SOME OBSERVATIONS ON THE SPECIFICITY OF BACTERIAL ALLERGY TO CERTAIN OF THE NEISSERIAE

By C. PHILLIP MILLER, M.D., and RUTH CASTLES

(From the Department of Medicine, The University of Chicago, Chicago)

Of the phenomena manifesting hypersensitiveness to bacteria or their products, the cutaneous reaction has received the greatest attention of investigators. The first example to be carefully studied was the tuberculin reaction, which was readily applied as a practical diagnostic procedure by the clinician and the veterinarian. The analogs of the tuberculin test: the typhoidin, mallein, luetin, abortin, gonococcin reactions, are similar examples of exaggerated inflammatory response in the skin of a host to the introduction of organisms with which it (the host) is infected.

Reports of the clinical application of this technique do not here concern us, and of the literature on the experimental work on the problem only a few studies need be cited; *viz*, those which have inquired into the underlying mechanism of the reaction and those bearing on its specificity. Zinsser and his coworkers have published several papers on the relation of bacterial allergy, as demonstrable by the cutaneous reaction, to anaphylaxis. A summary of his views is contained in a lecture delivered Jan. 5, 1928, before the New York Academy of Medicine (1). Using the method of Zinsser and Raymond (2) for producing chronic pyogenic foci in guinea pigs, Zinsser and Parker (3) studied the allergic cutaneous reaction to staphylococci. They also sensitized guinea pigs to typhoid bacilli by repeated intracutaneous inoculation. The cross-reactions which they encountered will be discussed below.

MacKenzie and Woo (4) found that guinea pigs repeatedly injected intracutaneously with pneumococcus protein developed during the 3rd week a hypersensitiveness which continued for about a week and then disappeared. The animals, however, showed no alteration in their susceptibility to intraperitoneal inoculation with pneumococci.

Julianelle and Avery (5) found that rabbits repeatedly injected intracutaneously with heat-killed pneumococci developed typical allergic reactions to pneumococcus nucleoprotein, but not to the soluble specific substance—the type-

specific carbohydrate of Avery and Heidelberger. Intravenously vaccinated animals failed to become allergic.

Zinsser and Grinnell (6) reported the production of allergy to hemolytic streptococci by means of agar foci infected with living organisms. The most extensive study of streptococcal allergy, however, has been made by Swift and his coworkers. Their interest in this phenomenon grew out of the observation of Andrewes, Derick, and Swift (7) that about half of their rabbits which had been inoculated intracutaneously with non-hemolytic streptococci suffered a recrudescence of the local inflammatory process 8 or 9 days after the inoculation. This was found (8) to be due to the development of a state of hypersensitiveness (analogous to that which evokes the tuberculin reaction) and has been studied in great detail by Derick and Swift (9). Intravenously immunized animals responded to intracutaneous injection by the formation of small hard nodules, quite different from the large, acutely inflamed lesions which appeared in the sensitized animals (10). Sensitization was effected by a variety of procedures (11), so long as small numbers of organisms were introduced at a time or a very low grade, chronic infection maintained.

In the literature on this subject of cutaneous hypersensitiveness to bacteria the specificity of the reaction seems to have been rather assumed than demonstrated. For with few exceptions mention is not made of reactions to organisms other than the ones with which the animals had been sensitized. In their recent study of the bacterial endotoxin of *Salmonella pullorum* Hanks and Rettger (12) mention the non-specificity of cutaneous reactions to *Salmonella pullorum*, *Proteus vulgaris*, and *Serratia prodigiosus* in rabbits rendered hypersensitive to any of these organisms.

In a study of the abortin reaction Stroem (13) states that he was unable to sensitize guinea pigs to heat-killed cultures of *B. abortus*, even when they were mixed with kieselguhr to stimulate local tissue reaction. He did observe cutaneous hypersensitivity in animals infected with *B. abortus* in which gross anatomical lesions were entirely absent. Tuberculous animals were found to give slight reactions to abortin, and he attributed this to heightened, non-specific reactivity.

Hanger (14) found that rabbits (presumably the same ones which he mentions as carriers of *Bact. lepisepticum* in their upper respiratory passages) reacted to intradermal inoculation with filtrates of *B. influenzae*, *B. coli*, and meningococcus as well as of *Bact. lepisepticum*. He also states that human beings who react to filtrates of the aforementioned organisms also react to those of *Bact. lepisepticum* and he concludes, therefore, that "there is apparently considerable antigenic relationship between many Gram-negatives of different biological groups."

