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Further Observations on the Reproduction of Bacilli from Large Bodies in *Proteus* Cultures.*

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In a previous paper¹ the question was raised as to whether certain phenomena observed in *Proteus* may be sexual, in spite of the fact that fusion of cells is not involved. The experiments described in this note were undertaken to study this question. No evidence was obtained to prove that the influence of *Proteus* strains on each other is sexual, but the observations give further information concerning the remarkable antagonism between the strains.

The spreading filaments of *Proteus* when they meet filaments of another appropriate strain go through a peculiar transformation. They develop into large, round bodies, which continue to enlarge and reproduce either the usual small bacilli or tiny pleuropneumonia-like colonies. The influence of the spreading filaments on each other is quite specific. The usual small bacillary forms of *Proteus* are neither influenced by, nor exert any influence on, the filaments or small bacillary forms of another strain. The strains survive and retain their identity indefinitely in mixed cultures in broth. The motile filaments, so sensitive toward other *Proteus* strains, grow without hindrance through the various bacterial and mold colonies which they may encounter on the plates. The filaments are transformed into large bodies by relatively slight physical or chemical injuries; for example, refrigeration and exposure to tap water. The small bacillary forms are not sensitive to these influences.

This reaction of the filaments may be accidental. The spreading filaments develop only on a suitable medium and only have a short existence before they break again into small bacilli. It is unlikely that during the brief period of spreading they are exposed to adverse conditions which would change them into large bodies. Hence, the formation of large bodies at the contact zone of two spreading strains may be a natural function of the filaments.

Although the usual mechanism of sexuality, the fusion of cells, is absent in these processes, it seemed desirable to determine whether the essential purpose of sexuality, the crossing or segregation of properties, is not accomplished by some other unknown mechanism. To study this question large bodies were isolated with a micromanipulator from the contact line of two spreading cultures, and their descendants were compared with the parent strains. The technique used for the isolation of the large bodies was similar in principle to that described by Dickinson.[†] The large bodies were transferred to small pieces of transparent agar, and their development into bacterial colonies was observed under the microscope. This procedure assured that the colonies developing on the agar were the descendants of the large bodies. From single colonies of each culture so obtained, several strains were cultivated for further study.

These strains were studied with respect to their serological properties and their antagonism toward other *Proteus* strains. Cultures from different *Proteus* strains planted beside each other on a plate do not cover the agar entirely, but a sharp line of demarcation re-

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¹ Dienes, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1947, **64**, 165.

[†] See article of Stoughton.²

² *System of Bacteriology*, London, published by His Majesty's Stationery Office, 1931, **9**, 105.

mains between the areas covered by different strains. Descendants of a single strain do not show this phenomenon. Approximately 70 strains isolated from different sources were examined in this respect. All combinations of these strains which were tried were antagonistic to each other. This antagonism between the strains offered a simple method to determine whether all descendants of a large body are similar and correspond to one or the other of the original strains. If the cultures are thinly planted and begin to grow in isolated colonies, even a single colony with strain specificity different from the others is clearly apparent in the plates.

Fifty-six large bodies were isolated from different combinations of 5 strains. Thirty-four of these developed into bacterial colonies and 18 did not grow. Their descendants were always similar to one of the original strains both in strain antagonism and serological properties. Neither segregation of properties nor crossing over from one strain to another was observed. However, it is of interest that with a single exception, in the combination of two strains, only large bodies belonging to one strain grew into bacillary colonies. For example, 34 large bodies were isolated from the combination of strain No. 3 with other *Proteus* strains. Fourteen large bodies did not grow. The 20 which did develop never reproduced strain No. 3 but always the other strain. Large bodies produced in strain No. 3 by refrigeration are fully viable. Twelve large bodies were isolated from the mixture of strains Sm and No. 14, both of which dominated over strain No. 3. Six large bodies were viable all of which corresponded to strain

No. 14. In a combination of strains No. 14 and No. 52, 6 large bodies were isolated; 3 of these developed colonies corresponding to strain No. 52, one to strain No. 14 and 2 did not grow out. Strain No. 14 dominated over strain No. 3 and strain Sm, and was apparently equal to or weaker than strain No. 52.

The dominance of one strain over the other is not genetic, but it is probably the result of the strain antagonism already discussed. When the strains come in contact, both respond by transformation into large bodies, but apparently only the large bodies of one strain survive. The numerical relationship observed between the viable and non-viable large bodies may be influenced by the fact that large bodies are not produced in equal numbers by the different strains. Also, some are destroyed by the manipulation necessary for their isolation.

The observations in their present status give no information as to the function of the large bodies or the reproductive processes connected with them in *Proteus*. It is possible that their real significance is in connection with pleuropneumonia-like colonies and not with the direct reproduction of bacilli. The antagonism which appears in certain phases of growth between *Proteus* strains has thus far not been observed in other species.

Summary. Single large bodies were isolated from the contact line of 2 spreading *Proteus* strains. Their descendants were similar to one of the strains with respect to the properties studied, and crossing or segregation of properties was not observed. With a single exception, only one strain was recovered from the large bodies formed at the contact of 2 strains.