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A THERMOPRECIPITATION REACTION IN TRYPANOSOMA EQUIPERDUM INFECTION IN LABORATORY ANIMALS

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Ascoli (1) showed that a thermostable substance associated with the anthrax organism would give a precipitate of diagnostic value, with the corresponding immune serum. Several other observers, among whom are Kraus (2), Piras (3), Philip and Hirst (4), Nai (5), Joos (6), Meuller and Tomcsik (7), Landsteiner and Furth (8), Dochez and Avery (9) and Zinsser and Parker (10) have found similar thermostable substances in bacteria and yeasts. The evidence points to the nature of these substances as carbohydrate. Since no such work has been done on trypanosomes, we decided to investigate *T. equiperdum* in regard to whether it possesses a thermoprecipitinogen.

EXPERIMENTAL

The precipitable substance was made as follows: Extracts of various organs and tissues of rats, guinea pigs and rabbits that were infected with *T. equiperdum* or had died from the infection, were tested by means of the precipitation reaction for the presence of a substance which reacts with the serum of recovered animals. Some of the organs and tissues tried were spleens, hearts, livers without the gall bladders, lymph nodes with their surrounding tissue, and blood drawn while the number of trypanosomes was as high as 1,670,000 per c. mm.

Extracts of each of these organs or tissues were prepared by cutting them into small pieces and then triturating them in the presence of talc. To this triturate was added five parts of physiological salt solution or water for each gram of the original organ or tissue. The suspension was then boiled for 5 minutes at 100°C. and permitted to cool without chilling. The liquid was separated from the residue of coagulated protein and talc by centrifugalization. The supernatant fluid was then removed by pipettes and kept cool until used.

The extracts were generally prepared and used on the same day. 0.5 cc. of the clear extract was layered upon an equal quantity of immune serum in a small agglutination tube 100 x 11 mm. The results were read after 30 minutes at room

or body temperature and again after 18 hours in the ice box. Frequently tests which were negative or only weakly positive at the end of the 30 minute period of incubation showed a markedly positive reaction after standing 18 hours in the ice box. We have, therefore, recorded in table form only the readings after 18 hours in the ice box.

Within the last 2 years we have done thermoprecipitation tests on the extracts and sera of 108 white rats. 20 of these rats were not infected and the extracts and sera of these normal animals were used as controls. Of the other 88 infected rats, the organs tested were from animals in which the infection following its usual course had terminated fatally in from 4 to 6 days. In a few instances the animals were killed while being bled for immune sera. When this occurred during the 4th or 5th day of the infection, the organs were still used even though the normal course of the infection had been interrupted.

The serum used in each instance was obtained by letting the blood drawn from the normal or infected animals clot and pipetting it off aseptically after centrifugalization. It was not inactivated. In most instances pooled serum from several animals was used. The serum was kept in the refrigerator until ready for use. In this way the same lot of serum was used over a period of 2 or 3 weeks.

The tests were further controlled by using, not only the extracts of the whole blood of normal rats and others at the height of infection, but also the residue of trypanosomes and red blood corpuscles separated from the serum by centrifugalization. While there was an occasional weakly positive reaction with the red blood corpuscles and the trypanosome extracts, no positive reactions were observed with extracts prepared from the clotted cells and organisms which had been washed free of the serum before extraction.

The sera of rats that have been repeatedly infected with *T. lewisi* gave some precipitation reaction with spleen extracts of animals infected with *T. equiperdum*. In the same manner the immune sera of rabbits infected with *T. equiperdum* gave some precipitation reaction with spleen extracts of *T. lewisi*-infected rats. These group reactions were never marked but generally they were of a one plus or an occasional two plus reaction.

Within the last 12 months we have supplemented the test on the 108 rats with 39 guinea pigs, 10 of which were used as normals and the other 29 were infected with *T. equiperdum*. The serum of the guinea pigs was generally taken during the 8th to 12th week of infection. The organs used for preparing the extracts were taken after death, which resulted from the infection or from the bleedings. Similarly 42 rabbits have been used in which 6 were controls, while the others were given mild infection. After the inoculation of small numbers of

living trypanosomes, some of the rabbits recovered from the infection within 12 to 16 weeks with only a slight evidence of having had the disease. If these animals were reinfected, they showed varying degrees of enhanced resistance to infection. The sera from the

TABLE I

This table summarizes the results of thermoprecipitation tests when 0.5 cc. of clear extracts of the spleens of normal animals and animals infected with *T. equiperdum*, respectively, is carefully layered onto the surface of an equal amount of immune serum from various animals. All the results recorded represent readings after 18 hours in the ice box.