The study here reported took its origin from an interest in the interrelation of certain of the Gram-negative diplococci (15) but gradually grew in scope by the inclusion of unrelated organisms, largely

for purposes of control. Since we had at our disposal a stock of snuffle-free rabbits[1] we were able to repeat Hanger's observations without the complicating factor which he mentions, the presence of *Bacterium lepisepticum* in the upper respiratory passages of the rabbits.

Methods and Materials

The observations reported below were all made on young adult rabbits from a snuffle-free stock protected at all times from possible contact with carriers of this disease.

Cultures.—The Neisseriae employed were gonococcus (6 strains), meningococcus (2 strains) and *Micrococcus catarrhalis* (1 strain), as well as *Bact. lepisepticum*,[2] and R pneumococcus originally derived from a Type I organism, and in a few instances *Staphylococcus aureus*, and scarlatina streptococcus. Except as noted to the contrary, the organisms were grown for 18 hours on an egg white digest agar similar to that described as control medium in a preceding publication (16). The liquid medium had the same composition as the solid less the agar.

Agar Foci.—Large tubes of 2 per cent agar were melted and cooled to about 45°C. The appropriate inoculum of organisms was then mixed with 20 cc. of melted agar and at once injected under the skin.[3] The regions usually selected were the outer surface of the upper leg or the flank near the rib margin. A long needle was used so that the agar was placed at some distance from the puncture wound. Pieces of ice were held about the margin of the injected mass to cool it quickly and prevent its spreading out into a thin layer and also to prevent its leaking back through the tunnel made by the needle.

Most of the rabbits tolerated their foci with little apparent difficulty. Some of them, particularly those bearing foci heavily infected, became emaciated and had to be sacrificed before they could be tested by intracutaneous inoculation.

The skin overlying the focus partook of the inflammatory reaction which

---

[1] This supply of snuffle-free rabbits was made available by the generosity of Harold H. Swift, Esq., who raised them under unusually favorable conditions at his farm near Lakeside, Mich. The original stock was obtained through the courtesy of Dr. Leslie T. Webster of The Rockefeller Institute for Medical Research and the late Professor Carroll G. Bull of the School of Hygiene and Public Health of the Johns Hopkins University.

[2] The strain of *Bact. lepisepticum* was very kindly sent to us by Dr. Leslie T. Webster of The Rockefeller Institute for Medical Research.

[3] This method seems to have been introduced by Dochez, who employed it for the immunization of horses to the toxins of scarlatina streptococci. It was adapted to the use which here concerns us by Swift in his experiments (referred to above) on the sensitization of rabbits to non-hemolytic streptococci. The procedure of Zinsser and Raymond was to fill a celloidal sphere with melted agar containing live organisms and place it in the peritoneal cavity of an animal.

developed about it, and, when the focus had been too superficially placed, under-went necrosis with resulting extrusion of the remnant of the agar mass. This occurrence, which was infrequent, did not seem to impair detectably the rabbit's development of hypersensitiveness, possibly because it did not take place until several days after the implantation.

The inflammatory reaction was appreciably more marked about foci containing gonococci and meningococci than around those containing *M. catarrhalis*.

Microscopic and bacteriological study was made only of foci containing gonococci. In these the sequence of events may be briefly described as follows: Within an hour or two after implantation, invasion by polymorphonuclear leuco-cytes begins. These cells ingest the bacteria which they encounter and in doing so are damaged or killed. But enough of them enter to constitute a steadily—and quite rapidly—advancing border which is followed by a second containing other phagocytic cells and a third consisting of capillary loops, which make their appearance on the 2nd or 3rd day. Section of a focus at this time or later reveals concentric rings of these elements. Gonococci can be cultivated from the center of a focus only so long as it is beyond the reach of the polynuclear leucocytes.

Eventually the periphery becomes encapsulated, the interior more or less liquified for a time, and as organization proceeds, the whole mass gradually con-tracts to a firm cyst-like structure with a caseous center.

Preparation of the Rabbits' Skins.—Epilation was accomplished by the use of impure barium sulfide[4] as recommended by Derick and Swift (9).[5] The ventral surface of the abdomen was alternately washed with soap and water and rinsed in warm running tap water several times to cleanse the fur of oil so that the barium sulfide could quickly penetrate to the skin. 10 to 15 gm. of the powdered barium sulfide were then dusted on to the fur and gently patted with a large pledget of cotton soaked with water. After a few seconds the rabbit was held under the tap and very throughly washed to remove all of the barium sulfide. The hair was removed by the stream of water. The animal was then dried by patting the epilated skin gently with a towel and rubbing the rest of the fur. The skin was then greased with vaseline. Epilation was usually performed 1 day before the intracutaneous tests were made so that areas which had been irritated by the process might be avoided in placing the inoculations. After a little practice, however, it is possible by this method to remove the hair from rabbits without doing any noticeable damage to the skin.

