ON THE NATURE OF BACTERIAL ALLERGIES.

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In the light of the experimental facts which have accumulated since Baldwin's (1) first investigation of the problem, it would seem clear that the tuberculin reaction cannot be regarded as a manifestation of typical protein anaphylaxis. While it is true that all the phenomena of protein anaphylaxis with all the demonstrable mechanism that this term implies can be simulated by experiments with bacterial substances, everyone who has worked on the subject will agree that it is a matter of considerable difficulty to accomplish this. Both active and passive sensitization with bacteria require special and intensive methods (2) and the results, while recognizable and valid, are never so clear-cut or so simple of interpretation as are the classical experiments so easily performed with, let us say, horse serum or egg white. This is probably due to the relatively small amount of coagulable protein present in the bacterial body. But, however this may be, the fact remains that the study of true bacterial anaphylaxis has been disappointingly sterile in its influence upon our comprehension of infection and immunity. Of much more immediate significance in these processes are those types of bacterial hypersensitiveness of which the tuberculin reactions are the classical examples, and of which the general significance is indicated by the observation of analogous phenomena in the mallein, typhoidin, and abortin reactions, and in such experiments as our own with typhoid- and staphylococcus-injected guinea pigs.[1]

It appears, therefore, that two distinct types of hypersensitiveness may result from the contact of bacterial materials with the animal

[1] The differences between the immediate skin reactions of protein anaphylaxis and the "delayed" tuberculin type have been discussed in one of our previous papers (6).

body. One of these, true protein anaphylaxis, in all likelihood plays a relatively slight rôle in the phenomena of bacterial invasion and resistance. The other, hypersensitiveness of the tuberculin type which appears early in the course of infection, has gained fundamental significance in view of the accumulating evidence which points to a definite relationship of this type of "allergy" with immunity and resistance to superinfection (3).[2]

It was, of course, natural that the earlier investigators of anaphylaxis should have hoped that the antigen-antibody mechanism revealed as basic to these phenomena might equally serve to explain the bacterial allergies (von Pirquet and Schick and others) (4). But this hope has gradually been abandoned for the following reasons.

1. The inciting substance (tuberculin) is not an ordinary coagulable protein, is not biologically altered by heat, and does not lose its properties on repeated alcohol precipitation. Recent studies in our laboratory (Mueller) indicate that it is probably contained in what we have hitherto spoken of as a nucleoprotein fraction of the bacterial extracts, a substance which in its immunological relations shows considerable differences from the coagulable proteins concerned in true anaphylaxis.

2. Anaphylaxis to tuberculoprotein may be induced in guinea pigs by repeated injections of tubercle bacillus extracts, but these animals need not, and usually do not, become tuberculin-sensitive. Conversely, infected guinea pigs are tuberculin-sensitive within 2 weeks after infection without showing any evidences of being anaphylactic (Baldwin (1); Zinsser (6)). Skin sensitiveness to tuberculin has, however, been obtained by us in a limited number of animals intensively treated with the so called nucleoprotein fraction of the bacterial extracts, but the identity of this reaction with true tuberculin allergy is unclear, owing to the mild and temporary nature of the reactions so obtained.

3. Passive transfer of tuberculin sensitiveness with sera containing antibodies has not been clearly demonstrated.

[2] This relationship was first suggested by Koch's work on superinfection. It was distinctly voiced by von Behring and definitely set down as a possibility by Krause in 1916. Our own as yet unpublished work with Petroff, Ward, and Jennings is tending to corroborate it.

4. It appears that the development of typical tuberculin sensitiveness is not dependent upon the successive injection of dissolved bacillary extracts which may be followed by antibody formation, but depends, rather, upon actual infection with bacilli. This fact, so clearly stated by Baldwin, Krause (7), and others, that there is no tuberculin sensitiveness without a tubercle, has vindicated itself in our own work, and although, with Petroff, we have succeeded in inducing perfectly satisfactory tuberculin reactions by the injection of dead tubercle bacilli, we have failed with all fractional preparations of these organisms except in the isolated nucleoprotein experiments cited above.

