

fragmentation of the nuclei is seen in those cells which were regarded as dead before fixation.

Further information as to the technical details, will be available later.

3088

Pseudobacteriophage of *Bacillus anthracis*.

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During the examination of some old stock cultures in November, 1924, there was found an agar slant of *Bacillus anthracis* which had the appearance of undergoing lysis by bacteriophage. The film of growth was interrupted by well defined partly confluent denuded areas, many of them containing small centrally located secondary colonies. Transplants of the culture to agar gave similar appearances after incubation for two days at 37° C. and one or more days at room temperature. Transplants to broth did not show clearing but the plaques again appeared when the broth cultures were transplanted to agar. An older agar slant culture from which the culture first mentioned above had been inoculated did not show plaques nor did fresh transplants of this culture, but when this "negative" strain was inoculated with material from one of the plaques of the "positive" strain plaques appeared in subsequent transplants. Filtrates of broth cultures of the positive strain were inactive when added to the negative strain.

Plaques on agar plates examined under the microscope were found to contain a nucleus of free spores, the remainder to the plaque being made up of pale remnants of bacilli and amorphous granular detritus. The matrix of growth surrounding the plaques was composed of typically curled but apparently spore-free anthrax filaments. Fishings from the centers of plaques yielded cultures of typical sporulating *Bacillus anthracis*. Fishings from the matrix yielded cultures of non-sporulating bacilli

otherwise like *B. anthracis*, hereafter referred to as the non-sporulating strain.

After prolonged growth on agar, on potato or in broth at 37° C. and at 20° C. the non-sporulating strain failed to produce visible spores and did not resist heating for twenty minutes at 75° C. Cultures of this sporulating strain resisted similar exposure to heat. Neither sporulating nor non-sporulating strain showed plaques when grown alone on agar. When the two were mixed plaques appeared. In the pure state the non-sporulating strain produced coarser curls than did the sporulating strain and in the mass the former growth had a somewhat more transparent appearance than the latter and was rather viscid to the touch of the needle. An agar slant inoculated over the entire surface with the non-sporulating strain and then touched in one or several places with the sporulating strain showed plaques where touched with the sporulating strain. Slants similarly inoculated with the sporulating strain, and then touched in spots with the non-sporulating strain, also showed plaques where touched with the latter. In the latter case, however, there were no spores in the centers of the plaques and central secondary colonies did not appear after further incubation.

When inoculated sub-cutaneously into mice both strains produced typical anthrax. For guinea pigs the sporulating strain was more pathogenic than the non-sporulating strain. Only the sporulating strain was pathogenic for rabbits. Passage of the non-sporulating strain through mice or guinea pigs and subsequent cultivation on agar or potato did not result in the appearance of spores. Neither did exposing bits of the infected spleens from animals in petri dishes at room temperature for several days induce spore formation.

When in mixtures of the two strains the sporulating strain was predominant, the non-sporulating colonies in young agar cultures presented the appearance of the well known so-called "pellucid spots" which have been described for a number of sporulating aerobes. Later these pellucid spots became sunken and had the appearance of plaques due to bacteriophage. On examining other strains of *B. anthracis* most of them were found to produce pellucid spots on agar. Another non-sporulating strain was isolated from one of these. In other cases there were obtained strains in which spore formation was greatly retarded

so that apparently the appearances described may be produced not only by mixtures of sporulating and non-sporulating strains but also by mixtures of rapidly and slowly sporulating strains.

The appearances described are similar to those described by Andrevont and Simon¹ and by Pesch². A more complete description and discussion of our observations and those of the authors cited will appear in a subsequent publication.

3089

Chemical nature of some substances required for the growth of fibroblasts and epithelial cells

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Pure strains of fibroblasts or of epithelial cells increase in mass in an unlimited manner, when they are cultivated in plasma and embryonic tissue juice. For 14 years, colonies of a strain of fibroblasts have doubled in size every 48 hours in such a medium. Pavement and thyroid epithelium also have been found to manufacture unlimited amounts of protoplasm from the constituents of embryonic juice. Neither epithelial cells nor fibroblasts multiply in serum proteins, egg albumin, crystallized egg albumin, amino acids from embryonic juice, or artificial mixtures of amino acids for a longer time than in Tyrode solution. So far, embryonic juice is the only material which has been found to maintain epithelial cells and fibroblasts in a condition of true cultivation.

Investigation of the chemical nature of the nutritive materials in the embryo juice has led to the conclusion that the nitrogenous substance utilized by the tissues is the protein itself. The amino acids and other ultra-filtrable and dialyzable constituents slightly stimulated the migration and multiplication of the cells, but failed to produce an increase in the mass of the tissues. Since the protein of the embryo juice is utilized by the cells, it seems evident

¹ Andrevont, H., and Simon, C. E., *Amer. J. Hyg.*, 1924, iv, 386.

² Pesch, K. L., *Centralbl. f. Bakt.*, Abt. I, Orig., xciii, 525.