Extracts of spleen	Rat serum		Guinea pig serum		Rabbit serum	
	Normal	Immune	Normal	Immune	Normal	Immune
Normal rats, saline and aqueous	−	−	−	−	−	−
Infected rats, aqueous	−	+	−	+	−	+++
Infected rats, saline	−	±	−	++	−	++
Normal guinea pigs, saline and aqueous	−	−	−	−	−	−
Infected guinea pigs, aqueous	−	−	−	+	−	++
Infected guinea pigs, saline	−	−	−	±	−	+
Normal rabbits, saline and aqueous	−	−	−	−	−	−
Infected rabbits, aqueous	−	−	−	+	−	+++
Infected rabbits, saline	−	−	−	±	−	+++
T. lewisi-infected rats, aqueous and saline	−	±	−	±	−	+
T. lewisi-infected rats, aqueous and saline extracts of red blood corpuscles and trypanosomes	−	−	−	−	−	±

+++, a gray ring of flakes 1 mm. or more in thickness at the junction of the two liquids, and sufficiently dense to be opaque when observed from above.

++, a gray ring about 1 mm. in thickness at the junction of the two liquids but not sufficiently dense to cut off vision when observed from above.

+, a distinct gray line at the junction of the two liquids when observed from the side of the tubes, but transparent when observed from the top.

±, a faint gray line at the junction of the two liquids when observed from the side, but transparent when observed from the top.

rabbits immunized in such a manner are the ones that showed the most pronounced reaction, generally given as three plus in Table I.

There were considerable variations in the reaction in each of the series of animals in which there were positive rings, but the table shows the predominating reaction for the combination noted.

associated with the tissue reaction to the organism. This substance was not necessarily antigenic.

Except for the fact that the antigen is a thermostable substance, little is known as to its exact nature. We do know, however, that it is free from all protein that can be coagulated by heat, either in the presence of water or saline solution. It may conceivably be a polysaccharide with immunologically specific properties.

SUMMARY AND CONCLUSION

There is a thermoprecipitinogenic substance in extracts of the spleen of rats, guinea pigs and rabbits infected with *T. equiperdum*. It does not appear to be within the body of the trypanosome itself.

Antibodies to this heat-resistant precipitable substance were found in the serum of infected animals.

The antibody strength seems to be relatively less in the serum of rats than in the other animals but the power of extracts from the spleen of infected rats appeared to be equivalent to the power of similar extracts of the other animals.

The antibody titer of the serum of rabbits was greater than in the case of the other two species investigated. This was shown not only by the reaction with the extracts of spleens of the same species, but also by the reaction with extracts of the spleens of similarly infected animals of other species.

I wish to express my appreciation to Dr. Claus W. Jungeblut of the Department of Bacteriology of the College of Physicians and Surgeons of Columbia University, whose preliminary criticisms of this article caused the control test with *T. lewisi* to be added.

BIBLIOGRAPHY

1. Ascoli, A., *Centr. Bakt., 1. Abt., Orig.*, 1911, **58,** 63.
2. Kraus, R., *Wien. klin. Woch.*, 1897, **10,** 736.
3. Piras, L., *Centr. Bakt. 1. Abt., Orig.*, 1913, **71,** 67.
4. Philip, W. M., and Hirst, L. F., *J. Hyg.*, Cambridge, Eng., 1917, **15,** 527.
5. Nai, D. D., *J. Immunol.*, 1930, **19,** 255.
6. Joos, A., *Centr. Bakt. 1. Abt., Orig.*, 1903, **33,** 762.
7. Mueller, J. H., and Tomcsik, J., *J. Exp. Med.*, 1924, **40,** 343.
8. Landsteiner, K., and Furth, J., *J. Exp. Med.*, 1929, **49,** 727.
9. Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26,** 477.
10. Zinsser, H., and Parker, J. T., *J. Exp. Med.*, 1923, **37,** 275.
11. Hughes, T. P., *J. Immunol.*, 1933, **25,** 275.