That this association of tuberculin type of sensitiveness with *infection* rather than with immunity and antibody formation finds its analogy in similar reactions with other bacteria is of the utmost significance in view of the general conclusions which we believe are developing from our present work. Thus Fleischner and Meyer (8), in an investigation of cutaneous reactions in guinea pigs treated with the bacillus of bovine abortion, found that typical skin reactions occurred only in animals which on autopsy showed characteristic lesions of infection. Immunized animals and normal ones gave no intracutaneous reactions, and "animals that had been intensively immunized by intraperitoneal injections of dead *B. abortus bovinus*" (or extracts) showed cutaneous hypersensitiveness in *no* single instance, although agglutination reactions were usually positive. This is in complete analogy with our own observations in tuberculosis, as well as those of others.

I.

It appeared to us that no great progress could be hoped for toward further comprehension of the tuberculin and similar reactions until the relationship of these occurrences to antibodies, free or sessile, had been more clearly defined. In approaching the problem experimentally, it seemed that, in addition to a direct investigation of the tuberculin phenomena, it would be helpful to make use of other bacterial antigen-antibody reactions in which potent antisera could be more easily obtained than in tubercle bacillus experiments.

It may be remembered that in previous studies of different bacteria we obtained two biologically active substances which were designated,

respectively, the "nucleoprotein fraction" and the "bacterial residues." The nucleoprotein fraction, so called because of precipitability with acetic acid in the cold, constituted the bulk of the material obtained by extraction, and, in the case of tubercle bacillus extracts, always gave potent skin reactions. The residue material, which was left after removal of nucleoprotein and coagulable protein, still gave skin reactions in tuberculous guinea pigs, and, in the case of the tubercle bacillus, as well as in that of all other bacteria from which such material was obtained, was actively precipitated by homologous antisera. Because, in our early experiments, both the nucleoprotein and the residue gave skin reactions, the nucleoprotein fraction always somewhat more potently than the residue, we suggested, at that time, the possible derivation of the residue, by cleavage, from the nucleoprotein, but were unable to demonstrate this satisfactorily by experiment. We attempted at that time to separate the antigenic substance responsible for skin reaction from that reacting with the antibodies *in vitro*, but failed to accomplish this. But, since the residue materials in all cases reacted strongly and specifically with homologous antibodies *in vitro*, there seemed no logical reason for questioning the capacity of this substance for similar reactions in the animal body. It was, therefore, at least conceivable that one possible explanation of the tuberculin type of reaction might consist in a union of these non-protein residue substances with sessile antibodies, a process entirely analogous to the mechanism of protein anaphylaxis, except that in this case the reacting antigen consisted of these residue materials instead of the coagulable proteins,—this circumstance perhaps accounting for the physiological differences between the two phenomena.

Experiments upon Passive Sensitization to "Residue" with Pneumococcus Antiserum.

Since Antipneumococcus Type I serum can be easily obtained, our experimental procedure consisted simply in injecting varying and considerable amounts of antipneumococcus serum into light colored guinea pigs not less than 400 to 500 gm. in weight, and testing them intracutaneously with the pneumococcus residue material at inter-

vals ranging from a few hours to several weeks. The methods by which the residue was made have been described in detail in a previous communication. It was invariably produced from Pneumococcus Type I, grown on pie plate blood agar cultures, rabbit blood being used to avoid confusion in connection with the horse antiserum employed in the experiments themselves. These reactions were, in every case, tested by precipitation against the same anti-horse serum used in the experiment.

