

The same experiments were repeated, using the blood of another patient† with chronic myelogenous leukemia and also that of a normal individual, and very similar results were obtained. (Lymphocytes and monocytes in normal blood were never seen to show any phagocytic activity, but the number of the latter cells encountered was too small to permit a conclusion).

We also tested the phagocytic activity of various types of leukemic blood cells on *pneumococcus type I* and *streptococcus hemolyticus* and our findings were in agreement with those reported by Strumia and Boerner.¹

Conclusion. Neutrophilic leukocytes beyond the myeloblastic stage, and occasionally the eosinophils, from the blood of patients with chronic myelogenous leukemia phagocytize L.D. bodies *in vitro*.

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Intracutaneous Immunization of Rabbits with Photodynamically Inactivated Type I Pneumococcus.

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Julianelle,¹ employing the endermal route of immunization of rabbits with heat-killed type I pneumococcus, could not demonstrate any significant agglutinative or precipitative response to the homologous organism and specific soluble substance respectively. He ascribed this failure to the fact that the pneumococcus undergoes disintegration in the skin of rabbits and this results in the destruction of the type-specific antigen. The rapid extracellular digestion by tissue-ferments of heat-killed pneumococci when injected into the skin, as demonstrated by Dubos and Macleod,² lends some support to this idea. In view of the fact that the pneumococcus inactivated by the photodynamic action of methylene blue is much more resistant to

† The second patient was a Chinese male of 28, who had been suffering from the disease for one year and 8 months. The total leukocyte count at the time of the experiment was 179,000 per mm³. He was also subsequently treated with deep X-ray irradiation which brought about a satisfactory remission.

¹ Strumia, M. M., and Boerner, F., *Am. J. Path.*, 1937, **13**, 335.

¹ Julianelle, L. S., *J. Exp. Med.*, 1930, **51**, 441.

² Dubos, R. J., and Macleod, C. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 696.

autolysis than that treated with formalin or heat,³ it was considered of interest to determine whether or not type I pneumococcus so treated could serve as an effective antigen when injected into the skin. The present investigation was therefore undertaken to study the comparative efficacy of methylene-blue-treated vaccine with those prepared by formalin or heat in immunizing rabbits by the endermal route.

Pneumococcus type I used for this experiment was one of high virulence; 0.5×10^{-9} cc of an 18-hour broth culture killed white mice regularly. The photodynamically inactivated and the formolized vaccines were prepared according to methods described previously,³ while the heat-killed vaccine was prepared by Julianelle's method.¹ The method of immunization was essentially the same as Julianelle's, except that daily rather than weekly inoculation was adopted. Each animal was given 6 consecutive injections of 0.2 cc each followed by a week of rest. The same course of immunization was repeated once and bleeding was done 10 days after the last injection. The amount of vaccine given in 4 weeks was more or less equal to that employed by Julianelle in 3 months. For agglutination and precipitation homologous organisms and soluble specific substance were used.

After 2 courses of immunization it was found that of a total of 10 rabbits immunized with photodynamically inactivated vaccine, the serum of one had a complete-agglutination titer of 1-128; three, 1-64; four, 1-32; and two, 1-16. On the other hand, out of a total of 6 animals immunized with formolized vaccine, the sera of 2 had a "complete" titer of 1-32; two, 1-16; and two, negative. Among 5 animals immunized with heat-killed vaccine, the serum of only one yielded a complete titer of 1-32; two, 1-16; one, 1-8; and one, negative. Precipitative reactions with soluble specific substance also showed higher titers of sera from animals immunized with photodynamically inactivated vaccine. Four sera of a higher agglutinative titer reacted with this polysaccharide up to the dilution of 1-500,000 and the rest 1-100,000. Two of the rabbits immunized with formolized vaccine had precipitative titers of 1-100,000 and another two, 1-10,000. The 2 sera that did not agglutinate also did not give any precipitative reaction. Sera of animals immunized with heat-killed vaccine had low precipitative titers, namely 1-50,000 in one, and 1-10,000 in two. Precipitin could not be demonstrated in the serum of the rabbit having the lowest agglutinative titer or of the one which gave no agglutination. Complement-fixation tests

³ T'ung, T., *Chinese Med. J.*, Supplement II, 1938, 291.

were also done on all sera and it was found that in general, the titer was about 10 to 20 times higher than that obtained by precipitation.

Only sera having the higher agglutinative and precipitative titers were tested for passive protection of white mice. It was found that whereas 0.2 cc of each of the 4 sera prepared with photodynamically inactivated vaccine protected mice against 10,000 to 100,000 MLD, the same amount of 3 sera from each of the other 2 series of animals protected mice against only 1000 MLD.

The superiority of the photodynamically inactivated vaccine over those treated either with formalin or by heat in the immunization of rabbits by the intracutaneous method was further substantiated by the fact that rabbits which had failed to respond to formalized or heat-killed vaccine yielded significantly high agglutinative and precipitative titers when these animals were given another 2 courses of immunization with photodynamically inactivated vaccine by the same route.

The results of these experiments offer further proof that photosensitized pneumococcal vaccine is more efficacious than vaccines prepared with formalin or heat.

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Antigenic Activity of Polysaccharides of *B. rhinoscleromatis*.

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Polysaccharide obtained from *B. rhinoscleromatis* through extraction of dried bacterial cells in weak acetic acid solution provokes in immunized rabbits the formation of complement-fixing antibodies and induces in guinea pigs active sensitization. In this experiment an attempt was made to demonstrate that the antigenicity of the polysaccharide of this bacterial species is analogous to that of the whole bacterial cell. It seemed also of interest to compare the immunological properties of this polysaccharide with that prepared by the method in which the treatment of the bacteria by heat was avoided.

A stock culture of *B. rhinoscleromatis* was passed through mice 3 times until its agar culture acquired markedly slimy appearance. The first type of polysaccharide was prepared in accordance with the