Intracutaneous Inoculations.—Injections of 0.1 cc. were made into the sub-stance of the skin itself by means of a very fine hypodermic needle and a tubercu-lin syringe. The suspensions of organisms were standardized by Gates' method (17), the density being such that the ring just disappeared at a depth of 1 inch

---

[4] We use Merck's preparation labeled "barium sulphide, gray powder, about 80 per cent."

[5] Derick and Swift (9), page 619.

(when checked by actual counts found to contain about 6.5×10^8 organisms per cc.). This simple method of standardization sufficed in these experiments because control animals were always injected with identical inocula and the relative, not the absolute, sizes of the resulting lesions were taken into consideration in judging the results of any experiment.

In the case of the Gram-negative diplococci, 18 hour cultures freshly removed from the agar medium and washed in saline were used. The other organisms were killed by heating to 60° for an hour.

Description of the Cutaneous Lesions

The reaction which followed the intracutaneous inoculations was of the so called delayed type. It began a few hours after the injection as a localized erythema which spread for 12 to 36 hours and then receded. The rapidity of its development was not always an index of its maximal intensity. Swelling of the skin began soon after the erythema and spread peripherally. In severe reactions edema of the subcutaneous tissues occurred, and in the most severe, necrosis of the skin, sometimes preceded by the formation of a small pustule at the site of the injection. The subcutaneous edema sometimes spread ventrally beyond the erythema (presumably by gravity) and persisted after the redness had disappeared.

By the end of 36 hours most of the lesions had begun to recede, but some did not attain their maximum size and intensity until this time.

In our earlier experiments the lesions were observed every few hours for the first 2 days and once a day thereafter for a fortnight. This routine was abandoned when it was found to yield no more information than daily readings for 3 or 4 days and biweekly readings thereafter.

Observation consisted of noting and recording two diameters (the maximum one and that at right angles to it) of the areas of redness and of swelling and the estimated depth of the latter; pustule formation; necrosis or healing.

At the onset of this study it was hoped that the state of hypersensitiveness might be demonstrated by the intracutaneous injection of a dose of organisms (or of a filtrate of liquid culture) which would evoke a reaction in the sensitized but not in the control animal; and a number of preliminary experiments were made with this end in view. It was found, however, to be impracticable in the case of gonococci and

meningococci, with the rabbit as the experimental animal, for an inoculum which would regularly give rise to a positive reaction in a sensitized animal, produced one also—though a much smaller one— in the controls. This method was therefore abandoned in favor of the one employed in all of the experiments herein reported; namely, the injection of an inoculum which was known to produce lesions in the controls as well as in the rabbits containing agar foci. The criterion of the sensitization was therefore the relative intensity of the reactions in both groups to an equal dose of the same inoculum. This neces- sitated the injection of controls every time tests were made, and in the analysis of our data, attention to comparative differences be- tween rather than to absolute sizes of lesions.

"*Secondary Reactions.*"—Among our control rabbits the "secondary reaction" described by Andrewes, Derick, and Swift (7) was observed but a very few times. In those few instances it occurred during the 2nd week and lasted only a day or two. It consisted merely of a temporary increase in the depth of color and the extent of the fading erythema. With one possible exception, it would have escaped de- tection but for careful measurement and comparison with data al- ready recorded. In that one instance only was it pronounced enough to have drawn attention to itself.

Development of Cutaneous Hypersensitiveness to Gonococci

Experiment 1.—A subcutaneous agar focus containing viable gonococci was implanted into each of four adult rabbits. Three of them contained gonococcus Strain 1, the other Strain M_6B_2. On the 18th day they, as well as three control rabbits, were tested by intracutaneous inoculation with:

Gonococcus, Strain 1, grown on solid media.

Gonococcus, Strain M_6B_2, grown on solid media.

Gonococcus, Strain 3, grown in liquid media for 18 hours, removed by centrif- ugation, and made up to the same concentration as the two preceding.

A Berkefeld filtrate of the supernatant of this 18 hour liquid culture of Strain 3.

Uninoculated agar medium.

