THE SIGNIFICANCE OF ANAPHYLAXIS IN PNEUMOCOCCUS IMMUNITY.

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That bacterial derivatives possess anaphylactogenic properties was demonstrated by the pioneers in the study of anaphylaxis. But from the numerous subsequent studies in bacterial hypersensitiveness the fact has emerged that these antigens are not highly endowed with anaphylactogenic properties. Compared to such substances as egg albumin and animal serums bacterial derivatives yield positive results less easily and less constantly. Nevertheless active and passive anaphylaxis and the phenomena of desensitization have been accomplished repeatedly either with intact bacterial cells or with extracts of many microorganisms. It has also been found that during the course of certain experimental infections, particularly guinea pig tuberculosis, anaphylaxis to the bacterial protein may sometimes be demonstrable (Baldwin (1), Krause (2), Zinsser (3), and Smith (4)). Hence anaphylaxis may be artificially produced with bacterial derivatives in the absence of infection, or it may occur as one of the phenomena of an infection. In contrast with the general agreement concerning the fact of bacterial anaphylaxis are the widely differing opinions which have been expressed concerning its significance:

In naming anaphylaxis, Richet (5) had in mind a condition the opposite of protection; Weil (6) on the basis of experiments with non-bacterial antigens believed anaphylaxis to be a preliminary phase in the development of immunity wherein cellular antibody is inadequately protected against antigen by circulating antibody; Arthus (7) from venom experiments has concluded that such may be the case and also that anaphylaxis and immunity may coexist; Bordet (8) suggests

that anaphylaxis may be merely a concomitant of immunity without any essential relation to it—a sort of complication; Krause (2) and others hold that in tuberculosis, at least, anaphylaxis to tuberculoprotein has no influence on tuberculosis immunity.

Rosenow (9) says: "The question of anaphylaxis to pneumococcus extracts in relation to susceptibility and immunity to pneumococcus infection has also been studied. It has been found from numerous tests that guinea-pigs which are sensitive to pneumococcus extracts resist small injections of virulent pneumococci (from 0.01 to 0.2 cc. of 24-hour broth culture, depending on the virulence), but are more susceptible to large injections than the normal controls." Rosenow gives no further details of these experiments. He was clearly working with an organism of much lower virulence than the one we have used and consequently had a much more restricted range for immunity titrations. As will transpire from the description of our experiments the results do not provide confirmation for Rosenow's findings.

Clough (10) obtained sensitization of guinea pigs inconstantly with extracts of pneumococcus. With this preparation the difference between the lethal dose for normal and the lethal dose for sensitized guinea pigs was not large. However, he states that: "Guinea pigs sensitized with extracts of pneumococci showed inconstantly a very slight grade of immunity to infection with living virulent cultures." As appears from this statement as well as from the data in his paper, Clough's experiments indicate that if sensitization plays any part in the immunity mechanism, it is only an inconstant and inconsequential rôle.

Recently Zinsser and Mallory (11) have reported successful experiments in active and passive anaphylaxis using the uterine horn method and killed pneumococcus suspensions as anaphylactogen. This report, however, records no observations bearing on the relation of anaphylaxis to pneumococcus immunity.

EXPERIMENTAL.

The present paper reports an attempt to subject one type of infection to an experimental analysis so planned that in the particular instance studied the immunological significance of anaphylaxis would be revealed.

The general plan of the experiments was to subject guinea pigs to immunizing injections of pneumococcus, titering the immunity at frequent intervals, and testing for the appearance and duration of allergy and anaphylaxis. With such data for analysis any significant effect of an alteration in reactivity shown by skin allergy or by anaphylaxis upon resistance of the animals to infection ought to be reflected in the immunity curve.

