TABLE II.
Serological Activity of Serum Prepared with Decapsulated Organism.

Polys. fraction	Ppt. titre	Complement-fixation titre (serum diluted 1:10)
RI	1:1,000	1:10,000
RII	1:5,000	1:50,000
RIII	1:10,000	1:100,000
SI, SII and SIII	1:2,000,000-1:4,000,000	1:16,000,000

acid the smooth organism was only partially decapsulated. Three facts are difficult to reconcile with this assumption: (a) The chemically decapsulated organism was agglutinated by anti-S serum to a titre of 1:256. (b) Further treatment of the decapsulated organism with acetic acid did not yield any specific substance. (c) Fraction RI prepared by acid hydrolysis of the rough organism reacts poorly with the serum prepared with decapsulated organism in contrast to the high activity with anti-S serum. The exact explanation of these findings is not possible at present. The possibility of Fraction RI being derived from the remains of the capsule can not entirely be ruled out, the available evidence shows, however, that the bacteria from which the substance was prepared possess no demonstrable capsule and, therefore, the substance must have been probably derived from the cell itself.

Summary. Two immunologically distinct polysaccharides have been obtained from a rough strain of *Bacillus rhinoscleromatis,* one serologically active and the other more or less inert. Their chemical and serological properties are described.

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Immunological Studies of Polysaccharides of Encapsulated Bacillus. III. Active Sensitization with Polysaccharide from *B. rhinoscleromatis.*

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Attempts to produce active sensitization of guinea pigs through the use of killed cultures of different Gram negative microörganisms, have been, so far, uniformly unsuccessful. Since the development of hypersensitivity in infections caused by certain Gram negative bacteria is well established, it appears inconceivable that these bac-

teria should be incapable of causing active sensitization under experimental conditions. The failure in this respect might be attributable either to insufficient dosage or to unsuitable preparations of the antigen. In the present work it will be shown that the specific polysaccharide derived from a Gram negative, capsulated strain of *B. rhinoscleromatis* may act as an efficient antigen in bringing about active sensitization of guinea pigs.

Two lots of the specific polysaccharide were used in this experiment. The first lot of the substance (Fraction SI[1]) was prepared by a 30 minutes' hydrolysis of a watery suspension of bacterial cells in the presence of one percent acetic acid which was followed by alcoholic precipitation. The second lot (Fraction SIII[1]) was obtained by a similar process; 0.5% potassium hydroxide was used instead of acetic acid. From chemical analysis it was found that both lots of the substance represent polysaccharide fractions of the bacterial cell showing identical properties and similar serological behavior when tested against rabbit sera prepared by immunization with killed cultures of *B. rhinoscleromatis*. The important difference between the 2 lots of the substance consisted in finding that the polysaccharide prepared by acid hydrolysis proved to be antigenic in rabbits while that obtained by alkaline hydrolysis showed either very feeble antigenicity or none at all.

Two series of young guinea pigs, weighing from 250 to 300 gm., were sensitized in the following manner: The first series received two intraperitoneal and one subcutaneous injection of Fraction SI (prepared by acid hydrolysis). The total dose of the polysaccharide given to each animal was equal to 20 mg. The injections were made at 5-day intervals and the test for hypersensitivity was performed 10 days after the last sensitizing injection. The second series of animals was sensitized in a precisely similar manner using Fraction SIII (obtained by alkaline hydrolysis). The dosage and time intervals were maintained the same as in the previous series of guinea pigs. Hypersensitivity in these animals was tested by intravenous injections of measured quantities of each type of the substance. The result of the experiment is given in Table I. It was found that all animals sensitized with Fraction SI and subsequently injected intravenously with 2 or 4 mg. of the homologous substance or of the heterologous substance, Fraction SIII, developed acute anaphylactic shock. The general picture of the shock corresponded to the classical manifestations of protein anaphylaxis. Within 3 to 5 minutes symptoms developed, the animals showing profound

[1] Wong, Sam C., in press.