Result.—The cutaneous reactions induced by all three strains of gonococci were much larger and appreciably more indurated in the rabbits with agar foci than in the controls. These results are pre- sented graphically in Text-fig. 1, in which are plotted the sums of the

diameters of these lesions.[6] The suspensions of all three strains produced lesions of equal intensity (within the limits of error of the method) in all of the sensitized rabbits. The filtrate of the 18 hour liquid culture of Strain 3 produced only a moderate reaction (not plotted in Text-fig. 1) in one out of the four sensitized rabbits and none in the others, and in the controls a minimal reaction in two. The agar media produced no reaction in any of the animals.
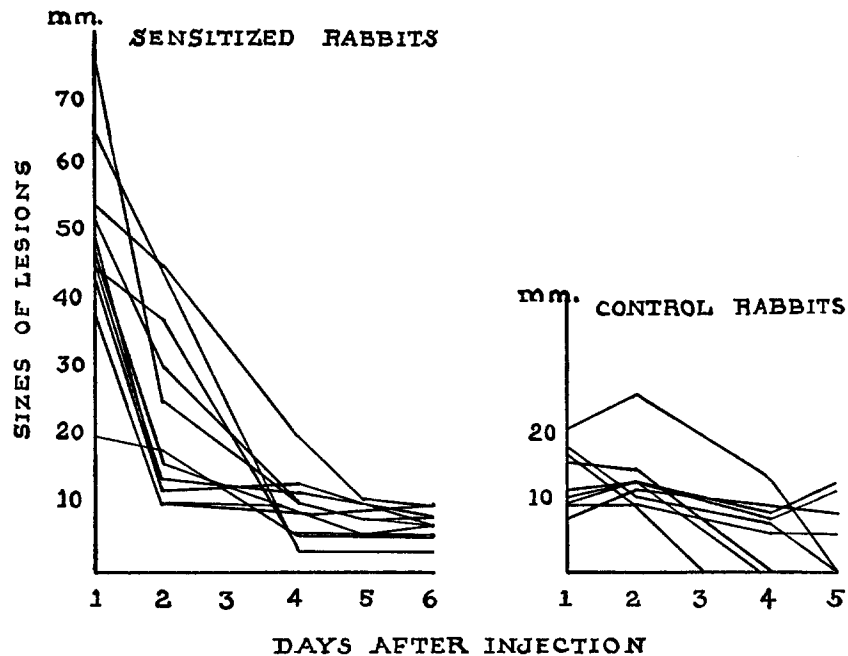
TEXT-FIG. 1. Sizes of individual lesions (each plotted as the sum of two diameters) produced by three different strains of gonococci in four sensitized and three control rabbits.

This experiment shows then that the two strains of gonococci cross-reacted; that in a young culture in liquid medium the reacting sub-

[6] This method of graphic representation is used for the sake of simplicity. It should be noted, however, that redness and swelling were invariably more marked in the positive allergic reactions than in the corresponding controls, the difference being usually more striking in appearance than the comparison of the figures recording the diameters of the lesions.

stance is contained entirely or almost entirely within the bodies of the organisms themselves rather than in the liquid medium surrounding; and that the cutaneous reaction is not produced by some substance in the agar medium which might conceivably adhere to the bodies of the organisms grown upon it.

It was likewise shown in subsequent experiments that in the development of hypersensitiveness the agar used in making the focus plays no rôle since rabbits containing uninoculated agar foci behaved exactly like controls.

Additional experiments similar to Experiment 1 failed to disclose any differences, by this method, among six strains of gonococci.

The Development of Hypersensitiveness by a Variety of Organisms

Experiment 2.—A series of rabbits was prepared by the implantation of agar foci. The focus in each of a pair contained one of the following organisms:

|	
Gonococcus, Strain 1.	*M. catarrhalis.*
Gonococcus, Strain 3.	Meningococcus.
Gonococcus, Strain 5.	*Bact. lepisepticum.*
Gonococcus, Strain M_6B_2.	

After an interval of 10 days each of these rabbits was injected intracutaneously with its homologous organism. The resulting lesions, plotted in Text-fig. 2, were much larger than those in the control, demonstrating that by this method cutaneous hypersensitiveness could be induced by each of these organisms.

The Specificity of the Cutaneous Reactions

Experiment 3.—Four groups of five rabbits each were prepared by the implantation of agar foci, containing in the case of each group gonococci, meningococci, *M. catarrhalis*, and *Bact. lepisepticum*, respectively. 10 days later each animal, as well as each of five controls, was inoculated intracutaneously with 0.1 cc. of a standard suspension of each of the four organisms mentioned.