150 guinea pigs were used in each experiment. Beginning with heat-killed 18 hour broth cultures of pneumococcus, and later using fresh unkilled 18 hour cultures, the animals during the course of 4 or 5 weeks were brought to a state of immunity in which they survived 10 million lethal doses of living culture. About 10 per cent of the animals died during the course of the immunization. The doses given intraperitoneally at 5 to 7 day intervals were: killed culture 0.25 cc., 0.5 cc., 1.0 cc., 1.5 cc.; living culture 0.001 cc., 0.01 cc., 0.1 cc.

Every 5 to 7 days during the course of the experiment twelve to fifteen animals were taken from the group to determine the degree of immunity attained, and to demonstrate by the methods outlined below the presence of specific hypersensitiveness whenever it existed.

Throughout the work we have used a Pneumococcus Type I isolated from the blood of a patient with lobar pneumonia. Upon isolation the lethal dose by intraperitoneal injection for guinea pigs of 200 to 300 gm. was 0.001 cc. By repeated passage through guinea pigs the virulence was raised. After it reached a point where 0.00000001 cc. regularly killed, the virulence was maintained by a weekly passage. In the intervals between passage it was stored in blood broth in the ice chest. During a year and a half there has been no apparent tendency for the virulence to decline.

Young guinea pigs, chiefly females, of 200 to 250 gm. have been used. Since each experiment extended over a period of several weeks the animals often gained 25 to 50 gm. between the beginning of the experiment and the time when they were employed for immunity titrations, but with only a few exceptions the weights of the animals at the time that graded doses of culture were given varied between 225 and 300 gm. We have not found that there is any significant difference in the natural susceptibility of guinea pigs to intraperitoneal injection of Pneumococcus Type I if the weights are between 200 and 300 gm. After intraperitoneal injection of broth cultures of pneumococcus guinea pigs die in 1 to 7 days with a diffuse peritonitis and bacteremia. When death occurs early, the peritoneal exudate is a thin purulent fluid with only a small amount of fibrin. Enormous numbers of pneumococci are present. If the animals live several days the exudate becomes more fibrinous. The typical pathology does not include pneumonia.

No attempt has been made to separate or purify the various antigenic substances of the pneumococcus. Interesting and important as are such studies as those of Zinsser and Parker (12) and Heidelberger and Avery (13) on the chemical properties of antigenic substances obtainable from the pneumococcus, such purified substances are not essential for a study of the particular problem under consideration.

In testing for skin allergy and anaphylaxis we have used as antigens: (*a*) 18 hour broth cultures killed by heating to 60° for 1 hour; (*b*) filtrate from fresh 18 hour broth cultures. This filtrate contains the soluble substance described by Dochez and Avery (14) and recently studied in more detail by Heidelberger and Avery (13). The filtrate was freshly prepared on the day it was used. (*c*) An extract prepared by centrifugalizing 18 hour broth cultures, removing the super-

natant broth, grinding the sediment with powdered glass, extracting, with frequent shaking, for 24 hours at room temperature with N/100 NaOH; after filtration the extract is standardized by nitrogen content; 0.5 per cent phenol has been added as preservative. During the course of the work, this extract, to which we shall refer as pneumococcus protein, has been freshly prepared each week. It is obviously a mixture of the soluble cellular constituents, but since it has been found to be antigenic, the fact that it is a mixture in no way invalidates the results.

