zol and picrotoxin. Overdosage of the stimulants either had no effect or caused greater depression of diuresis. Properly balanced dosages of the antagonists, however, frequently increased diuresis to a marked degree over that observed with the depressant alone and most frequently in those cases where the degree of depression was lessened.

Paraldehyde was used throughout at dosages of 2.0 cc./kg. Eight experiments with Metrazol indicated that 35 mg./kg. intraperitoneally was the most suitable dosage for this antagonist. By injection a few minutes before the paraldehyde, the period of depression was delayed 30 to 40 minutes but the time of recovery was not particularly affected. By injection a few minutes after the paraldehyde, there was no particular effect on the time of appearance of the depression but the period was considerably shortened. Two experiments with picrotoxin (2 to 3 mg./kg.) increased diuresis over that with paraldehyde alone but was not a suitable antagonist at this dosage because of the convulsions produced.

Three experiments were made with sodium luminal at dosages 110 to 150 mg./kg. Diuresis was increased during the period of stimulation but the effect on recovery was unfavorable, apparently because of the unusually prolonged action of this drug.

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Attachment of Marine Bacteria to Submerged Slides.

C. E. ZOBELL AND ESTHER C. ALLEN. (Introduced by M. L. Tainter.)

From the Scripps Institution of Oceanography, University of California, La Jolla.

Winogradsky,[1] Cholodny,[2] and Conn[3] have shown that a different bacteriological picture is presented when the microorganisms which attach themselves to glass slides incubated in the soil are observed directly, than when the same soil is studied microbiologically by the conventional cultural methods. Henrici[4] studied freshwater bacteria by such a direct microscopic method. We used a somewhat similar procedure to study marine bacteria on the coast of southern California.

[1] Winogradsky, S., *Soil Sci.*, 1928, **25**, 37.

[2] Cholodny, N., *Arch. f. Mikrobiol.*, 1930, **1**, 620.

[3] Conn, H. J., *Z. f. Bakt.*, 1932, **87**, 233.

[4] Henrici, A. T., *J. Bact.*, 1933, **25**, 277.

Our experiments were designed to supplement the work of Coe and Allen (Coe[5]) at the Scripps Institution of Oceanography on "fouling organisms" so destructive to navigation equipment and other marine structures (Visscher[6]).

Standard slides, bacteriologically clean, were submerged off the end of the Institution pier, and after one to 7 days' submergence the slides were taken with aseptic precautions to the laboratory for examination. Results indicate that attachment of bacteria and, to a lesser extent, diatoms and actinomycetes, usually precedes the attachment of barnacles, hydroids, bryozoa, and other "fouling organisms" by several hours or even days.

Many forms of bacteria which have not been recovered by concurrently plating samples of sea water on culture media have been observed directly on these slides. However, not all marine bacteria attach themselves to submerged glass slides.

Seventy-three pure cultures isolated from the sea and differing morphologically or physiologically were tested under controlled conditions for their attachment propensities. The bacteria were inoculated into wide-mouth bottles containing nutrient sea water. Sterile glass slides were inserted vertically. After 2 days' incubation at 25°C. the slides were examined. With no fixation the slide was stained and dried. Only 24 of the 73 cultures attached themselves to the slides, although those which did not attach had multiplied until the medium had become turbid. The bacteria which had firmly attached to slides will be termed "attachment bacteria". Some of them appeared to be definitely thigmotactic because they grew only in adherent films on surfaces. Among the 24 attachment bacteria 3 such thigmotactic cultures formed macroscopically visible films on bottles and slides before the broth itself became perceptibly turbid. On the other hand, in some pure cultures enough cells attached themselves to the slides to warrant being classified as "attachment bacteria" although the sea water broth was very cloudy with free-floating cells.

Only some marine bacteria attach themselves to submerged slides. The direct microscopic technic is not applicable to a quantitative study of marine bacteria because the submerged slides will detect only the bacteria which attach themselves to surfaces, and these seem to be in the minority. Incidentally, if the same relationship exists among soil or fresh-water bacteria, this would have to be con-

[5] Coe, W. R., *Bull. Scripps Instit. Oceanog., tech. ser.*, 1932, **3**, 37.

[6] Visscher, J. P., *Bull. Bur. Fish.*, 1927, **43**, 193.

sidered in the interpretation of results obtained by the Cholodny-Conn technic.

It usually requires 2 to 4 hours for appreciable numbers to fix themselves to slides firmly enough so that they will not be washed off by running water and the staining processes. Thus, the submergence of a slide in broth already cloudy with bacteria followed by its immediate removal does not result in the attachment of bacteria. Under these conditions a few cells of both the attachment, as well as the non-attachment types, may accidentally adhere to the slide, but the number is small and the arrangement irregular. Attachment occurs more readily during the logarithmic growth phase than during the phase of negative acceleration or later. The best preparations of attachment bacteria are obtained by inserting the slides at the same time the media are inoculated, or shortly thereafter.

All stages of bacterial development from individual cells, dividing cells, chains, and micro-colonies have been observed. It is quite evident that many of them are multiplying on the surface of the slide. Hence, such adherent films are invaluable for observing the stages in the processes of cell division or multiplication, because the intricate details are not disrupted as is frequently the case in the preparation of smears in the usual way. Thus, 2 forms have been noted which, though otherwise indistinguishable from the bacilli, were found to multiply by fragmentation actinomyces-like.

Most, if not all, of the attachment bacteria are capsulated. Some of them appear to develop a definite holdfast, although it has been difficult to find a way to ascertain the nature of the attaching mechanism. Certain other forms produce a film of faintly staining material extending beyond the cells 2 or 4 times their diameter, and on this the bacteria are grouped making them appear to be arranged on islands of this material. The attachment tendency has been found to be affected by the composition of the media, glucose favoring it. As stated above, water does not destroy the holdfast. Neither is it destroyed by alcohol, but xylol removes many of the attached cells.