Result.—As all of the reactions were maximal at the end of 24 hours, the readings made at that time are plotted in Text-fig. 3. It will be seen that the foci containing meningococci evoked almost as great a sensitivity to gonococci as to meningococci, and *vice versa*, but that cross-reactions in the case of *Micrococcus catarrhalis* and *Bacterium lepisepticum* did not occur. In several other experiments, however,
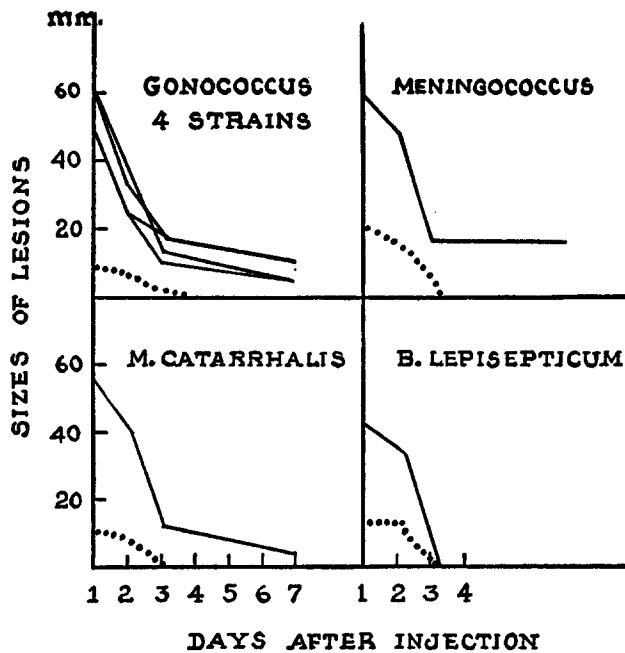
TEXT-FIG. 2. Cutaneous reactions in sensitized and control rabbits. Each line represents the average of the sizes (plotted as the sums of their diameters) of the lesions in two rabbits. Solid lines, sensitized rabbits. Dotted lines, control rabbits.
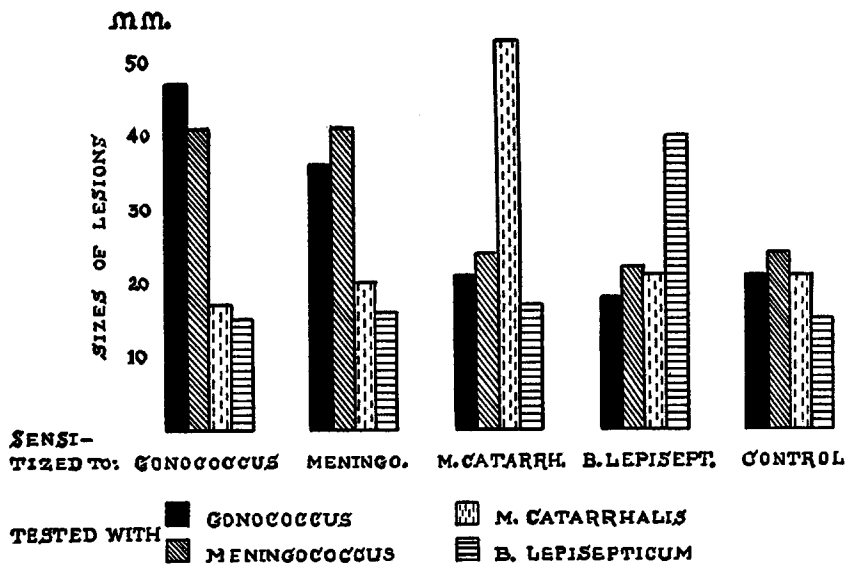

TEXT-FIG. 3. Comparison of the cutaneous reactions to four organisms in rabbits sensitized to one of each. Each column represents the average of the lesions in five animals at the end of 24 hours.

