certain controls are included:

(1) An untreated culture, which normally grows more opaque over the 48 hr. period.

(2) A culture heated to 115° C. for 20 min. This gives approximately the opacity at the time of adding antiseptic, but in fact it always becomes slightly more opaque.

(3) A culture to which is added a large multiple of the concentration of the antiseptic used in the test proper.

Organisms

These fall in three classes. Advanced lysis was found with staphylococci, pneumococci, *B. subtilis* and several strains of *B. coli*. Less-marked lysis was found with *B. dysenteriae* (Flexner and Sonne). Very little evidence of lysis was found with *Streptococcus haemolyticus* and *non-haemolyticus*.

Antiseptics

Lysis was found with formalin, 0·012 %; phenol, 0·032 %; mercuric chloride, 0·0008 %; sodium hypochlorite, 0·005 %; and merthiolate, 0·0004 %. In general, a concentration approximately double that of the minimal bacteriostatic value was found to be effective. Lysis was also found with penicillin, at concentrations varying widely with the organisms tested.

RESULTS

Bacterial counts

These are most unsatisfactory. Owing to the fact that lysis sets in at about the third hour, disintegrated forms soon appear which prevent any reliable counting, either by dark-ground or in stained preparations.

Viable counts

There is a rapid fall in the viable count, but cultures treated with concentrations of antiseptics yielding good lysis often continue to give growth on sub-culture.

Photometric analysis

A Leifo extinction photometer was used. In this instrument the light reflected from a culture is measured. This however bears no direct relationship to bacterial numbers. An increase in the opacity of a culture may be due to an increase in numbers, size, or refractive index of the organisms, or to changes in their state of aggregation or in the medium. Owing to the difficulty of counting organisms undergoing lysis correlation with luminosity is of little value, but there are indications that cultures become more transparent before a fall in numbers is appreciable.

Photometric measurements have, however, a certain value. An initial luminosity figure of 0·0672 fell in 24 hr. to 0·0497, with well-developed naked eye lysis. The most interesting results were those found by heating cultures, and by adding small amounts

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of acid, or strong formalin. One drop of concentrated H_2SO_4 gave an immediate rise from 0·0687 to 0·1650, although no other change in the nature of the culture was distinguished. Stock solutions of formalin are acid, and a similar change is found when even weak solutions are added; this does not occur with weak neutralized formalin. Strong neutralized formalin, and the heating of cultures, gave increased luminosity.

The indications are, therefore, that weak antiseptics tend to produce increased translucency of bacteria, and that heat, strong formalin and acids tend to produce increased opacity.

Stained films

5 c.c. of the cultures were centrifugalized for 5 min. at constant speed. The deposit was resuspended in saline, and again centrifugalized. The deposit was stained for 5 min. in Carbol Fuchsin diluted one part in ten.

By this means an excellent picture of the lytic process is given. For 2 hr. no changes are observed. In the case, for example, of *B. coli*, vacuoles then make their appearance, and from the third to the fifth hour the bacilli lose their affinity for the stain, and give a beaded appearance. In 24 hr. nothing but debris is visible, although the cultures are often not sterile on sub-culture.

Streptococci are exceptional. With formalin no lytic changes were observed. With mercuric chloride about 5 % of the organisms were found in 3 hr. to be shrunken to a quarter or less of the average volume, and poorly staining units were found. In 5 hr. small intensely stained cocci were present in small numbers and persisted for 48 hr. The shrunken cocci were not noted in 24 or 48 hr. specimens, and may have lysed.

With both formalin and mercuric chloride the average number of cocci in a chain was greater than with the control.

Photography of cultures

The lysis is well recorded by photography. A thick yellow line is drawn on the back of the bottle, which is illuminated from below through a slit. Halation is produced, in unlysed cultures, obscuring the yellow line. This method offers an alternative approach to photometric analysis.

Lysis by this method, with a suitable organism, is well established in 24 hr. with mercuric chloride, penicillin and merthiolate. With phenol and formalin it is usually most marked in 48 hr.

Ineffective anti-bacterial agents

No lysis was found with boric acid, 0·5 %, or with sulphamezathine at any concentration.

Effect of antiseptics on older cultures

When control cultures are examined after 24 hr. incubation, a fair percentage of the organisms which show lysis under the conditions here discussed gave poor staining. No increase in this respect is shown when antiseptics are added, and no visible clearing takes place. Young organisms which are boiled or autoclaved show no clearing or microscopic changes.

Effect of high antiseptic concentrations on young bacteria

In the case of penicillin, an enormous increase in the concentration was still followed by lysis, and no concentration was found at which lysis did not occur.

With merthiolate, lysis occurred both at 0·04 and 0·0004 %, and no higher concentration was used.