The presence or absence of cutaneous allergy was determined by the intracutaneous injection of whole killed cultures, filtrate, and pneumococcus protein. Approximately 0.02 cc. of each substance tested was injected; the test sites were watched for 30 minutes and then read at 2, 6, 18, 24, 48, and 72 hours. Intact animal anaphylaxis was determined by the intravenous method. When the pneumococcus protein was used it was injected in amounts of 0.5 to 1.5 cc. of a solution containing 50 mg. of nitrogen per 100 cc. This substance produces in untreated control animals immediate clonic muscular contractions and a sharp drop in temperature of 2° or 3°C. The animal shivers violently for 4 or 5 minutes, then lies still and appears sick for perhaps 10 minutes more, but with the amounts of pneumococcus protein used death was not observed in any of the control animals. After 20 minutes recovery was complete. Although there is a certain similarity between anaphylaxis and these symptoms from a first injection of pneumococcus protein, they should not be confused. The characteristic bucking movements, respiratory embarrassment, and fatal outcome of anaphylactic shock have not occurred after the injection of pneumococcus protein. Animals were also injected intravenously with filtrate from fresh 18 hour cultures in amounts of 1.5 to 2.5 cc. These amounts produce no symptoms in normal controls. The third method for the detection of specific hypersensitiveness was the Schultz-Dale uterine strip technique. A 200 cc. bath of Locke's solution was used. The antigens added to the bath were pneumococcus protein in amounts from 4 to 8 cc. (nitrogen = 50 mg. per 100 cc.) and filtrate from fresh 18 hour cultures in amounts of 1.0 or 2.0 cc. Owing to the fact that broth alone in amounts as small as 2.5 cc. (in 200 cc. bath) may with some normal animals produce a uterine contraction, one inclines toward caution in interpreting the results with the Schultz-Dale technique when filtrate is added to the bath. On two occasions, when testing immunized animals, we obtained uterine contractions with 1.5 and 2.0 cc. but owing to the narrow margin between the sensitiveness to filtrate of the uterine horns from these immunized pigs and those from normal controls we have discarded these results and have not concluded that any anaphylaxis to filtrate has been demonstrated by the Schultz-Dale method. With the pneumococcus protein the case is different. The uterus of normal guinea pigs showed no contraction after amounts up to 12 cc. of a solution containing 50 mg. of nitrogen per 100 cc. were added to the 200 cc. bath. Larger amounts were not tried.

As has been stated the virulence of the pneumococcus was raised by repeated passage through guinea pigs. Eventually 0.00000001 cc. injected intraperitoneally

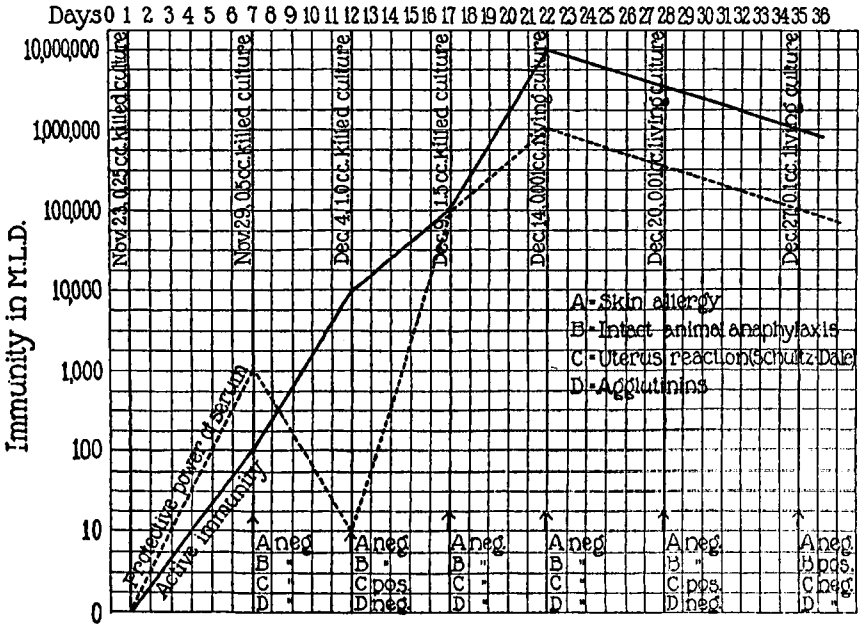
			peritoneally.				
		gm.	cc.				
Actively immunized.	309	263	1.0	Died 40 hrs.	Typical.	Pneumococcus.	Pneumococcus.
	310	262	0.1	Survived.			
	313	261	0.01	"			
	314	256	0.001	"			
	315	254	0.0001	"			
	319	248	0.00001	"			
Passively immunized, 0.5 cc. serum from actively immunized animals 6 hrs. before injection of culture.	415	268	1.0	Died. 40 hrs.	"	"	"
	416	265	0.1	" 5 days.	"	"	"
	417	253	0.01	Survived.			
	418	250	0.001	Died 5 days.	"	"	"
	419	242	0.0001	" 4 "	"	"	"
	420	248	0.00001	Survived.			
Controls.	424	270	0.001	Died. 11 days.	No peritonitis.	No growth.	No growth.
	425	261	0.00001	" 4 "	Typical.	Pneumococcus.	Pneumococcus.
	426	258	0.000001	" 40 hrs.	"	"	"
	427	250	0.0000001	" 4 days.	"	"	"
	428	245	0.00000001	" 4 "	"	"	"
	429	239	0.000000001	Survived.			

