The negative or depressing effect of all kinds of nutrient material and the stimulating effect of earth on the expansion of the halo on non-nutrient agar plates are observations which probably will be of interest for the further study of the halo organisms and their relationship to the bacteria. Our strains were obtained usually from contaminated blood cultures and originated probably from dust. Numerous colonies showing halo formation grew from dust collected under the roof of the laboratory building. Adding earth to the agar probably makes the medium more similar to the natural habitat of our strains and the addition of nutrient material less similar. It seems rather probable from this observation that the peculiar process which we observed is a regular process in the natural life of our strains, which is usually not apparent in artificial growth but under the influence of saccharose and other appropriate conditions will be noticeable. The halo formation on nutrient agar is a peculiar case of growth and expansion of the halo substance, the conditions of which are entirely different from the conditions which regulate the extension of the halo substance into the medium of non-nutrient plates.

7506 P

Morphologic Elements in the Halo of Subtilis Colonies.*

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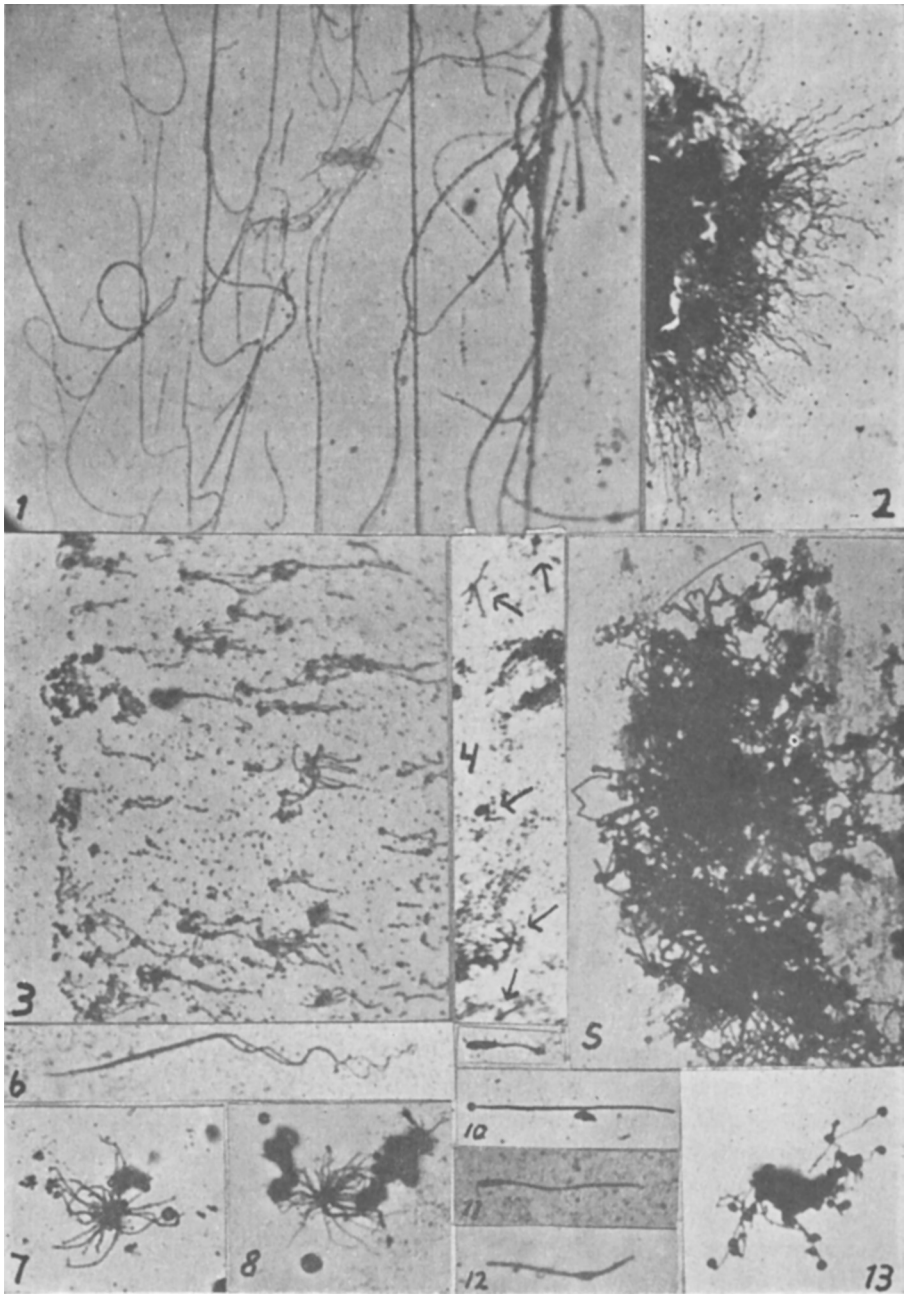
The most interesting question in connection with the phenomena which are the subject of the previous note[1] is whether they are life phenomena produced by the growth of living organisms or whether they represent only a peculiar combination of diffusion phenomena and enzymatic action. The number of successive transfers in which the halo substance so far was grown is not sufficiently high to exclude the supposition that the mucoid substances are synthesized in the medium by an enzyme excreted from the bacteria. The observations which will be described in this note show that the halo consists of organized elements and is not an amorphous substance.

