

charged Ca compound of the saliva and accelerate the precipitation.

8. Examinations of the mixed saliva collected from patients with pyorrhea have shown that in the majority of instances the composition reveals a marked increase of the cations over the values established for the individuals with mouths free from pyorrhea. In particular the amounts of K and Na are higher while correspondingly the other values are decreased. The Ca and PO₄ percentages are reduced approximately 25% and the amount of Mg even 40%.

9. Examination of the saliva during pregnancy has shown that the Mg is increased (in contrast to pyorrhea) and the Ca content reduced to approximately 50% of the normal value.

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Studies on the Heat Resistance of Spores of *B. Subtilis*.

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This report presents in summary form the results of a study of the survival time of *B. subtilis* spores produced under a wide variety of nutritive and environmental conditions and exposed to high temperatures. The basic resistance has been defined as the resistance of a suspension of a definite density, produced and tested under certain standard conditions. The basic resistance of the strain employed was 6 minutes at 105° C., 14 minutes at 100° C., and 35 minutes at 95° C. Most of the determinations were made at 100° and 95°.

Pronounced deviations from the basic resistance have been produced by changing the nutritive substratum. The kind of peptone used has been found to be significant, but the resistance appears to have been independent of the concentration of the peptone.

Spores of diminished resistance were formed in all digest media tested with the exception of casein digest, which yielded spores of an enhanced resistance. The explanation of this probably rests on the difference in phosphate content of these media. Very resistant spores were formed in isoelectric gelatin. Evidence which has been acquired indicates that this was probably due to the low salt content of this medium. Infusions of vegetable matter were found to be excellent media. Spores of high resistance were formed in these media in all cases. Slightly more resistant spores were formed in

liquid media than in solid media. Cultivation in the presence of a suitable concentration of either phosphate or magnesium resulted in the formation of spores considerably more resistant than the normal spores. Spores slightly more resistant than normal were formed in the presence of calcium or iron, and also by cultivation in physiologically balanced solutions. The increase in these cases, however, was not pronounced. A number of inorganic salts were found to be without influence on the resistance of spores produced in media to which they had been added. Media enriched by the addition of certain organic substances were found to yield spores greater in resistance than the normal. Of especial significance in this connection may be mentioned available carbohydrates and certain organic and amino acid radicles.

No correlation was apparent between the extent of growth or sporulation and the resistance of the spores produced. The initial reaction of the medium in which the spores were formed was found to be without influence. The temperature of cultivation, however, was found to be of considerable significance, the higher temperatures resulting in spores of an enhanced resistance. Cultivation in a medium with the surface tension reduced by the addition of bile resulted in spores of normal resistance. It was found that the time necessary to sterilize a suspension at a given temperature increased, as the concentration increased. With uniform concentrations the time necessary to sterilize decreased as the temperature increased.

No effect was apparent as a result of heating in a solution of increased density, or in tubes of different lengths. Exposure of the cultures to X-rays before incubation had no effect on the resistance of the spores produced. Evidence has been acquired which emphasizes the necessity of using strained suspensions in heat resistance tests. Irregular and erroneous results will be obtained unless this precaution is observed. Confirmation of the results of previous workers on the disinfecting action of the hydrogen and hydroxyl ions has been obtained.

Desiccated spores exhibited a resistance in excess of that exhibited by young, moist spores. Spores stored for an equal length of time in a moist condition were not, however, appreciably changed in resistance. The course of mortality, as determined by quantitative plating at various time intervals, was not of such a nature that it could be described by the equation for a first order chemical reaction. Dormancy, either of heated or unheated spores, was not exhibited by the culture used in this study.

The resistance of the spores was increased by a process of selection of resistant cells. Efforts to increase the resistance by cultiva-

tion in media which regularly yielded spores of an enhanced resistance were not successful. Attempts to produce spores in any appreciable quantity in synthetic media prepared from highly purified chemicals met with failure.

Evidence gleaned from the literature and accumulated during the progress of this work supports the idea that the cause of death in cells exposed to a high temperature is the coagulation of bacterial protein. Conditions which render protein more difficult to coagulate consequently result in an increased resistance to heat. The water and the ash content of the cell appear to be especially important in this connection. However, cultivation under certain nutritive conditions which do not appear to be intimately related to either the water or ash of the cell has invariably resulted in the production of resistant spores.

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An Endeavor to Adapt a Trypsin Susceptible Bacteriophage to the Action of Trypsin.

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It is well known to students of the d'Herelle phenomenon that bacteriophages, with rare exceptions, resist the action of trypsin for indefinite periods of time. Recently, one of us¹ described the trypsin susceptibility of 2 races of staphylococcus bacteriophages; one a monovalent, the other a polyvalent race. These 2 races were found to be highly susceptible to the action of trypsin, complete inactivation following an exposure to this enzyme within 48 hours at incubator temperature. In view of the difference of opinion entertained by various investigators on the question of whether bacteriophages actually possess powers of adaptation, a property indicative of life, it occurred to us that this susceptibility to tryptic activity might possibly serve as a basis for determining further the capacity of bacteriophages to adapt themselves to deleterious or inhibiting agents.

Two methods of arriving at an answer were employed. In one series of experiments, the bacteriophage was exposed to the action

¹ Schultz, E. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, xxv, 280.