

that the allergic antibody was alone responsible for the patient's skin sensitivity, generalized urticaria and other allergic symptoms, and was entirely unrelated to the patient's resistance to insulin.

*Conclusions.* Evidence is presented indicating the presence of two antibodies for crystalline insulin in the serum of a patient who was both allergic and resistant to crystalline insulin. It was also shown that the patient was allergic but not resistant to a preparation of human insulin. The relation of the two antibodies to the patient's response to crystalline and human insulin is discussed.

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### Collodion-Agglutination Test in Human Tuberculosis.

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In human tuberculosis the precipitin-reaction, even utilizing a highly purified and reactive antigen, has been found positive in only about 12% of cases.<sup>1</sup> It was thought that the collodion-agglutination technic<sup>2</sup> would demonstrate precipitins in a much higher percentage of tuberculous humans. Previous reports concerning this latter method in human and experimental tuberculosis had suggested its accuracy and extreme delicacy.<sup>3, 4</sup>

The technic as outlined by Cannon and Marshall<sup>2</sup> was rigidly adhered to in all details. As antigens, Old Tuberculin and a purified tuberculo-protein were used. The protein had been previously shown to be a superior antigen, *in vitro*, to Old Tuberculin.<sup>1</sup> For adsorption, 1 cc of OT in 19 cc of water was allowed to stand overnight with 20 cc of a dense suspension of collodion particles. For adsorption of the TPT, 20 mg of this was substituted for the 1 cc of OT, and the pH of the solution was adjusted to 5.8 with sodium-acetate acetic-acid buffer.

Over a period of several months, coated particles prepared in this manner were repeatedly tested with tuberculous human and rabbit sera, and normal human and rabbit sera. Some undoubtedly true and specific reactions were obtained in the animal sera, better reactions

<sup>1</sup> Seibert, F. B., *Am. Rev. Tuberculosis*, 1930, **21**, 370.

<sup>2</sup> Cannon, P. R., and Marshall, C. E., *J. Immun.*, 1940, **38**, 365.

<sup>3</sup> Weir, J. M., personal communication.

<sup>4</sup> Weir, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 47.

being obtained with TPT-coated particles than the OT-coated ones. However, the data could not be considered significant since many false reactions were also obtained in both animal and human sera. The unsatisfactory reactions were due to several causes, *viz.*, the use of suspensions of particles older than 24 hours, the use of non-inactivated sera, or sera more than 24 hours old, and to the varying effects of different speeds of centrifugation, time of incubation, etc. In other words, the collodion particles, particularly when mixed with sera in low dilutions, are liable to become flocculated because of the action of a variety of non-specific elements. It is for this reason, therefore, that the system must be rigidly controlled at all stages.

Fewer false positive reactions were obtained when fresh serum and freshly made particles were used. To enhance adsorption the tuberculo-protein was adjusted to a pH close to its isoelectric point before it was mixed with the suspension of particles. Even with these particles, titers below 1:60 were not considered reliable.

When these precautions were observed, it could be readily demonstrated that the coated particles were specifically agglutinated in high dilutions (1:3000) by antiserum from a goat immunized with dead tubercle bacilli. The OT-coated particles, however, when tested against 8 sera from tuberculous patients of different types gave no positive reactions. The TPT-coated particles, when tested against 24 sera from tuberculous patients of all types showed only one probably true reaction (titer 1:128) and 5 doubtful reactions below 1:60. Nor could positive reactions be obtained with either type of particle with sera from humans with positive cutaneous reactions but no active tuberculosis.

*Conclusion.* After several of the causes for false positive reactions in this method were eliminated, about 4% of human tuberculous sera gave specific positive reactions.