443

M. catarrhalis was found to cross-react to a certain degree with gono-coccus and meningococcus.

The Influence of the Size of the Focal Inoculum on the Development of Cutaneous Hypersensitiveness in the Case of Gonococcus

Experiment 4.—Two groups of four and three rabbits respectively were injected with agar foci. The foci in the animals of the former group (four rabbits) contained 1 cc. of a 1:10 suspension of gonococci; in the latter group, 2 cc. of the same suspension. After an interval of 10 days all the rabbits, as well as three normal controls, were injected intracutaneously with standard suspensions of gonococci and meningococci.
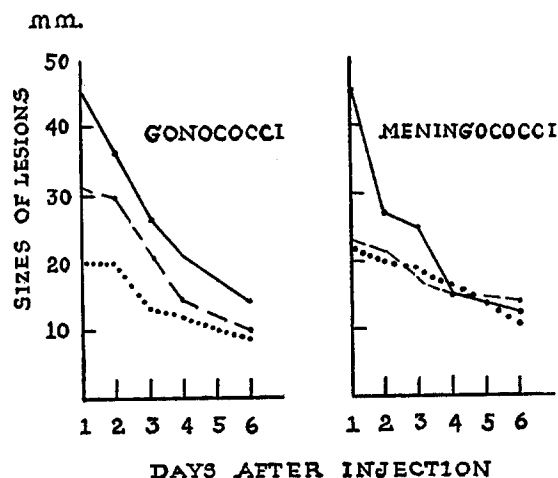
Text-Fig. 4. Differences in degree of hypersensitiveness effected by focal inocula of different quantities. Solid lines, average sizes of lesions in four rabbits bearing agar foci containing 1 cc. of 1:10 suspension of gonococci. Broken lines, average sizes of lesions in three rabbits bearing agar foci containing 2 cc. of 1:10 suspension of gonococci. Dotted lines, average sizes of lesions in three control rabbits.

Result.—As shown in Text-fig. 4, the cutaneous reactions were greater in the animals which had been prepared by the smaller focal inoculum (1 cc.) than in those which had received the larger focal inoculum (2 cc.) or in the controls. The meningococcal reactions of the second group differed not at all in size from those of the controls, but only in their somewhat deeper color and greater swelling.

Experiment 5.—Two groups of three rabbits each were injected with agar foci containing respectively 2.5 and 5 cc. of a 1:10 suspension of gonococci. After an interval of 10 days these rabbits, as well as two normal controls, were injected intracutaneously with standard suspensions of gonococci and meningococci.

Result.—The result was comparable to that in Experiment 4 in that there was a marked difference (more marked, in fact, than in Experiment 4) between the lesions produced in the two groups by both gonococci and meningococci. In this instance also the animals prepared by the smaller focal inoculum developed the larger cutaneous lesions. The control rabbits developed lesions of the same size, though not quite so indurated as those which had received the focal inoculum of 5 cc.

These two experiments showed clearly that the degree of hypersensitiveness was related to the size of the focal inoculum. The obvious discrepancy between the two experiments lay that in the fact that in Experiment 5, 2.5 cc. of the bacterial suspension sensitized the rabbits very well, whereas in Experiment 4, 2 cc. was much less effective than 1 cc. It was at first thought that this discrepancy could be easily explained as failure of centrifugation to pack the organisms as closely in one instance as in the other. (The experiments were performed several days apart.) But subsequent repetitions of these experiments, made in the hope of establishing an optimum sensitizing dose, showed only that very small and very large focal inocula were either ineffective or much less effective than the doses already mentioned, and also that the animals vary considerably in the degree of sensitiveness which they develop. The interval between sensitization and cutaneous inoculation is considered in a subsequent section.

The Duration of the Hypersensitive State Produced by This Method

In the case of the three Gram-negative diplococci studied, it may be said that although rabbits were occasionally found to be hypersensitive 1 week after the implantation of the infected agar focus, the best reactions usually occurred after an interval of 10 days. By the end of the 3rd week the hypersensitive state produced by this method had usually passed. In the following experiment the influence of the

size of the focal inoculum was controlled by sensitizing rabbits with agar foci containing three different amounts of gonococci.

Experiment 6.—Three groups of two, three, and three rabbits each were injected with agar foci containing respectively 1, 2.5, and 5 cc. of a 15 per cent suspension of gonococci. At (approximately) weekly intervals these animals as well as two controls (new controls being introduced at each testing) were injected intracutaneously with standard suspensions of gonococci, meningococci, and *M. catarrhalis.*

Result.—The result of this experiment may be briefly summarized as follows: The most intense reactions occurred in the first group on the second testing (15 days after implantation of the focus), and moderate ones on the next (the 22nd day), but by the fourth test (on the 32nd day) the reactions were no greater than those in the controls. In other words, hypersensitiveness had not developed by the 6th day, was maximal on the 14th, and had ceased by the 32nd. The reactions were sharpest to gonococci, less so to meningococci, and least of all to *M. catarrhalis; i.e.,* cross-reactions with meningococci were less pronounced in this experiment than was the case in some of the foregoing. The second group of rabbits (containing foci of 2.5 cc.) reacted less strongly but in the same general way as those first described, while those in the third group showed no evidence of hypersensitivity.