TABLE II.

Titration of Active Immunity and Protective Power of Serum 25 Days after the Beginning of Immunization.

	Guinea pig No.	Weight.	Amount of 18 hr. culture intra-peritoneally.	Result.	Gross pathology.	Culture from peritoneum.	Culture from heart's blood.
		gm.	*cc.*				
Actively immunized.	349	247	1.0	Survived.			
	350	265	0.1	"			
	352	210	0.01	"			
	354	207	0.001	"			
	355	190	0.0001	"			
	357	254	0.00001	"			
Passively immunized. 0.5 cc. serum from actively immunized animals interperitoneally 6 hrs. before injection of culture.	445	247	1.0	"			
	446	267	0.1	"			
	447	220	0.01	"			
	448	215	0.001	"			
	449	200	0.0001	"			
	450	252	0.00001	"			
Controls.	451	247	0.001	Died 60 hrs.	Typical.	Pneumococcus.	Pneumococcus.
	452	268	0.0001	" 60 "	"	"	"
	453	217	0.00001	" 60 "	"	"	"
	454	209	0.000001	Survived.			
	455	199	0.0000001	Died 84 hrs.	"	"	"
	456	240	0.00000001	" 60 "	"	"	Gram-negative bacillus and pneumococcus.

quite constantly killed guinea pigs of 200 to 300 gm. Frequently 0.000000001 cc. was lethal. In the immunity titrations there was therefore available a wide range of dosage. For each titration at least six animals and six normal controls were used. Dilutions of fresh 18 hour broth cultures were made with plain broth so that 1.0 cc. of the dilution contained the desired fraction of a cubic centimeter of the undiluted culture. While the results of these titrations were on the whole unequivocal, occasionally an animal survived a larger dose than one which was fatal to other animals of the same group previously treated in exactly the same way. This occurred with almost the same frequency in control animals as in the im-



TEXT-FIG. 1.

munized. At best, such titrations, however, can attain only approximate accuracy, but with a range of dosage from 1.0 to 0.000000001 cc. significant alterations in susceptibility are revealed. Tables I and II illustrate the method and results of the immunity titrations.

At the same time that active immunity was measured, the protective power of the serum was quantitatively determined. Two of the animals whose serum was to be tested were bled to death and the serum pooled. 0.5 cc. of this pooled serum was injected intraperitoneally into each of six normal guinea pigs. 6 hours later these animals were injected with graded doses of culture. Preliminary experiments had shown that the amount of serum used and the interval between

serum administration and culture injection conferred maximal protection for that serum. A larger quantity of serum or a longer interval yielded no additional protection.

Results.

Text-fig. 1 shows the results of the first immunization experiment. On the 22nd day the animals survived 10 million lethal doses of culture. On the same day the protective power of the serum was 1 million M.L.D. The protective power of the serum shows a drop on the 12th day, 5 days after the second immunizing dose of killed culture. The significance of this is not clear but it is worth noting that the decrease in protective power of the serum occurs just prior to the

TABLE III.