Inasmuch as the technique of these experiments is simple, and since a detailed description of the individual protocols would needlessly prolong this report, we believe it best to summarize this part of our work, as follows:

In the course of 8 months some 89 experiments on individual guinea pigs were done. In all cases Antipneumococcus Serum Type I, obtained from the New York State Department of Health by the courtesy of Miss M. B. Kirkbride, and from Dr. Benjamin White's Laboratory in Jamaica Plain, was injected. The quantities ranged from 4 to 6 cc. intravenously to as much as 12 cc. intraperitoneally, and, in a few cases, individual guinea pigs received as much as 30 cc. of the Type I serum subcutaneously and intraperitoneally, in consecutive injections within 24 hours.

Intracutaneous injections of 0.2 cc. of the unconcentrated pneumococcus residue were made on these pigs both before injection of the serum, in order to guard against non-specific reactions, and from a few hours after the injection until, in many cases, 2, 3, or 4 weeks later, at intervals varying from 3 days to a week. In the belief that there might be some interference with local reactions on the part of antibodies still left in the circulation, titrations of circulating antibodies were made from time to time in some of the pigs, by the agglutination of pneumococci and the precipitation of residue.

It should be mentioned that in experiments of this kind the greatest care must be taken to avoid non-specific reactions in normal animals, since every now and then a control guinea pig would show a marked reaction to pneumococcus residue, without preceding serum injection. Controls to which normal horse serum had been given were uniformly negative. Non-specific reactions were in many cases associated with pregnancy in the pigs, and in later experiments male animals only were employed.

Young guinea pigs less than 400 gm. in weight were entirely unsuitable for the experiments, because their normal reactions to the pneumococcus residue were irregular.

In the large majority of the animals no indications of delayed skin reactions of the characteristic tuberculin type were obtained. This

occurred, to a mild degree, which might be designated as from + to ++ reactions, in about 5 per cent of the animals, never, however, with any degree of edema, the reactions consisting of flat, reddened areas. Whenever such reactions did occur, as in Guinea Pig A on March 10; Guinea Pig B, March 12; Guinea Pig C, March 21, and a few others, they were obtained by injection of the residue from 48 to 72 hours after the serum administration. In a large majority of the guinea pigs no reactions whatever of this type occurred, but in a considerable number of them marked swelling and local edema were apparent 4 to 6 hours after the residue injection, but almost completely faded by the following morning; *i.e.*, a variety of "immediate" reaction comparable to that of true anaphylaxis. In many of the pigs no reaction of any kind resulted.

We continued with our pneumococcus experiments for a much longer period than we had anticipated, largely because the occasional indications of mild, delayed skin reactions of the tuberculin type caused us to persist by increasing the quantities of serum and by varying the intervals of testing.

We were finally forced to conclude, however, that the administration of antipneumococcus serum, potent in specific antibodies and capable of powerfully precipitating the pneumococcus residue *in vitro*, does not render guinea pigs allergic to this residue.

Information Obtained by Attempts to Produce the Tuberculin Reaction Passively.

While the foregoing experiments with pneumococcus materials were in progress we were, at the same time, preparing rabbits in various ways with human and bovine tubercle bacilli in order to carry out analogous experiments directly with tuberculin. Before proceeding to these experiments, however, it will be advisable to review briefly the more important investigations that have dealt with this problem.

In 1909 Helmholz (12), using the cutaneous method of determining tuberculin sensitiveness in guinea pigs, injected a series of pigs intraperitoneally with 4 to 5 cc. of the defibrinated blood of strongly reacting tuberculous guinea pigs. He obtained moderate skin reactions 3 and 4 days after the injection of the defibrinated blood, the maximum reaction 4 to 6 days later. These experiments have not been subsequently confirmed.

In 1910 Bail (13), employing the systemic tuberculin reaction as an index, injected guinea pigs with suspensions of ground uncaseated tuberculous organs and glands of infected animals. He injected quantities of 1.5 to 2 cc. into very light guinea pigs (200 gm.) and found that, when he injected these animals 20 hours later with 0.6 cc. of tuberculin intraperitoneally, they died within 48 hours, while animals similarly treated with normal organs hardly reacted to this amount of tuberculin. Later he found that when large amounts of the tuberculous organ suspension were introduced, namely 5 cc., as little as 0.2 cc. of tuberculin would kill.

