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Inhibition of Typhus and Spotted Fever by Intradermal Inoculation of Antiorgan or Certain Normal Sera.*

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The present report includes one phase o the study on the mechanism of action of antiorgan sera (ACS=REIS) on infectious processes,[1] namely: on the course of typhus and spotted fever† in guinea pigs after intradermal inoculation into an area previously infiltrated with the antiserum. This involves primarily the role of the skin as a natural portal of entry and as dermal barrier for these infections.

Methods. A square area (1 cm along each side) on the shaved abdominal skin of the guinea pig is delineated and injected intradermally at 4 corners toward the center with the serum in 1:5 or 1:10 dilutions (0.1 ml at each corner). The center of this area is injected 2 hours later with 0.1 ml of a 10% brain suspension of a typhus or spotted fever guinea pig (Anigstein *et al.*[2]) The antiorgan serum was prepared in rabbits with guinea pig spleen and bone marrow as antigen according to the original technic of Marchuk,[3] and its modifications (Anigstein *et al.*[4]). Each series of test guinea pigs was accompanied by controls injected either with infective brain tissue alone, or in addition, with normal rabbit serum substituted for the immune serum. The potency of the antisera was based on complement fixation titers with homologous antigen (spleen and bone marrow), and by action on the outgrowth of tissue explants (Pomerat[5]). In one series, the globulin fraction of the antiguinea pig serum in dilution 1:10 was used. Absorption tests with boiled sheep cells of both antiorgan and "normal" rabbit sera showing antisheep hemolysins were carried out to study the possible role of the Forssman antibodies. Groups of 6 to 12 afebrile guinea pigs of both sexes (400-500 g body weight) were used in each series of experiments involving various combinations of the test sera. Body temperatures of test guinea pigs were recorded daily over a period of 18 days.

Results. The first series of 6 guinea pigs were treated with antiguinea pig rabbit serum (dilution 1:10) and 2 hours later injected at the site of serum inoculation with the brain suspension of a typhus infected guinea pig. Of these, 4 animals remained afebrile; in one the course of the disease was markedly attenuated, and one showed typical typhus. All controls reacted with typical fever after 6 days incubation. Of 6 test animals in another series, 5 remained afebrile, while only one responded with fever of 2 days duration. Significant results were obtained with antiorgan serum No. 91 (complement fixation titer 1:150; hemolysin titer 1:1000) used in a dilution 1:5. Of 5 guinea pigs, 4 remained afebrile, one developing fever after 11 days. The controls treated with serum (diluted 1:5) from the same rabbit (No. 91) bled before immunization ("normal" serum), reacted with typical typhus.

Occasionally, sera from untreated ("normal") rabbits attenuate the course of the

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1 Anigstein, L., and Pomerat, C. M., *Tex. Rep. Biol. Med.*, 1945, **3**, 545.

2 Anigstein, L., *et al.*, *J. Immunol.*, 1944, **48**, 69.

3 Marchuk, P. D., *Am. Rev. Sov. Med.*, 1943, **1**, 113.

4 Anigstein, L., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 279.

5 Pomerat, C. M., unpublished data.

infection, showing abortive fevers of one to 2 days duration, in contrast with the afebrile course of the animals treated with the antiserum. The serological analysis of these "normal" sera gave evidence of lysins against sheep cells with titers of 1:20 or 1:40, and in some cases as high as 1:200. This has been recorded by other workers and attributed either to constitutional genetic factors (Witebsky and Neter[6]), or to past infections of heterogeneous origin (Hyde[7]). Attempts to investigate the possible role of the hemolysins by their absorption from both normal and immune sera with boiled sheep cells do not suggest correlation of heterophile antibodies with the observed protective action.

Experiments with guinea pig antiserum No. 91 also showed protective qualities when challenged with the highly virulent spotted fever, namely, abortive fever reactions, or complete absence of fever were observed. No greater protection was afforded by the globulin fraction of antiguinea pig serum (Complement fixation titer 1:300; hemolysin titer 1:1250) used in dilution 1:10. Slight elevations of body temperatures not exceeding 40°C for 1-2 days were recorded in 5 guinea pigs of the 10 used in the series. The temperatures of the other 5 animals remained normal over the 18 days observation. On the contrary, the 3 untreated controls developed typical spotted fever. As observed in the typhus series, the course of spotted fever was occasionally attenuated by certain "normal" rabbit sera.

Conclusions. Antiguinea pig rabbit immune sera, when injected intradermally into guinea pigs inhibited the clinical manifestations of typhus and spotted fever after the infective material was inoculated at the site of the serum administration. Highly protective properties against typhus and spotted fever, previously absent in the serum of untreated rabbits, were demonstrated in the antisera of the same animals after their immunization with guinea pig spleen and bone marrow. However, an attenuation of typhus and spotted fevers occurred when serum of certain "normal" rabbits was used. The presence of Forssman antibodies in the rabbit immune sera and of sheep cell lysins in the "normal" sera seems of no significance. Among the factors involved in the observed phenomena, the barrier effect of the skin and its modified permeability are probably decisive. The role of the spreading factor in the invasiveness of rickettsiae is under systematic experimental study.

The authors make no suggestions as to the possibilities of utilizing the methods and results described as a practical application for protection against or treatment of typhus or spotted fever.

[6] Witebsky, E., and Neter, E., *J. Exp. Med.*, 1935, **61**, 489.

[7] Hyde, R. R., *Am. J. Hyg.*, 1928, **8**, 205.

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Autographic Localization of Radio-Iodine in Stained Sections of Thyroid Gland by Coating with Photographic Emulsion.*

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Radioactive materials emit radiations which have the same effect as light on a photographic emulsion. This property has been used to locate radio-elements in histological sections of tissues, and especially radio-iodine in the thyroid gland.[1] The radioactive section is usually placed in contact with a photographic film or plate.[2,3,4] The image or "autograph"

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[1] Gross, J., and Leblond, C. P., *Canad. Med. Assn. J.*, 1947, **57**, 102.