8965 C

Dynamics of Dissociated Bacterial Cultures.

MICHAEL DOUDOROFF. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, Calif.

In order to obtain material for a study of the alleged specific racial stabilizing or antimutation hormones[1] in bacterial cultures, a 7-day broth culture of *Staphylococcus aureus,* grown from a single agar-plate colony, was plated out on Martin's agar. Plates thus obtained showed from 97 to 99.7% *aureus* colonies with a 0.3 to 3% *albus* dissociation (average dissociation about 1.5%). Pure-line *albus* strains grown from these white dissociates showed no demonstrable tendency to revert to the ancestral orange type. Highly pigmented orange strains similarly selected usually underwent approximately 1.5% *albus* dissociation by the end of 7 days' growth in peptone-broth.

Flasks containing 50 cc. peptone-broth were inoculated with 0.5 cc. of 24-hour broth cultures of pure-line white and orange dissociates thus obtained. The resulting growth-curves of 2 typical contrasting strains are recorded in Fig. 1.

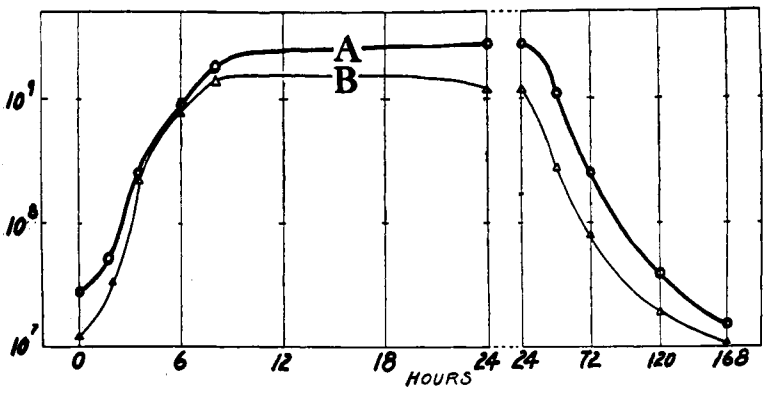

FIG. 1.

Growth of Pure-line Staphylococcal Dissociates.

A, 50 cc. peptone broth inoculated with 0.5 cc. of a 24-hour broth culture of a pure-line orange dissociate. The curve records change in viable count per cc. of broth culture on incubation at 37° C. for 168 hours. The flasks were constantly stirred during the incubation period to prevent sedimentation and the samples thoroughly shaken with glass beads before plating. B, control data in flask inoculated with a pure-line white dissociate.

[1] Etinger-Tulezynska, R., *Z. f. Hyg.*, 1932, **113**, 762; Neufeld, F., and Kuhn, H., *Ibid.*, 1935, **116**, 95; Mohr, Werner, *Ibid.*, 1935, **116**, 288.

From this figure it is seen that the *albus* growth-curve is characterized by a shorter lag-phase than the *aureus* control. The *albus* dissociate also showed more rapid proliferation during the logarithmic phase of population increase. Moreover, there was a stabilization of the *albus* viable population at a lower level than in the *aureus* control, and an earlier and more rapid development of the senescent phase, or terminal fall in viable count.

Identically the same relative differences in "growth-vigor" and "longevity" were noted when the same white and orange dissociates were reinoculated into filtrates or centrifugates from 24-hour to 5-day *albus* or *aureus* broth cultures. Varying the volume or the age of the inoculum caused no demonstrable changes in the relative growth rate or longevity.

From the observed differences in inherent "growth-vigor" and longevity one would predict marked fluctuations in the relative percentage of *albus* and *aureus* individuals in a mixed (or dissociated) staphylococcal culture as the culture increased in age. Two graphs confirming the predicted fluctuations are recorded in Fig. 2.

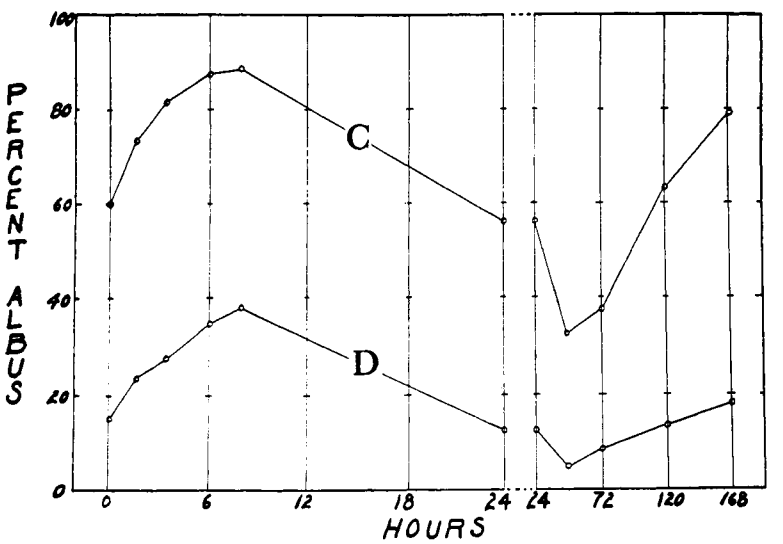

FIG. 2.
Fluctuations in Albus Percentage in Mixed Cultures.

D, 50 cc. peptone-broth inoculated with 15% pure-line *albus* and 85% pure-line *aureus* dissociates. The curve records changes in *albus* percentage during the first 168 hours incubation. C, control flask inoculated with 60% pure-line *albus* and 40% pure-line *aureus* dissociates.

Curve D in this figure records the percentile fluctuations in a mixed *albus-aureus* broth culture, whose initial viable count showed 15% *albus* individuals. The *albus* percentage increased to nearly

40% by the end of 8 hours' incubation (37° C.), then gradually fell to approximately 5% by the end of 36 hours. After the 36th hour the *albus* percentage again rose, reaching about 18% by the 168th hour. Curve C in the same figure records data from a mixed broth culture originally containing 60% *albus* individuals. This curve shows the same type of fluctuations in *albus*-percentage. Identically the same fluctuations were noted when mixed cultures were grown in filtrates or centrifugates from 24-hour to 5-day *albus* or *aureus* broth cultures.

Since all of these fluctuations are predictable from the observed differences in "growth-vigor" and longevity in pure-line *albus* and *aureus* dissociates, such percentile fluctuations furnish no evidence of existence of a specific hormonal antagonism between white and orange dissociates of *S. aureus*.

8966 P

Action of Compounds Related to Cysteine on the Regression of Jensen's Rat Sarcoma.

JESSE L. CARR. (Introduced by C. L. Connor.)

From the Division of Pathology, University of California Medical School, San Francisco.

Since the report[1] of the effect of cysteine hydrochloride on Jensen's sarcoma in rats, attempts have been made to determine the active portion of the cysteine structure responsible for the tumor regressing action. This has been studied by injecting into the tissue of the Jensen rat sarcoma several acids with the same pH as the cysteine hydrochloride solutions, fractions of the cysteine molecule, and compounds built up from the cysteine molecule.

In the first group, hydrochloric acid, sulphuric acid, and acetic acid were tried, and also because of its sulphur content rather than acid properties, sodium thiosulphate. The acids were adjusted to the same pH as the solutions of cysteine hydrochloride and were injected in equivalent volumes. All the acids were rapidly absorbed from the tumor tissue. Except for a little central hyperemia in the tumor and occasionally a small focus of necrosis in the center of the tumor mass, no cytological changes were noted. There was no influence

1 Connor, C. L., Carr, J. L., and Ginzton, L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 374.