In 1921 McJunkin (14) injected large amounts of glycerol broth cultures of tubercle bacilli suspended in 2 per cent gelatin solution, intraperitoneally into tuberculous guinea pigs. These animals died on the following day, and the considerable amounts of sticky exudate obtained from the peritoneal cavity were filtered. These filtrates were injected subcutaneously into other guinea pigs. He prepared other series by injecting filtrates from extracts of crushed abdominal walls and tuberculous organs of infected animals. In such guinea pigs he obtained positive tuberculin reactions, but not until the 8th or 12th day after injection, and, in his third series, not until 23 days after injection. He himself suspects that his animals may have been actively sensitized.

The most recent and interesting work was published by Lange in 1924 (15). Lange succeeded in producing positive cutaneous tuberculin reactions in guinea pigs injected with filtrates of tuberculous foci of previously infected animals. Such animals were repeatedly injected during the course of testing and skin reactions became positive usually after 8 days. In some of her experiments positive reactions did not appear until the 10th day; in others not until the 15th. Her work is particularly interesting in that she carried out controls with guinea pigs injected with filtrates of extracts of sterile inflammatory foci produced in guinea pigs by injection of Kieselguhr, and these non-specific filtrates seemed to give reactions as easily and almost as marked as those appearing in the animals treated with extracts of tuberculous foci.

Before discussing the apparent significance of these results, some of which have not been confirmed by those who have attempted to repeat them, we will submit our own results, citing only the experiments which seem to us particularly important.

Experiment 1.—

Rabbits M-1, M-2, and M-3 had received intraperitoneal injections of tubercle bacilli, human strain, No. H 37, dehydrated with alcohol and ether and autoclaved for an hour, as follows:

May 5. 0.2 gm. dry weight.
 " 13. 0.35 "   "   "
 " 20. 0.5 "   "   "
 " 27. 0.5 "   "   "

June 6. Bled; strong precipitation with precipitated O.T.[3]

The guinea pigs used below were all tested and found negative on intracutaneous injection of O.T. 1/10 before use.

1/10 O.T. injections on dates mentioned—intracutaneous throughout—approximately 0.1 cc.

Guinea pigs injected June 9.	June 10.	June 12.	June 16.	
Guinea Pig D. 6 cc. Serum M-1 intravenously.	+ + in 4 hrs. Faded next day.	0	Strong +	This reaction mild but typical after 24 hrs.
Guinea Pig E. 6 cc. Serum M-2 intravenously.	0	0	+	
Guinea Pig F. 6 cc. Serum M-3 intravenously.	0	0	±	

Titration of the Rabbit Sera against O.T. Precipitated with Alcohol and Taken Up in Salt Solution.

	Serum M-1.	Serum M-2.	Serum M-3.
Serum concentrated.	++++	++++	++++
" diluted 1:5.	++	++	++++
" " 1:10.	+	+:+	+++(slow).

The above experiment is notable in that on the 7th day after injection we obtained a mild but typical delayed reaction in Guinea Pig D. In this animal, also, there was an immediate reaction in 4 hours comparable to those described above in the case of the pneumococcus experiments. In spite of a relatively potent precipitating titer in these sera, therefore, little if any tuberculin sensitization occurred. It is noticeable also that the animal receiving the strongest precipitating serum, M-3, developed the weakest sensitiveness to tuberculin.

Experiment 2.—In this experiment we attempted passive sensitization to tuberculin by injecting the serum of an intensively treated rabbit into guinea pigs, controlling this by a similar injection of normal rabbit serum into a control.