With sodium hypochlorite, the effect of higher concentrations is complicated by the chemical lysis of the reagent.

With three antiseptics, formalin, phenol and mercuric chloride, low concentrations were found at which lysis occurs, and higher concentrations at which it does not occur. For *B. coli*, these values are as follows: lysis occurs with formalin at 0·045 %; mercuric chloride, 0·0009 %; and phenol, 0·045 %. Lysis does not occur with formalin, 11 %; mercuric chloride, 0·001 %; and phenol, 0·54 %.

The formalin values are percentages of a neutralized 40 % solution of formaldehyde. It was noted that the effect of these three reagents, in the higher concentrations, was quite different; stained films showing distinctive morphological and tinctorial appearances. Similarly, the lytic process studied at hourly intervals showed characteristic distinctions in the three cases.

DISCUSSION

The phenomenon of bacterial lysis has many aspects, and many agents such as bacteriophage and lysozyme have been much studied. No lytic agent is demonstrable in the filtrate of the lysed cultures here studied. For example, when *B. coli* is treated with 125 units per c.c. of penicillin, lysis in 24–48 hr. is apparently complete; but since penicillinase is produced, no penicillin remains. Filtrates of the lysed cultures fully support growth when reinoculated with *B. coli*. Similarly, low concentrations of sodium hypochlorite disappear in 24 hr., and an identical experiment may be performed. Indeed, the lysed cultures become completely opaque again in 72 hr., without reinoculation, since living bacteria persist.

With other reagents chemical procedures are needed to remove the antiseptics, and results would not be reliable. But in all cases if a drop of filtrate be placed on a plate of agar, and a drop of a culture of the organism superimposed, normal growth occurs. There is thus no suggestion of a bacteriophage, or presence in the medium of any effective

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lytic agent. Todd (1945) has ascribed the lysis found with penicillin to autolytic enzymes, and suggests that lysis occurs after the organisms have been killed by penicillin. His arguments are applicable also to the lysis here studied, using other antiseptics.

Lysis can be studied very simply by making cover-slip preparations of surface cultures when penicillin or formalin diffuses from a focus. In both cases there is an initial growth up to the focus, and in 2 hr. lysis sets in, so that only a 'mush' is visible. This shows as a scum on the plate, and with shake cultures in chicken plasma clotted with embryo extract is still more striking. If the experiment is performed in sealed containers, a densely opaque zone appears at the edge of the zone of apparent inhibition in 24 hr. when the culture is cooled. This opaque zone disappears on warming, and is reversible at will. It does not appear when the seal is broken. The phenomenon is probably due to a change in refractive index of lysed organisms when the vapour pressure is altered. Incidentally, the zone of inhibition on fibrin plates, and in fibrin cylinders, is much smaller than in 2 % agar; and the zone of inhibition in agar is inversely proportioned to the concentration of agar within certain limits. Since the penetration of penicillin into fibrin is a material factor in certain cases of infection, such as bacterial endocarditis, the point is not without clinical implications.

There are many antiseptics, and many bacteria, and it would be foolish to suggest that any general law can be deduced. But it may be safe to suggest that certain antiseptics produce more than one type of lethal effect. If used in minimally effective concentrations, they act on any one of complex enzyme systems, and the organism dies. If the antiseptic is used at a concentration without effect on autolytic enzymes, lysis follows. If used at higher concentrations, lysis is inhibited either by action on the autolytic enzymes, or on the substance of the organism.

Analysis of the range of lytic concentration involves more work than we have done. It is perhaps significant that we find the range with penicillin and merthiolate to be very wide.

Quastel & Wooldridge (1927) have shown that toluene has a different effect on different bacterial enzyme systems, leaving certain reactions unaffected, while entirely inhibiting others. It is theoretically only necessary to interfere with one system essential to life; so long as autolytic enzymes are not affected, lysis will then occur. On the other hand, when boric acid is used, reproduction ceases, but no lysis occurs. Thus a purely bacteriostatic agent, with no lethal qualities, does not induce lysis.

Finally, these experiments appear to show that only very young and actively dividing organisms autolyse when killed.

SUMMARY

When a number of organisms are exposed in early rapidly multiplying broth cultures to minimally effective bacteriostatic concentrations of a number of antiseptics, almost complete lysis may be demonstrated.

When older cultures are exposed to low concentrations, or young cultures to strong concentrations, lysis does not occur in many cases.

The lytic effect of penicillin is apparently identical with that of the antiseptics tested. The phenomenon appears to depend on the nature of the effect of the antiseptic on metabolic enzymes, autolytic enzymes, or both.

