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[Reprinted from THE MEDICAL NEWS, September 6, 1890.]

**A PRELIMINARY STUDY OF THE PTOMAINES
FROM THE CULTURE-LIQUIDS OF THE
HOG-CHOLERA GERM.¹**

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DRS. SALMON and SMITH, of the Bureau of Animal Industry, U. S. Department of Agriculture, were the first to demonstrate that the substances produced by bacteria in their growth in culture-media could be used for purposes of preventive inoculation. This they proved by a number of experiments upon pigeons, in 1887. They succeeded in producing immunity from hog-cholera by inoculating pigeons with sterilized culture-liquids of the hog-cholera germ. With a view to learn more of the products of these and other disease-germs, a chemical laboratory was recently attached to the Bureau of Animal Industry, and for a short time I have been engaged in studying the products in the culture-liquids of the hog-cholera germ. The bacteriological work was done by Dr. Moore, of the Bureau of Animal Industry. The advice of Drs. Salmon and Smith has also been very valuable, and it was at their suggestion that the work was under-

¹ Read before the Chemical Section of the American Association for the Advancement of Science, August, 1890.



taken. Although the experiments are not complete, we think it best to make now a preliminary statement in regard to some of the results obtained, reserving for the near future a more extended account of the work.

The researches of Brieger, Baginsky, Salkowski, Selmi, and others, have shown that the multiplication of a number of different bacilli produces basic substances of an alkaloidal character, called *Pto-maines*. Some of these bodies are very poisonous, and a number of them have been shown to be identical with certain of the artificially prepared amines. In general, the best results have been obtained by allowing the germs to multiply in beef-bouillon, and after a number of experiments we have found an acid bouillon containing 2 per cent. of peptone to be the most satisfactory medium for the growth of the hog-cholera germ. Erlenmeyer flasks of 500 c. c. capacity were used, the mouths of the sterilized flasks being closed with a cotton plug. After inoculating the liquid with the germ the flasks were allowed to stand in the incubator for from two to three weeks, at a temperature of about 37° C. At the end of this time the liquid had become cloudy, and considerable precipitate, due to the growth of the germ, was found at the bottom of the flask and in suspension. The fluid had a decidedly alkaline reaction. Careful examination showed that the culture-liquid had not become contaminated with foreign germs.

In endeavoring to isolate the chemical products from these liquids, the methods, with some slight

modifications, by which Brieger obtained such brilliant results, were followed.

The culture-liquid, after being acidified with dilute HCl, was evaporated on the water-bath. The residue was then extracted with 98-per-cent. alcohol, and the filtered solution treated with mercuric chloride. A heavy crystalline precipitate was formed which increased upon standing. After filtration this precipitate was treated with water, and decomposed with sulphuretted hydrogen, and the mercury sulphide removed by filtration. From the filtrate, after removal of the excess of H_2S and concentration, I was able to isolate cadaverine, and a primary amine which I have not yet identified. The filtrate from the mercuric chloride precipitate was freed from excess of mercury by sulphuretted hydrogen, and the mercury sulphide filtered off. The residue, after concentrating this filtrate, was extracted with absolute alcohol, the solution thus obtained showing the presence of a salt of an alkaloidal character. The reactions were as follows:

With phosphomolybdic acid—light-yellow precipitate.

With bismuth-potassium iodide—red needles.

With phosphotungstic acid—a white precipitate.

With potassium iodide and iodine—brown-red precipitate.

With platinum chloride—yellow crystalline precipitate.

With gold chloride—yellow-red crystalline precipitate.

The double salt obtained with $Pt Cl_4$ was submitted, after crystallization from 96-per-cent. alcohol, to a

preliminary analysis, giving results which correspond to the formula $C_{14}H_{34}N_2PtCl_6$.

The free base I have not yet succeeded in obtaining in a pure form, and will not be ready to give this ptomaine a name until more is learned of its constitution. The hydrochloride of this base is soluble in absolute alcohol, but I have obtained the salt only as a thick syrup, which so far will not crystallize over sulphuric acid in vacuo.

By treating the original culture-liquids of the hog-cholera germ with a large excess of absolute alcohol a white flocculent precipitate was obtained, a portion of which was soluble in water, and could again be precipitated by alcohol. By repeated treatment in this manner with water and alcohol a small quantity of an albuminoid body containing C, H, N, O, and S was finally obtained. This substance, which we will call albumose, when dried over sulphuric acid in vacuo, crystallized in white translucent plates. After drying, it was still soluble in water, though it dissolved with more difficulty. The water solution gave with $PtCl_4$ an almost insoluble precipitate, appearing under the microscope as needle-like crystals.

The toxic effects of this albuminoid body and of the ptomaine were tested by subcutaneous inoculation of guinea-pigs. At the point of the inoculation with the albumose there was swelling and the formation of a hard lump, which disappeared after four or five days. There was also a rise in the temperature of the animal for a few days, but in other respects it seemed to suffer no inconvenience.

After subcutaneous injection of a small quantity

($\frac{1}{2}$ c. c. or about 0.005 gramme) of the neutral solution of the hydrochloride of the new base before referred to, a rise of temperature in the animals was noted for a few days, and also necrosis and slight ulceration at the point of inoculation, otherwise the animals appeared well. The salt of this base, as well as the albumose, are, therefore, not virulent poisons. It may be added that special attention was given to keeping the solutions sterile, by means of a Pasteur filter, and that the absence of germs was determined by making plate-cultures.

According to an article in the *Berliner klinische Wochenschrift*, March, 1890, Brieger and Fränkel have succeeded in isolating from culture-liquids (containing 10 per cent. of blood-serum) of the diphtheria, typhoid fever, and cholera-infantum germs, albuminoid bodies which they call tox-albumose, and which are very poisonous in small doses.

From culture-liquids of beef infusion with peptone and 2 per cent. of blood-serum, we have also obtained with the hog-cholera germ an albumose, having chemical properties similar to those described by Brieger, but not virulently poisonous to guinea-pigs when given subcutaneously, though there was considerable ulceration at the point of inoculation.

The detailed results of the experiments in preventive inoculation which have been conducted are not quite ready for publication. The statement may be made in advance, however, that by inoculating guinea-pigs with certain chemical compounds which I have prepared, the animals have been rendered immune from hog-cholera.

Having, therefore, made considerable progress in the study of the culture-liquids of this hog-cholera germ, and also in preventive inoculation with chemical compounds, the results of this line of work are reserved for the present, and until more detailed results of the experiments can be given, which are promised for the near future.