Rabbit A had been intensively treated since December 28. Between that date and February 1 it received eight injections of a suspension of defatted tubercle

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[3] Ordinary O.T. was precipitated with 10 volumes of alcohol and redissolved in salt solution to its original volume.

bacilli (Dreyer), then was allowed to rest, and between March 25 and May 5 it received five intraperitoneal injections of 4 to 5 cc. of a suspension of living H 37 bacilli. The rabbit was bled on May 20, 15 days after the last injection of H 37. The serum at this time gave a $+ + + +$ ring test with precipitated O.T.

Tests on dates mentioned with O.T. 1/10—intracutaneous—approximately 0.1 cc.

May 21.	May 23.	May 28.	June 1.	June 3.	June 11.	June 17.	July 11.	July 25.
Guinea Pig G. Intravenously 7 cc. serum, Rabbit A.	++	+	+++	++	±	0	0	0
Guinea Pig H. Duplicate of above.	++	++++	+++	±	±	Flat redness.	+++	
Guinea Pig I. Intravenously 7 cc. normal rabbit serum.	0	0	0					

We obtained definite intracutaneous tuberculin reactions in the above guinea pigs on the 3rd day after the intravenous injection of the serum. In one of these pigs the reaction became intensified after 8 days; in the other it did not intensify until the 12th day. It seems unquestionable that a certain amount of passive transmission of the tuberculin reaction occurred in these pigs on the 3rd day, which is far too early for the development of active sensitization by infection. Furthermore, the fading of the reaction in Guinea Pig G, which was negative on July 25, would indicate definitely that, whatever may happen in the future with this pig, its sensitization by the serum was temporary and not progressive. This animal is still in our possession and gaining weight.

Experiment 3.—In the next experiment there is a comparison between the precipitating properties and passive sensitizing capacities of the sera of rabbits treated in different ways. Descriptions of the rabbits are sufficiently stated in the protocols except for Rabbit B. To this animal, suspecting the importance of the tissue reactions in producing the sensitizing substance, whatever it may be, we administered multiple subcutaneous injections (in this case twelve) of small amounts of living bovine bacilli. A little more than 3 weeks later the rabbit was bled and the tissue excised for further use, as indicated in subsequent experiments.

Description of Rabbits.

Rabbit W. B.   Infected intravenously with bovine tubercle bacilli on June 8.

Rabbit W. L.   Treated as Rabbit W. B.

Rabbit A.   Intensively treated with dead and then living H 37 human tubercle bacilli since December, 1923 (see Experiment 1).

Rabbit C.   Treated as Rabbit A.

Rabbit B.   Multiple injections (twelve) of living bovine bacilli under skin on June 18—see above.

All of these rabbits were bled on July 8.

Titrations of Sera against Alcohol-Precipitated O.T.—July 11.

Ring Tests.

Rabbit Serum W. B.   0   precipitate.
"   "   W. L.   0   "
"   "   A.   +++   "
"   "   C.   +++   "
"   "   B.   ± ?   "

July 9.	July 10.	July 11.	July 14.	July 15.	July 16.
Guinea pigs.	O.T. 1/5	O.T. 1/5	O.T. 1/5	O.T. 1/5	O.T. 1/5
Guinea Pig J. 4 cc. Rabbit W.B. serum intravenously.	0	0	Good ++	0	0
Guinea Pig K. 5 cc. Rabbit W.L. serum intravenously.	0	0	±	± to +	0
Guinea Pig L. 5.5 cc. Rabbit A serum intravenously.	0	0	±	0	0
" " M. 5.75 cc. Rabbit A serum intravenously.	0	0	±	0	0
Guinea Pig N. 6 cc. Rabbit C serum intravenously.	0	0	0	0	0
" " O. 6 " " B " "	0	0	Strong +++	Strong ++	0

All of the guinea pigs tested with O.T. 1/10 and found negative before use.

