

the dilute alcohol extract of *B. typhosus* or *B. enteridis*. This crude preparation of *B. paratyphosus* B produced strongly agglutinating and precipitating sera when injected into rabbits.

A second extract was obtained also from *B. proteus* HX19 with saline solution from the bacilli previously treated with dilute alcohol. In contrast to the dilute alcohol extract this preparation had a high N content and was digested by trypsin. Both the dilute alcohol and the saline extracts reacted with common HX19 and OX19 immune sera but gave practically no precipitations with typhoid and paratyphoid antisera. The relation of these substances to that described by Przesmycki³ is being studied. This is a preliminary report.

¹ Landsteiner, K., and Furth, J., *Proc. Soc. Exp. Biol. and Med.*, 1927, **xxiv**, 379.

² Douglas, S. R., and Fleming, A., *Brit. J. Exp. Path.*, 1921, **ii**, 131, 175.

³ Przesmycki, F., *Soc. Biol.*, 1926, **xv**, 744.

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Antigenic Composition of the Watery Extracts of the Tubercle Bacillus.

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The differences found between the eggwhite hypersensitiveness of tuberculous guinea pigs and the tuberculin hypersensitiveness induced us to continue our former work¹ on the protein antigens of the tubercle bacillus. We publish this preliminary report because the detailed examination of the observations made requires considerable time. The following extracts of a virulent freshly cultivated strain of tubercle bacilli were examined in their relation to the antigen antibody reactions and the tuberculin reaction: Direct extract was obtained with distilled water. The growth on glycerin bouillon was washed without being broken up with distilled water, and filtered quickly, then suspended and slowly ground for two days in distilled water with the addition of some chloroform. If the culture is washed longer with distilled water and filtered slowly one obtains an extract of very slight efficiency and very little protein content. This was the reason that in our first publication, we overlooked the interesting properties of this extract. After being filtered and

washed again with distilled water the bacillary mass was dried and extracted with ether (2 days) and alcohol (twice during 3 hours), then extracted twice with distilled water, being thoroughly ground (5 days) (extract after defatting), and six hours (including filtration) with 0.2 per cent NaOH (NaOH extract). With every extract several sera were obtained from tuberculous guinea pigs in the first weeks after the infection. The complement fixation, precipitation, and tuberculin effect of the extracts and different precipitates of them with HCl, alcohol, NaOH, etc., were examined. None of the sera has given complement fixation with the alcohol extract of the tubercle bacillus, or precipitation with the purified carbohydrate precipitable substance, but they gave strong precipitation with the HCl precipitates of the extracts.

With these sera and with rabbit sera obtained with tubercle bacillus emulsion, three different antigens were clearly distinguished in the extracts, and the presence of two more made probable. There is a distinct antigen present in the direct extract HCl precipitate, in the filtrate from the HCl precipitate of the same extract (beside the carbohydrate precipitable substance) and in the HCl precipitate of the NaOH extract. From the HCl precipitate of the extract, after defatting of the bacteria, it could not yet be decided whether it contains an independent antigen or whether it is a mixture of the others, and there is indication that the filtrate of the direct extract contains two antigens.

The differences in the reaction of the different preparations are marked. For instance the filtrate after HCl precipitation of the direct extract gives a complete reaction in the amount of .004 cc. with the corresponding serum, and in .12 cc. is negative with the serum of the NaOH extract. The HCl precipitate of the NaOH extract gives a complete reaction in .0005 cc. with its own sera, and only in .03 cc. with one serum obtained with the direct extract, and in .015 cc. with the other serum.

All extracts and all preparations obtained from them have tuberculin activity. From the extract after defatting, and from the NaOH extract, both the antigens for complement fixation and the substances with tuberculin effect are precipitated to a large extent with HCl together with the protein substances. From the direct extract about half of the tuberculin effect remains in the filtrate, after the precipitation with HCl. The precipitate which contains about 14.3 per cent N, after two precipitations and washing with acid-distilled water, contains only a small fraction of the original activity of the extract. The tuberculin effect and the antigenic

effect in the complement fixation, demonstrable with the sera we have had at our disposal, are not bound to the same substance in the filtrate. The antigens are precipitated with the addition of 1 volume of alcohol, and the largest part of the tuberculin active substances are precipitated only with the addition of 5 to 9 volumes of alcohol. The properties of the direct extract filtrate are very similar to the properties of the residue material described by Zinsser,² and the effective substance may be similar to the water-soluble protein isolated by Long and Seibert³ from the culture fluid of tubercle bacilli.

In the hypersensitiveness obtained with eggwhite there is present an independent sensitization against egg albumen and globulin. We tried in several ways to decide whether there is present a specificity of the different fractions of the bacillus in the tuberculin effect. Two preparations, the direct extract HCl filtrate and the NaOH extract HCl precipitate, were compared. Certain animals are more sensitive to repeated tests with one or the other of the preparations. In the first weeks after the infection the sensitiveness appears first toward the direct extract filtrate; sensitiveness toward the NaOH extract seems to develop later.

If tuberculous guinea pigs are injected subcutaneously with large doses of the NaOH precipitate, the sensitiveness toward the NaOH precipitate is much more diminished than the sensitiveness toward the direct extract filtrate. The subcutaneous injection of the direct extract filtrate, which causes more violent general reaction, lowers the sensitiveness considerably toward both preparations, but decidedly more towards the filtrate. The subcutaneous injection of NaOH precipitate causes usually a much larger infiltration than the injection of the filtrate. On the other hand, according to our present observations from solutions of equal strength in the skin test, much less is needed from the filtrate of direct extract to kill a tuberculous guinea pig than from the NaOH extract. These observations point to a specificity of the different fractions also in the tuberculin effect, but they might also be the result of the circumstance that the active group is in different binding in the different fractions of the bacillus. This is a preliminary report.

¹ Dienes, L., Schoenheit, E. W., and Scheff, L. D., *Am. Rev. Tuberc.*, 1925, xi, 151.

² Zinsser, H., *J. Exp. Med.*, 1921, xxxiv, 495.

³ Seibert, Florence B., and Long, E. R., *Am. Rev. Tuberc.*, 1926, xiii, 400.