* The publication of the figures was made possible by a grant from the DeLamar Research Fund of Harvard University.

1 Peirson, O., and Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1208.



The magnification in all figures is 1:1000.
The preparations were stained by Zettnow's flagellar staining method.
FIG. 1. Long fine filaments from halo.
FIG. 2. Short fine filaments from halo transplant.

These observations furnish definite evidence for the living nature of the halo.

Direct examination of the halo under the microscope and examination of preparations stained with crystal violet reveals no formed elements in the halo. If a small piece of agar containing the deep colonies of halo is cut out and stained with crystal violet the colonies remain unstained in the deeply stained agar. These colonies often contain stained granules and sometimes fine bacillary forms but these can not be distinguished from the impurities present in the agar. Examined with dark field illumination the halo consists of a great number of faintly visible granules. The elements which build up the halo can be seen best in preparations stained by flagellar staining methods. For a long time our attempts to stain the halo elements with these methods remained unsuccessful and only after much experimentation were successful preparations obtained.

The elements found in the preparations show an extreme polymorphism. There are larger elements which consist of a straight or bizarre shaped filament which is often pointed or fusiform. They may run out into a fine filament or end in a coccus-like body. These filaments may form large interwoven masses. They are not stained with crystal violet and dark background preparations show that they are much thinner than bacteria. Another larger element is represented by coccus-like bodies which form sometimes with the filament asteroid forms. Sometimes in the halo transplants definite bacillary or coccus forms are found which are faintly stained with crystal violet.

Besides the larger elements bundles of very fine seemingly rigid filaments are found rarely and the mass of the halo substance is built up of fine short filaments or of tiny granules. The staining of the short fine filaments and granules is usually unsatisfactory and their differentiation from artefacts in the preparation is not always clear. The larger forms are visible with dark field methods and their form and arrangement, like the arrangement of the long fine filaments, is so characteristic that no doubt can remain that they represent actual structures.

The larger elements of the halo and halo transplants, though in the latter definite bacterial forms are rarely found, differ in essential

FIG. 3. Short fine filaments from halo, also a spore-like body in the right lower corner of the figure.
FIG. 4. Asteroid forms from halo.
FIG. 5. Large mass of thick filaments from halo transplant.
FIG. 6. A thick filament from halo running out into fine filaments.
FIGS. 7 and 8. Asteroid forms from halo.
FIGS. 9, 10 and 11. Thick filaments from halo transplants, 12 from halo.
FIG. 13. Filaments and coccus-like bodies from halo transplant.

points from the usual forms of bacteria. First of all they do not reproduce bacterial growth while the bacteria grow profusely in our media. They are extremely pleomorphic, very fragile, and remain unstained by crystal violet. In the halo the larger elements are only sparsely present and as the transplants give rise to thousands of tiny colonies the growth must originate from very small forms. It seems probable that the elements of the halo originate from the bacteria as from a heated spore emulsion all spores reproduce the halo. None of the filamentous structures in the halo correspond closely to the filaments which were described in the bacterium cultures.[2]

It is interesting to note that the elements of the halo which are so different from the bacteria resemble closely the elements from which the cultures of the virus of pleuropneumonia bovis are built up.

7507

Hereditary Variations in Litter-Size of Rabbits.

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An analysis of 569 pregnancies occurring in our breeding colony during the 5-year period from 1929 to 1933 has indicated the presence of wide variations in the mean gestation periods of different breeds.[1] These differences were attributed to hereditary factors. The present report is concerned with an analysis* of the size of the litters resulting from these 569 pregnancies, the particular purpose being to ascertain whether breed had any influence on litter-size.

The pregnancies were the result of matings made in all months with the exception of July and August. Eleven breeds consisting of 10 standard bred strains and one intensely inbred line of albinos

[2] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 388.

[1] Rosahn, P. D., Greene, H. S. N., and Hu, C. K., *Science*, 1934, **79**, 526.

* The symbols employed below are defined as follows; Var. = Variance; F = the ratio of the larger to the smaller variance; n = number of observations; M_n = mean of n observations; D = difference between two means; t = the ratio of the difference to its standard error; P = probability. For a description of the methods of analysis, see Fischer, R. A., Statistical Methods for Research Workers, Oliver and Boyd, London, 1930, and Snedecor, G. W., Calculation and Interpretation of Analysis of Variance and Covariance, Collegiate Press, Inc., Ames, Iowa, 1934.