This experiment is instructive in that the only powerful reaction obtained developed in Guinea Pig O, which had received intravenously 6 cc. of the serum of the "multiple lesion" rabbit (Rabbit B); a fair reaction was obtained on the same day in Guinea Pig J, which had been injected with the serum of a rabbit infected with living bovine bacilli intravenously on June 8.   These reactions were definite, with some edema, but no necrosis, averaging from 1.5 to 2.5 cm. in diameter, and well developed after 24 hours.   Neither of the sera

that induced reactions contained any appreciable amounts of precipi-
tating antibodies, whereas Rabbits A and C, giving practically no
tuberculin sensitization, were strong in precipitating power. It is
worth noting that in this experiment the serum of Rabbit A did not
induce allergy. The serum of the first bleeding of this rabbit on
May 20 induced definite skin reactiveness, but the animal received
further injections after that, and was bled for Experiment 3 on July
8, this time 5 instead of 15 days after the last injection. These facts
indicate the fluctuating character of the allergic substance in the serum
of these rabbits.

Experiment 4.—In order further to illustrate this fluctuation of the
sensitizing property at different times, we have put together results
obtained with the serum of three separate bleedings of Rabbit A.

Injection of guinea pigs.	O.T. injections.				
June 30.	July 1.	July 2.	July 3.	July 4.	July 5.
Rabbit Serum A. Bleeding of June 30, 4 cc. intravenously.	O.T. 1/10 negative.	O.T. 1/10 +	O.T. 1/10 +++ in 4 hrs. ++ next a.m.	Negative.	Negative.
July 9.	July 10.	July 11.	July 14.	July 15.	July 16.
Rabbit Serum A. Bleeding of July 8, 5.5 cc. intravenously.	O.T. 1/5 and O.T. 1/10 negative.	O.T. 1/5 negative.	O.T. 1/5 ±	O.T. 1/5 negative.	O.T. 1/5 negative.
July 24.	July 25.	July 28.	July 29.	July 30.	
Rabbit Serum A. Bleeding of July 23, 20 hrs. after injection of 2 cc. 1/5 O.T. intravenously.	O.T. 1/5 negative.	O.T. 1/5 good +++ after 20 hrs.	+++	+++	

It will be seen that practically negative results were obtained with
serum of the bleedings of June 30 and July 8. On July 22 we conceived
the idea of perhaps stimulating the tuberculous foci of this animal by
an intravenous injection of 2 cc. of 1/5 O.T. solution. That we were
close to the fatal dose of O.T. for tuberculous rabbits was apparent
from the fact that another rabbit similarly injected on the same day
was dead on the following morning. The serum, taken 24 hours after
the O.T. injection, sensitized the guinea pig so that a strong tuberculin

reaction was obtained on the 5th day. Two almost identical reactions were obtained on July 29 and 30. We have not yet repeated this experiment, but it at least suggests that stimulation of the foci with tuberculin may give rise to an increased passage into the blood stream of the substance responsible for passive allergic sensitization.

Experiment 5.—We wish to add one further compilation from our experimental protocols which shows that the passive sensitization obtained with the sera of a number of rabbits in which tuberculous foci had been produced could also be obtained with tissue filtrates of the tuberculous lesions made practically in the same manner in which Baïl produced them, except that, like Lange, we passed them through a Berkefeld filter, to remove tubercle bacilli and tissue fragments.

Rabbit B has been described in a preceding experiment. It was one of those that received multiple injections of bovine tubercle bacilli on July 8. The tissue filtrate was prepared by carefully excising the subcutaneous lesions under sterile precautions, cutting them up into small bits, grinding in a mortar with sand, shaking in salt solution for 4 to 5 hours, and filtering through a Berkefeld filter.. The tissue extract from Guinea Pig P was made in a manner analogous to that just described above from a guinea pig given multiple injections of B. H 37 on July 8.

Rabbit M.B.-2, the serum of which was injected into Guinea Pig Q, was prepared exactly as was Rabbit B.

Tested intracutaneously with O.T. 1/5, 0.1 cc.