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EXPLANATION OF PLATE 3

Fig. 1. Effect of various procedures on a 4-hr. broth culture of *Staphylococcus aureus*, incubated for 24 hr. after addition of reagents. (Left to right.) (*a*) 1 c.c. of 40 % formaldehyde added. Increased opacity. (*b*) Autoclaved at 115° C. for 20 min. Increased opacity. (*c*) Control. (*d*) 0·012 % formalin added. Partial lysis. (*e*) 0·5 units per c.c. penicillin added. Almost complete lysis. (*f*) 0·0008 % mercuric perchloride added. Complete lysis. After a further 24 hr. incubation, bottles (*d*) and (*e*) gave complete lysis.

Fig. 2. *B. coli*, 2 hr. broth culture.

Fig. 3. *B. coli*. Same culture, 3 hr. after adding 0·0008 % mercuric perchloride. Vacuolation marked.

Fig. 4. *B. coli*, 5 hr. after addition of mercuric perchloride. Staining capacity much diminished.

Fig. 5. *B. coli*, 23 hr. later. Almost complete lysis. Stained in carbol fuchsin diluted one part in five for 3 min.

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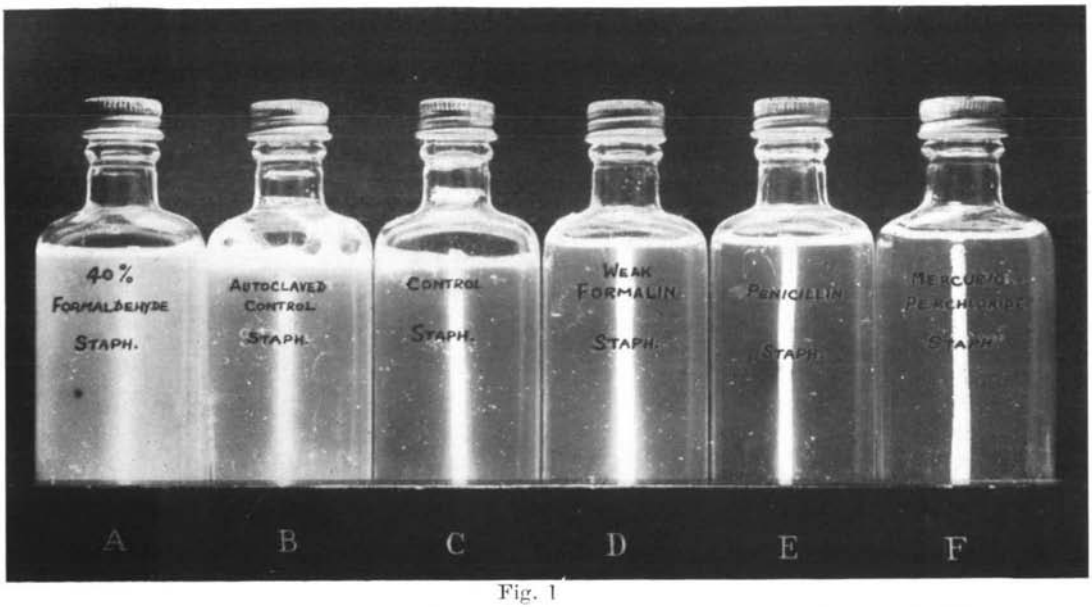

Fig. 1

Fig. 2

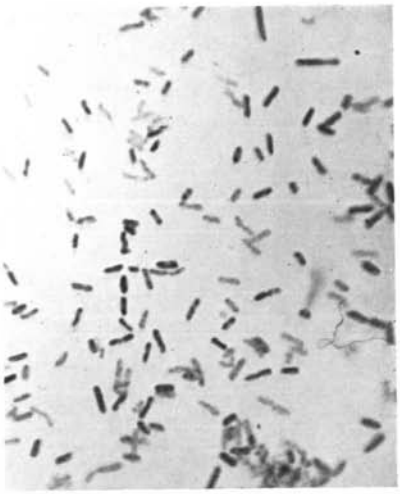

Fig. 3

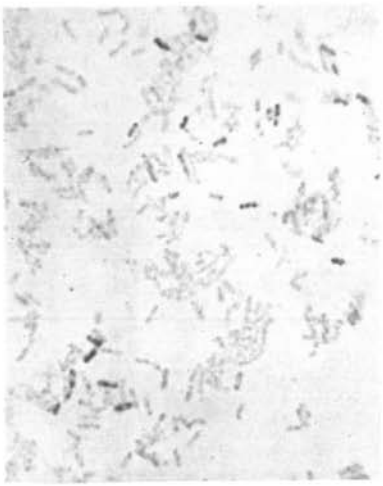

Fig. 4

Fig 5

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