It must not be concluded from the foregoing experiments that the hypersensitive state is always passed by the end of the 3rd week, for rabbits have been found to be somewhat allergic $2\frac{1}{2}$ and even 4 months after the implantation of their foci. Such animals, however, had had not more than two preceding cutaneous tests.

Cutaneous Reactions in Rabbits Intravenously Immunized to Gonococci

From time to time small groups of rabbits which had survived repeated intravenous injections of living gonococci were tested by intracutaneous inoculation with gonococci, meningococci, and *M. catarrhalis.* Of a total of seventeen animals so tested, all developed lesions indistinguishable from the appropriate controls.

DISCUSSION

The experiments here reported demonstrate the practicability of rendering rabbits hypersensitive to three common Neisseriae by implanting into their subcutaneous tissues masses of agar containing living organisms. Mention may again be made of the point that our only criterion of hypersensitiveness has been the local response to intracutaneous injection of bacterial suspensions. The hypersensitive state was found to develop about the beginning of the 2nd week, to be maximal on the 10th to 12th day, and to be gone in most instances by the 4th week. This agrees with the findings of Swift and his coworkers in the case of streptococci, of Julianelle and Avery and of MacKenzie and Woo in the case of pneumococci. Exceptions to our generalization were occasionally encountered, but the statement covers the usual observations.

No relationship could be established between the size of the focal inoculum and the rate of development or duration of the allergic state. But the degree of allergy developing was materially affected by the numbers of organisms contained in the agar focus; for very small and very large inocula both failed to elicit the desired effect. It is possible that in the latter instance the hypersensitive state was of unusually short duration and that we chanced to miss it on each trial; but numerous attempts with this point in mind were unsuccessful.

Although in each of the experiments herein described living bacteria were employed to sensitize the animals to gonococci, meningococci and *M. catarrhalis*, a few experiments which were made indicated that heat-killed organisms functioned almost as effectively. It might be noted in passing that agar foci containing nucleoprotein of the gonococcus[7] rendered rabbits even more highly allergic than (approximately) equivalent quantities of living organisms.

In several of our experiments efforts were made to correlate the precipitin titers of rabbits' sera with their cutaneous reactions, but the results were too inconsistent to justify any conclusion. Precipitins began to be demonstrable in the serum about the same time that

[7] For these preparations of gonococcal nucleoprotein the authors are indebted to Dr. Alden K. Boor.

hypersensitiveness developed, but among a group of rabbits which seemed, by cutaneous tests, to be equally allergic, some yielded sera containing, and some sera not containing, precipitins for the homologous organism.

As regards the specificity of the allergy engendered by the agar focus method our experiments seemed to warrant the following statements. Animals sensitized to gonococci usually but not always reacted equally or nearly so to meningococci; and *vice versa*. Animals sensitized to gonococci reacted appreciably less strongly to *M. catarrhalis* (and *vice versa*) but more strongly to it than to such organisms as streptococci and staphylococci. Among the Gram-negative diplococci, therefore, gonococci and meningococci are, by this criterion, more closely related to each other than to *M. catarrhalis*. This finding parallels the observation of Boor and Miller on the immunological relationships of their nucleoproteins and carbohydrate fractions.

Animals sensitized to *B. lepisepticum* were not allergic to any of the Gram-negative diplococci employed, and *vice versa*. In comparing these observations with those of Hanger it should be pointed out that all of the suspensions of *Bact. lepisepticum* employed in our experiments were heat-killed because we feared the possibility of contaminating our animal quarters with this organism.

The specificity of reaction observed in our experiments was rather surprising in view of certain reports in which this point is considered. The observations of Hanks and Rettger (12), of Stroem (13), and of Hanger (14) have already been cited. In addition should be mentioned Meyer and Christiansen's (18) experimental study of the typhoidin reaction in rabbits, wherein they report non-specific reactions even with extracts of organisms not at all related to the typhoid group. Zinsser and Parker (3) noted that tuberculous guinea pigs reacted in some instances to pneumococcus residue antigen. And Zinsser and Tamiya (19) found skin tests to be specific only when their animals were moderately hypersensitive, and encountered overlapping when a high degree of allergy was present. The methods employed by these authors to engender the hypersensitive state were not the same as ours, and the difference may account for the discrepancy in result.

