duced penicillinase; none of the susceptible strains had this property. (3) Many of the penicillinase-producing staphylococci produce coagulase indicating the importance of the former in infection. (4) Staphylococci naturally resistant to penicillin appear to be resistant as a result of their ability to produce penicillinase.

15090

Relationship Between Precipitative and Protective Antibodies of Type III *Shigella paradysenteriae* (Flexner) Immune Serum.

ELY PERLMAN* AND A. J. WEIL.

From the Laboratories of the Rockefeller Institute for Medical Research and the Lederle Laboratories, Inc., Pearl River, N.Y.

The isolation and properties of the specific somatic antigens of several types of *Shigella paradysenteriae* (Flexner) have been described in detail in a previous publication.[1] These substances appear to be single chemical entities constituted from a phospholipid, a protein and a polysaccharide. The antigens are toxic and as far as has been determined, account for all of the immunological properties of the microorganisms from which they are derived. Thus, when small quantities of antigen are injected into experimental animals they give rise to bacterial agglutinins and precipitins which are similar in all respects to those evoked by the microorganisms themselves. In addition, they elicit antibodies in mice which protect them against lethal infections with homologous bacilli. The polysaccharide portion of the complex is neither toxic nor is it antigenic in rabbits; it is this constituent, however, which is responsible for most of the serological properties of the somatic antigen.

The present study was undertaken to determine whether the specific lipocarbohydrate-protein complex of Flexner dysentery bacilli is the only antigen which stimulates the production of protective antibodies or whether the microorganisms contain additional constituents which are important in the immune response. Experiments designed to ascertain the immunological role of the polysaccharide hapten are included as well.

In the following experimental account we shall compare the protective antibody present in the sera of rabbits immunized with Type III Flexner bacilli with that remaining after absorption with the homologous specific antigen and its polysaccharide hapten.

Materials and Methods. The Type III specific antigen used in this study was isolated as described previously.[1] The polysaccharide hapten was obtained by dissociation of the complex with acetic acid.[1] The immune sera were prepared by subjecting rabbits to 3 prolonged courses of injections with formol-killed *Sh. paradysenteriae* (Flexner) Type III (Andrewes and Inman,[3] Type Z, Boyd,[2] and Weil *et al.*,[4] Type III). The rabbits were bled after each course of injections and the sera obtained were pooled. Passive protection tests were performed on Swiss mice weighing 18-20 g. 0.1 ml of 5-fold dilutions of the sera to be tested were injected intra-

* Part of the work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.

1 Goebel, W. F., Binkley, F., and Perlman, E., *J. Exp. Med.*, 1945, **81**, 315.

2 Boyd, J. S. K., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1940, **33**, 553.

3 Andrewes, F. W., and Inman, A. C., *Med. Research Council*, Special Report Series, No. 42, London, 1919.

4 Weil, A. J., Black, J., and Farsetta, K., *J. Immunol.*, 1944, **49**, 321.

TABLE I.
Results of Mouse Protection Tests of Type III Immune Serum Before and After Absorption with Specific Antigen and Hapten.

Exp. No.	No. of micro-organisms in challenging dose	Ml of serum required to protect 50% of the mice		
		Serum absorbed with specific antigen	Serum absorbed with hapten	Unabsorbed serum
1	16×10^6	—	—	0.00003
2	4×10^6	—	—	0.000025
3	1.4×10^6	0.003	—	0.00005
4	9×10^6	0.0008	0.00015*	0.000035
5	32×10^6	0.0008	0.00015*	0.000015
6	4×10^6	—	0.00025†	0.000075
7	4×10^6	—	0.0001†	0.00003
Mean		0.0015	0.00016	0.000037 ± 0.000018

50% of untreated control mice were killed by 1 to 9 microörganisms of the virulent Type III Strain No. 79-118-5Z.
* Serum absorbed with slight excess of hapten.
† Serum absorbed with great excess of hapten.

peritoneally into each of 6 to 8 mice together with the challenging dose of microorganisms. The test dose consisted of 0.4 ml of a 10^{-2} dilution of a 6-hour culture of mouse virulent Type III (strain 79-118-5Z†) organisms diluted in a 6.25% suspension of Wilson's granular mucin (Type 1701-W). The details of the method are described in a separate communication.[5] Each experiment was repeated at least once. The results given in the accompanying table are expressed in terms of the 50% survival endpoint.[6]

