the pH 6 antigen under the same conditions as *P. pestis*. The electrophoretic mobility of the original cells, devoid of the pH 6 antigen, was remarkably slow; thus, it was not possible to check correlation between the formation of this antigen and change in electrophoretic mobility.

The pH 6 antigen could be isolated from crude extracts of strain 27/C by the same method used for *P. pestis* (Bichowsky-Slomnicki and Ben-Efraim, J. Bacteriol. **85**:101, 1963). The purified fraction possessed the three biological activities described for *P. pestis*. As in the case of *P. pestis*, fractions of the pH 6 antigen devoid of agglutinating activity against red blood cells, but possessing the other biological activities, could also be isolated. The minimal effective dose of the purified fraction was 30 μg of protein for cytotoxic and agglutinating activity and 100 μg of protein for skin reactivity.

It may be concluded that only part of the *P. pseudotuberculosis* strains can synthesize the pH 6 antigen, whereas this property seems to be shared by all the strains of *P. pestis*. As in the case of *P. pestis*, three kinds of biological activities were related to the presence of the pH 6 antigen isolated from a strain of *P. pseudotuberculosis*.

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ELIMINATION OF PLEUROPNEUMONIA-LIKE ORGANISMS FROM EMBRYONIC HUMAN LUNG TISSUE CULTURE WITH TETRACYCLINE

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An attempt to eradicate pleuropneumonia-like organisms (PPLO) from an embryonic human lung tissue culture (Davis and Bolin, Federation Proc. **19**:386, 1960) by constant exposure to 2.5 μg/ml of tetracycline as suggested by Carski and Shepard (J. Bacteriol. **81**:626, 1961) was unsuccessful through 11 weekly passages.

Minimal doses of antibiotics having toxic effects on these cells, as evidenced by altered morphological appearance and reduced proliferation within 7 days, were determined to be 20 μg/ml for tetracycline, 1,500 μg/ml for kanamycin, and 30 μg/ml for oxytetracycline. Routine passages at 7-day intervals showed an apparent cumulative toxicity in cells exposed to these concentrations of kanamycin or oxytetracycline, with resultant loss of cultures in 2 to 3 weeks. Minimal doses of kanamycin and oxytetracycline that would permit successful serial passage were 375 and 7.5 μg/ml, respectively, but at these concentrations they were ineffective in eliminating PPLO. Tetracycline at 20 μg/ml showed no cumulative toxicity through 11 serial passages of tissue culture. The cultures were positive for PPLO after the first two passages but negative thereafter.

ISOLATION OF *BACILLUS ANTHRACIS* FROM SOIL STORED 60 YEARS

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Recently we found a small, rubber-stoppered bottle that had been in storage in this Department for many years. The bottle contained a small amount of dry, tan, powder-like soil with

a few lumps in it, and had a label in faded ink that read, "Dirt containing anthrax from La-Crosse outbreak. Aug. 1902, Dirt dried at 38 C," with the initials EGH (for E. G. Hastings) in one corner. We suspended 0.5 g of the "dirt" in 20 ml of sterile saline, and then injected 1 ml of the suspension intraperitoneally into each of five guinea pigs. One of the guinea pigs died in about 48 hr, but the others survived. Smears made from the heart blood, liver, and spleen of the dead guinea pig revealed large, capsulated, gram-positive, rod-shaped organisms. Cultures on Trypticase Soy Agar (BBL) slopes were made from the heart blood, and incubated for 18 hr at 37 C. The growth was typical of *Bacillus anthracis*. A suspension of these cells was made by washing one of the slopes with 5 ml of saline and injecting 1 ml intraperitoneally into each of three guinea pigs. These animals were dead within 48 hr, and smears from the heart blood, liver, and spleen contained large, capsulated, gram-positive, rod-shaped organisms. Further study of the culture, by the methods described by Leise et al. (J. Bacteriol. **77**:655, 1959), showed that it was nonhemolytic on sheep blood agar, and gave a positive "string-of-pearls" reaction. The cells were nonmotile. Subsequently, we sent a transplant of the culture to K. L. Burdon (Baylor University College of Medicine, Houston, Texas), who subjected it to a number of differential tests (Burdon, J. Bacteriol. **71**:25, 1956) and concluded that it was "a typical, virulent culture of *B. anthracis*."

Although the wording on the label of the bottle of soil might be interpreted to mean that a culture of *B. anthracis* isolated during an outbreak of anthrax at LaCrosse in 1902 had been added to dried soil at some subsequent time, this is not true. Both W. C. Frazier and E. B. Fred of this Department knew that Professor Hastings had a bottle of dried soil from a region where an anthrax outbreak had occurred, and that he could isolate *B. anthracis* from this material. Moreover, Professor Frazier stated that in 1920 he used soil from this same bottle to inject into guinea pigs to demonstrate anthrax for a class.

From the information given, it is concluded that *B. anthracis* survived for 60 years in dried soil kept at room temperature that varied from 50 to 100 F.

PLAQUE MORPHOLOGY OF MONKEYPOX VIRUS AS AN AID TO STRAIN IDENTIFICATION

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The ability of the poxviruses to form plaques on monolayers of susceptible tissue cells, and the differentiation of variola from other members of the group by plaque morphology, has been reported by other investigators (Noyes, Proc. Soc. Exptl. Biol. Med. **83**:426, 1953; Younger, J. Immunol. **76**:288, 1956; Porterfield and Allison, Virology **10**:233, 1960; Mika and Pirsch, J. Bacteriol. **80**:861, 1960). In the course of identifying a newly isolated strain of monkeypox virus (McConnell et. al., Nature **195**:1128, 1962), it was noted that the plaque size of this isolate was smaller than that of a commercially available strain (Com Vac) of vaccinia vaccine virus (smallpox vaccine lot no. 5124A14, The National Drug Co., Philadelphia, Pa.). This finding suggested an additional possible method for differentiation of vaccinia strains of poxvirus by their plaque characteristics.

The Dulbecco plaque technique (Dulbecco, Proc. Natl. Acad. Sci. U.S. **38**:747, 1952), modified in minor detail, was employed. Monkey kidney cell cultures (MKCC) and rabbit kidney cell cultures (RKCC) were grown in 4-oz prescription bottles, essentially as described by Melnick and Riordan (Proc. Soc. Exptl. Biol. Med. **81**:208, 1952). Several strains (obtained

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