de Schweinitz (E.A.)

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THE ENZYMES OR SOLUBLE FERMENTS OF THE HOG-CHOLERA GERM.[1]

BY E. A. DE SCHWEINITZ, Ph. D.,
BIOCHEMIC LABORATORY, BUREAU OF ANIMAL INDUSTRY, DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

IN the *Archiv für Hygiene*, 1890, vol. x, pt. 1, p. 1, Fermi describes the detection and isolation of soluble ferments from the cultures of a number of different germs. Some of these germs belong to the class that produce gas (principally CO_2 and H) when grown in media containing glucose or glucose-forming substances, and as a rule the gas-producing germs when cultivated upon proper media have been found to be ferment builders.

As has been shown by Smith and other investigators, the hog-cholera germ produces large quantities of gas when grown in peptonized beef-broth containing 2 per cent. of glucose. About one-fourth of this volume is hydrogen and three-fourths carbon dioxide, the remaining liquid containing acetic and succinic acids, as I have found by analysis.

To detect and isolate the soluble ferments of the hog-cholera germ, if any, several different culture-media have been tried, but the one that has given

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the most satisfactory results is sterilized milk. In plain peptonized beef-broth media but very small amounts of these ferments were produced, sometimes none.

Upon agar-agar the growth of the germ is rapid, and from the watery extract of the surface growth a mixture of two ferments has been obtained that will convert starch into glucose and liquefy gelatin—in other words, ferments that act as diastase on the one hand and as trypsin on the other.

If now we take either skimmed or fresh milk, carefully sterilize it, and then inoculate it with the hog-cholera germ, after about three weeks the milk has become thin and watery in appearance, a curd-like coating has formed on the surface, and the reaction, if the growth has been normal, has become either neutral or alkaline. The germs may now be removed by filtering the milk through a carefully sterilized Pasteur tube, or they may be killed by heating the solution to 54° C. for several hours, or still better, by the addition of a saturated solution of thymol.

From the cultures sterilized in this way the addition of absolute alcohol throws down a voluminous precipitate consisting of albumose, a little peptone and soluble enzymes.

A purification of this precipitate and partial separation of enzymes from the accompanying albuminoid matter is attained by a re-solution in water and precipitation with basic calcic phosphate. The enzymes are carried down mechanically by this, and can be dissolved out by water and reprecipitated with alcohol. After repeated treatment of this sort

a small quantity of white powder is finally obtained, which will liquefy gelatin, digest fibrin and albumin, and convert starch into glucose.

To separate the diastase-like ferment from the liquefying ferment, the mixed ferments were rubbed up with glycerin and filtered. The solution in glycerin contained the hydrating ferment, while the undissolved portion taken up again in water and precipitated by alcohol does not act as a hydrating agent, but as a digestive agent.

The trypsin-like ferment can also be precipitated by means of saturated salt-solution. This latter ferment seems to be formed in much smaller amount than the former.

The action of both of these ferments is destroyed above 55° C. They contain nitrogen, but when pure do not give the general albuminoid reactions.

In order to try and obtain these ferments in a purer form, and possibly larger quantity, an artificial culture-medium proposed by Fermi, in the *Archiv. für Hygiene*, vol. xiv, for use with other germs, was tried for the hog cholera bacillus. This contained 1 per cent. ammonium phosphate, 0.1 per cent. acid potassium phosphate, 0.02 per cent. magnesium sulphate, and from 4 to 5 per. cent. glycerin. The hog-cholera germ grows vigorously in this liquid, and after from three to four weeks the cultures become alkaline in reaction. They give also the peculiar amine odor characteristic of the hog-cholera cultures. From these artificial sterilized cultures I have so far been unable to separate the diastase-like ferment, but a small amount of the

tryptic ferment, showing that the ferment-production is dependent upon the medium.

As for the physiologic effects of these ferments, Fermi holds that in general the soluble ferments are without great physiologic importance in the process of disease.

The initial experiments that I have made upon guinea-pigs show that, so far as this particular disease at least is concerned, the ferments are of great importance in the production of immunity.

Guinea-pigs about a pound in weight each have been injected with a sterile solution in water of these ferments, the amount taken varying from 0.003 gram up to from 0.02 to 0.05 gram. The injections below 0.01 gram were without ill-effect, beyond that there was a rise of temperature for several days. The injection of 0.05 gram was in several instances sufficient to kill the animals. The autopsies showed some injection of the bloodvessels of the intestines, but in other respects the animal was normal in condition.

A single injection of 0.04 gram of the ferments was sufficient to make the animals immune to an inoculation with the hog-cholera germ that was sufficient to cause the checks to die in ten days.

It is probable, therefore, that the soluble ferments exert in their way, at least in some diseases, a potent action in rendering the animal insusceptible to disease.

It is possible, too, that to the indirect action of these specific ferments we may trace the protective and curative influence of the blood-serum of immune animals.