TABLE I.
Table Showing the Result of Sensitization of Guinea Pigs with Specific Polysaccharides of *B. rhinoscleromatis.*

No. of animals	Polysaccharide used for sensitization Prepared by	Polysaccharide used for shocking Prepared by	Shocking dose in mg.	Result
2	—	acid hydrolysis	2-4	No response—control normal animals
2	—	alkaline hydrolysis	2-4	No response—control normal animals
6	acid hydrolysis	acid hydrolysis	2-4	Anaphylactic shock, survived
3	" "	alkaline hydrolysis	2-4	Anaphylactic shock, survived
3	alkaline hydrolysis	acid hydrolysis	2-4	No response
3	" "	alkaline hydrolysis	2-4	" "

respiratory distress, spasmodic cough, urination, defecation and occasional jumping. Mild convulsions developed rarely. All the symptoms lasted for about 20 minutes, after which the animals showed a more or less prolonged state of general prostration. No fatal outcome has been observed in any of the animals tested. In contrast to the above described findings, the animals sensitized by the injection of the polysaccharide Fraction SIII did not respond to the intravenous administration of either type of the substance. From this experiment it follows that only the polysaccharide prepared by acid hydrolysis of bacteria (Fraction SI) possessed both sensitizing and shocking properties, while the polysaccharide obtained by alkaline hydrolysis (Fraction SIII) exhibited only shocking power.

Since the shock produced in guinea pigs which were sensitized by Fraction SI showed certain minor deviations from the typical anaphylaxis, *e. g.*, its course was markedly prolonged and no fatality occurred, it was thought necessary to corroborate our *in vivo* findings by an *in vitro* experiment. Accordingly 6 young virgin guinea pigs were sensitized, some received one fraction, the rest the other fraction; by the use of either polysaccharide the uterine horns of these animals were tested for hypersensitivity by the Schulz-Dale method. It was found that the addition, in concentrations ranging from 1:10,000 to 1:50,000, of each type of polysaccharide to the bath containing the uterine horns from the animals sensitized by Fraction SI resulted in a very marked and prolonged contraction of the horns. On the other hand, an entirely negative result was obtained when a similar test was performed with the uterine horns taken from the animals sensitized with Fraction SIII prepared by alkaline hydrolysis. The results indicate that our findings obtained *in vivo* are in perfect agreement with those seen in an *in vitro* experiment.

Our experiment shows that certain immunological characteristics which with a few exceptions have been attributed only to protein antigens may also be demonstrated in some bacterial polysaccharides, at least this is true with the soluble substance derived from *B. rhinoscleromatis*. Through the use of properly prepared polysaccharides it was possible in our hands to induce the formation of antibodies in rabbits and to induce active sensitization in guinea pigs.

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Relationship Between Female Sex Hormone and Dewlap in the Rabbit.

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Frazier and Mu[1] noted that prolonged administration of an estrogenic substance, extracted with butyl alcohol from the urine of pregnant women, feminized male rabbits. Among the physiological and anatomical changes observed was the development of a ruff, a prominent fold of loose skin encircling the anterior part of the neck. In well-developed cases the skin was so redundant that it could easily be pulled over the nose of the animal. A similar condition in normal rabbits is known to breeders as dewlap.[2] In order to ascertain the significance of this formation of skin, we have examined the rabbits in our breeding colony for incidence of dewlap, and have also made some observations on adult male rabbits and on immature male and female rabbits treated with estrogenic substance. Another series of female rabbits was subjected to ovariectomy. This we did on the assumption that dewlap is related physiologically to the female sex hormone of the animal.

The rabbits of the breeding colony were examined on 3 occasions, 6 months apart, in the spring and fall. Both male and female animals and their young were included in this study. They were of different breeds with a variation in age from birth to 3 years. In the case of the adult females, the physiological status (resting, pregnant, or lactating) and the number of pregnancies were taken into account.

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1 Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.

2 *Guide Book and Standard*, Chicago, Ill., The Am. Rabbit and Cavy Breeders Assn., 1932-1933.