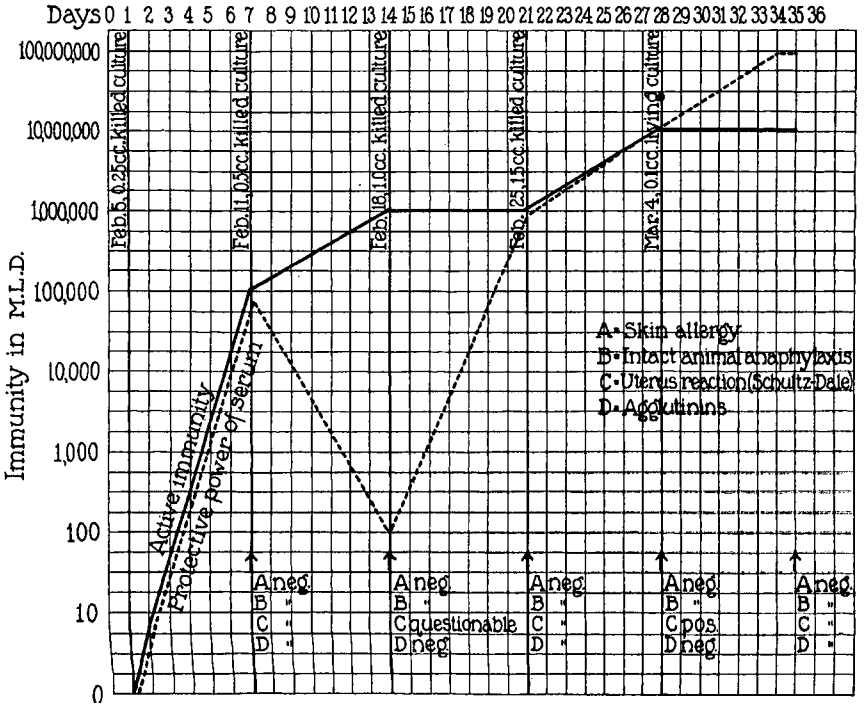
Intact Animal Anaphylaxis 35 Days after Beginning of Immunization.

	Guinea pig No.	Weight.	Amount of filtrate intravenously.	Result.
		gm.	*cc.*	
Immunized.	79	223	1.5	1 min. after injection scratches nose; violent bucking movements; lies on side; very sick. Survived.
	98	210	2.0	Typical anaphylactic shock. Death in 4 min.
	100	200	2.0	Typical anaphylactic shock. Death in 4½ min.
	113	218	2.0	Typical anaphylactic shock. Death in 5 min.
Controls.	222	225	2.0	No symptoms.
	230	192	2.0	" "
	240	220	2.5	" "

period when anaphylaxis becomes demonstrable. On each day that the protective power of the serum of the immunized pigs was titrated, precipitin and agglutinin tests were set up, using for the precipitin tests both filtrate and pneumococcus protein. Despite the presence of a high degree of active immunity and strong protective power in the serum, it was not possible at any time during the course of the experiments with immunized animals to demonstrate the presence in the serum of either precipitins or agglutinins. This lack of parallelism between protective power and other familiar antibodies has of

course been observed before, but what has been observed in these experiments is a particularly striking example.

On the 12th day and again on the 28th day, the animals were found by the Schultz-Dale method to be anaphylactic to pneumococcus protein. 4.0 or 8.0 cc. of a solution containing 50 mg. of nitrogen per 100 cc. gave strong contractions. An example of such an anaphylactic response with complete desensitization is shown in Text-fig. 3.

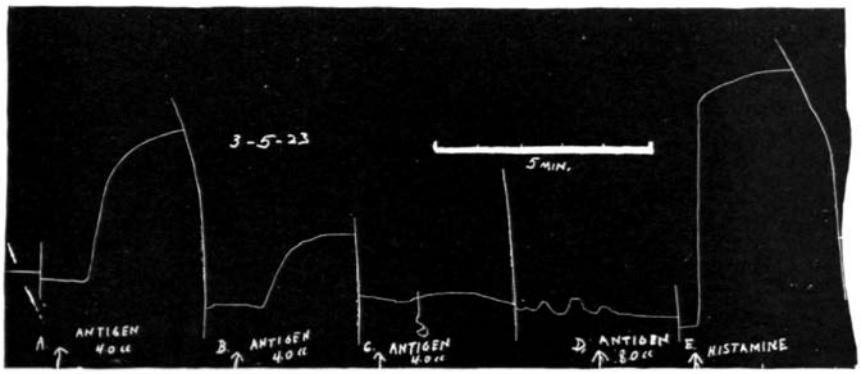


TEXT-FIG. 2.