The failure to demonstrate hypersensitiveness by the intracutaneous injection of an inoculum or saline extract which would cause no reaction in controls can be explained, we believe, on the basis of two facts. One is the inability, in our hands at least, to obtain the same degree of allergy to the Gram-negative diplococci as is possible to certain other antigens. The other is the toxicity of the proteins of the organisms dealt with. In connection with these two points it is appropriate to comment on the rarity of the occurrence of the "secondary rise" observed by Andrewes, Derick, and Swift to follow intracutaneous injections of streptococci. One possible explanation, which has not yet been subjected to experimental verification, is that the cellular reaction which results from the introduction of such toxic proteins alters in some fashion their composition so that they cease to exist as antigenically identical substances.

No evidence was obtained that gonococci "excrete" the substance responsible for the cutaneous reaction, for filtrates of cultures in liquid medium, filtered immediately after the phase of most rapid multiplication, usually evoked no cutaneous reaction, or at most a feeble one. It was from old liquid cultures, in which autolysis had begun, that reactive filtrates were obtained, or from filtrates of cultures lysed by means of dilute alkali.

SUMMARY AND CONCLUSIONS

By means of the reaction to intracutaneous inoculation with bacterial suspensions in amounts of 0.1 cc., bacterial allergy was demonstrated in rabbits into which had been implanted agar foci containing either gonococci, meningococci, *M. catarrhalis*, or *Bact. lepisepticum*. The criterion of hypersensitiveness was the relative size and intensity of reaction evoked by an identical dose in "agar focus" and control rabbits. Rabbits sensitized to gonococci or meningococci usually reacted indistinguishably to either of these organisms, but were less allergic to *M. catarrhalis*. Similarly, animals sensitized to *M. catarrhalis* gave moderate but not maximal responses to the two former organisms. Cross-reactions did not occur between *Bact. lepisepticum* and any of the three Neisseriae. Animals sensitized to the four organisms mentioned reacted no more intensely than did controls to hemolytic streptococci, staphylococci, and rough pneumococci.

The hypersensitive state was found to begin early in the 2nd week and to end usually by the 4th week, being at its height in most instances on the 10th to 12th days. The number of organisms contained in the agar focus determined the success of the sensitization only to this extent, that very small and very large inocula failed to evoke the allergic state.

Rabbits immunized by intravenous injection of live organisms developed cutaneous reactions indistinguishable from those in controls.

The "secondary rise" of Andrewes, Derick, and Swift was rarely observed.

BIBLIOGRAPHY

1. Zinsser, H., *Bull. New York Acad. Med.*, 1928, **4**, series 2, 351.
2. Zinsser, H., and Raymond, E. H., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1920–21, **18**, 121.
3. Zinsser, H., and Parker, J. T., *J. Exp. Med.*, 1923, **37**, 275.
4. MacKenzie, G. M., and Woo, S. T., *J. Exp. Med.*, 1925, **41**, 65.
5. Julianelle, L. A., and Avery, O. T., *Proc. Soc. Exp. Biol. and Med.*, 1928–29, **26**, 227.
6. Zinsser, H., and Grinnell, F. B., *J. Immunol.*, 1925, **10**, 725.
7. Andrewes, C. H., Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1926, **44**, 35.
8. Derick, C. L., and Andrewes, C. H., *J. Exp. Med.*, 1926, **44**, 55.
9. Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1929, **49**, 615.
10. Swift, H. F., and Derick, C. L., *J. Exp. Med.*, 1929, **49**, 883.
11. Derick, C. L., Hitchcock, C. H., and Swift, H. F., *J. Exp. Med.*, 1930, **52**, 1.
12. Hanks, J. H., and Rettger, L. F., *J. Immunol.*, 1932, **22**, 283.
13. Stroem, A. C. S., *J. Infect. Dis.*, 1931, **48**, 167.
14. Hanger, F. M., *Proc. Soc. Exp. Biol. and Med.*, 1927, **25**, 230.
15. Boor, A. K., and Miller, C. P., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 1050.
16. Miller, C. P., Hastings, A. B., and Castles, R., *J. Bact.*, 1932, **24**, 439.
17. Gates, F. L., *J. Exp. Med.*, 1920, **31**, 105.
18. Meyer, K. F., and Christiansen, C. R., *J. Infect. Dis.*, 1917, **20**, 391.
19. Zinsser, H., and Tamiya, T., *J. Exp. Med.*, 1926, **44**, 753.