Experimental. I. Absorption of Type III antiserum with somatic antigen. The pooled Type III antiserum was titrated quantitatively with varying amounts of the purified specific antigen. Each ml of serum was found to contain a total of 0.312 mg of precipitin nitrogen. It was also observed that at the equivalence point 395 μg of antigen were required to precipitate 0.296 mg of antibody nitrogen from each ml of serum. A test of the supernatant liquid after precipitation with this quantity of antigen revealed that it still retained 6% of the original precipitin, but contained only a very slight excess of specific antigen amounting to 2 μg/ml. In order to avoid the toxic effects of excess antigen, each ml of full strength serum was absorbed by the addition of an equal volume of a solution containing 395 μg of somatic antigen per ml. The mixture was incubated at 4° C for 48 hours and then centrifuged in the cold. The supernatant serum was used for the mouse protection tests as described above.

The results of the mouse protection tests performed with the serum before and after absorption with the specific antigen are summarized in Table I. In Table II these results are compared with those of the quantitative studies described above. From the data presented it can be seen that absorption of Type III antiserum with homologous antigen removes approximately 95% of the precipitin and approximately 97% of the protective antibodies. The amount of protective antibody which remains after absorption is roughly equivalent to the remaining precipitin.

II. Absorption of Type III antiserum with the homologous polysaccharide hapten. The pooled Type III antiserum was absorbed with a slight excess of specific Type III hapten. The addition of an equal volume of a solution containing 375 μg/ml of the polysaccharide sufficed to remove all of the antibody precipitable by hapten. After absorption, the supernatant was found to contain an excess of polysaccharide amounting to 84 μg/ml. The amount of antibody removed by

† Kindly supplied by Capt. C. V. Seastone, Army Medical School.

5 Weil, A. J., and Farsetta, K., *J. Immunol.*, in press.

6 Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

TABLE II.
Precipitative and Protective Antibodies Removed from Immune Serum by Absorption with Specific Antigen and Hapten.

Immune serum	Antibodies removed as determined by: Quantitative estimations on immune precipitates %	Quantitative estimations on supernates %	Mouse protection tests %
Absorbed with specific antigen	95	94	97
Absorbed with hapten	—	90	77

the polysaccharide hapten corresponded to 90% of that precipitable by the complete antigenic complex. From the mouse protection tests recorded in Tables I and II it can be seen that absorption of the serum with hapten removed approximately 77% of the protective antibodies.

The pooled antiserum was also absorbed with a large excess of Type III hapten (3000 μg per ml of serum). The results of the mouse protection tests with this serum are recorded in Table I. These results do not differ significantly from those obtained with the serum absorbed with a slight excess of hapten.

From the above experiments it is evident that the immune bodies in dysentery antiserum which confer passive immunity on mice are those primarily directed against the somatic antigen. The fact that some 77% of the protective antibodies can be absorbed with the polysaccharide hapten suggests that the major portion of these immune bodies are directed against the carbohydrate component of the complex antigenic molecule.

Summary. 1. Antisera prepared by prolonged immunization of rabbits with Type III *Shigella paradysenteriae* (Flexner) contain mouse protective antibodies which can be removed by absorption with the chemically purified type specific antigen. Absorption of the serum with the polysaccharide portion of the antigenic complex removes approximately 80-90% of the precipitating and protective antibodies.

2. Most, if not all, of the protective antibodies present in Flexner Type III antiserum are directed against the homologous specific antigen. Any protective antibodies reactive with other constituents of the bacterial cell appear to have little or no significance in the immune response. The polysaccharide hapten is the component of the antigenic complex most important in orienting the protective and precipitative antibodies.‡

‡ Similar results are reported by Smolens, J., Halbert, S. P., Mudd, S., Doak, B. W., and Gonzalez, L. M., *J. Immunol.*, in press.

15091

Subtilin—An Antibiotic Produced by *Bacillus subtilis*. I. Action on Various Organisms.*

A. J. SALLE AND GREGORY J. JANN.
From the Department of Bacteriology, University of California, Los Angeles.

Although it has been known for many years[1,2] that cultures of *Bacillus subtilis* are antagonistic to the growth of other organisms,

* This and subsequent investigations on subtilin are part of a cooperative study undertaken by the Department of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco, under the direction of Dr. H. H. Anderson; the Department of Bacteriology, University of California, Los Angeles, under the direction of Dr. A. J. Salle; and the Biochemical