On the 17th and 22nd days no positive contractions were obtained. The animals were apparently desensitized by the immunizing injections. With the filtrate from broth cultures no positive reactions were obtained at any time during the experiment. In testing for intact animal anaphylaxis with the pneumococcus protein and the filtrate, the results were different. At no time was there a definitely positive response with the protein extract, but on the 35th day fatal anaphylactic shock was obtained by intravenous injection of amounts of filtrate

quite innocuous for control animals. Table III shows these results. At no time was it possible to demonstrate any cutaneous allergy to protein extract, filtrate, or whole killed cultures. Experiments dealing with the production of a cutaneous reaction by repeated intracutaneous injections of pneumococcus protein and the relation of this allergy to resistance to infection are reported in a separate paper.

Text-fig. 2 shows the results of a second experiment carried out in the same way as that shown in Text-fig. 1. The intervals between test



TEXT-FIG. 3. Contractions of uterine horn from Guinea Pig 353 immunized to pneumococcus. At *A* 4.0 cc. of a solution of pneumococcus protein containing 50 mg. of nitrogen per 100 cc. were added to the 200 cc. bath of Locke's solution. At *B* and *C* this was repeated, the bath having been changed between each addition. *C* and *D* indicate that the preparation was desensitized. At *E* a small amount of histamine was added to show that the muscle strip had not lost its capacity to contract.

days were slightly different, but otherwise this experiment was a repetition of the first. The results were very similar. No skin allergy and no agglutinins were demonstrable. Questionable uterine contractions were obtained on the 14th day and undoubted responses on the 28th day. In this experiment, however, intact animal anaphylaxis was not observed. The curves of active immunity and protective power of the serum are very similar in the two experiments.

When we come to examine these results for some significant relation between anaphylaxis and active immunity we find none. The immunity curve rises from week to week and shows no change in its trend when anaphylaxis to pneumococcus protein appears, nor is

it altered by the temporary suppression of anaphylaxis occuring during the 3rd and 4th weeks as a result doubtless of the immunizing injections. The reappearance of anaphylaxis on the 28th day fails to produce any detectable effects upon the active immunity. The abrupt decrease in protective power of the serum observed on the 12th and 14th days occurs at the time when anaphylaxis first becomes demonstrable. What this means is not clear. With the reappearance of anaphylaxis on the 28th day no similar decrease occurs. From the fact that the active immunity curve does not participate in the temporary decline, there is justification for believing that whatever effect the development of anaphylaxis to pneumococcus protein may have on the protective power of the serum, it does not alter importantly the active resistance of the animal to pneumococcus infection.

CONCLUSIONS.

1. Intraperitoneal injections of killed and living broth cultures of a virulent pneumococcus produce in guinea pigs a high degree of active immunity and a serum with strong protective power.

2. Despite the protective power of such serum no agglutinins for the homologous organism and no precipitins for soluble derivatives were demonstrable.

3. Guinea pig immunity to pneumococcus infection produced by the method described is not attended by cutaneous allergy to derivatives of the pneumococcus used for immunization.

4. During the course of an artificially produced active immunity, anaphylaxis may at times be present and at times absent without any measurable effect upon the resistance of the animal to infection by intraperitoneal injection.

5. In the particular instance studied, the experiments indicate that anaphylaxis to pneumococcus protein has no important effect upon the resistance of the animal to infection. It appears to be a concomitant without any significant rôle in the immunity mechanism.

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