		July 18.	July 19.	July 22.	July 23.	July 25.
Intraperitoneal injections.	Guinea Pig R. Tissue extract filtrate from Rabbit B, 6 cc.	0	++		++ O.T. Pneumococcus residue negative.	++ O.T. Slight edema.
	Guinea Pig S. Serum B, 6 cc.	0	++		++ O.T. Pneumococcus residue negative.	+ to ±
	Guinea Pig Q. Serum from Rabbit M.B.-2, 4 cc.	0	++		++ O.T. Pneumococcus residue negative.	0
	Guinea Pig T. 6 cc. unfiltered tissue extract from Guinea Pig P.	0	++		++ O.T. Pneumococcus residue negative.	++
	Guinea Pig U. 6 cc. normal rabbit serum.	0	0		Both 0.	0

In this experiment passive sensitization to tuberculin was obtained on the 5th day after injection with tissue filtrates of the lesions of Rabbit B; with the serum of Rabbit B; with the serum of Rabbit M.B.-2, treated and bled exactly as was Rabbit B; also with the tissue extracts of a guinea pig infected with multiple lesions of H 37, 10 days before the experiment. It should be added that a number of experiments in which the defibrinated blood and serum of similar guinea pigs were injected were entirely negative, as were some experiments with tissue extracts of similarly treated guinea pigs.

We believe that the irregularity of our results, both with tissues and with sera, indicate that the substance which sensitizes to tuberculin fluctuates in amount at different times and under conditions for the determination of which the necessary criteria are as yet lacking.

It is interesting to note that repetitions of experiments with the same positive extracts and bleedings of rabbits like Rabbit B always gave identical results, thus removing the possibility of explaining the results by accidental peculiarities of the injected guinea pigs.

In the foregoing experiments we have observed that the injection of antipneumococcus sera potent in precipitating and other antibody effects rarely gives more than a slight sensitization to the tuberculin type of skin reaction when pneumococcus residue is subsequently injected, and, in most cases, is completely negative, whatever the quantities or serum injected may be, or at whatever time intervals the test injections are performed. In a number of these cases, however, an immediate edematous and evanescent reaction appears within 4 to 6 hours after the administration of the serum. The passive transmission of the tuberculin type of skin reaction in these cases seems to have no relationship whatever to the antibodies capable of precipitating the residue antigen.

Our experiments with passive transmission of tuberculin sensitiveness to guinea pigs indicate that it is possible to transfer this form of allergy with the sera of individual rabbits treated with tubercle bacilli; that such capacity for passive transmission of tuberculin sensitiveness is unrelated to the antibody contents of the serum as determined by precipitation of O. T. or of residue material; and that reactions similar to the above may be obtained upon injection of filtrates of crushed tuberculous tissues from rabbits furnishing the serum. A few experi-

ments have also been successfully performed with crushed tissues from tuberculous guinea pigs. We have not controlled these with tissue extracts produced from mechanical inflammatory foci, as has been done by Lange. This part of the problem will need further investigation. We have on one occasion, however, injected pneumococcus extracts into pigs passively rendered allergic to O.T., and obtained nothing, showing that a certain amount of specificity adhered to our method of sensitization. Normal rabbit sera never conferred such allergy.

II.

It is consistently apparent from the preceding experiments that high precipitating power for residue antigen, either in pneumococcus serum or antitubercle bacillus serum, is not in any way related to capacity for the passive production of allergy in injected animals. This seems to answer in the negative the query we had originally set ourselves, in that it indicates that allergy such as the tuberculin phenomenon is not due to reaction within the animal body between the antibodies in the serum and the non-protein residue material with which they cause precipitation in the test-tube. Such results naturally encouraged a resumption of chemical studies of the antigen, inasmuch as they again suggested the possibility of there being two separate materials in the crude tuberculin—one concerned with the precipitin reaction as described above, the other particularly important in regard to specific tuberculin hypersusceptibility or allergy.

In a previous communication made by one of us in 1921, unsuccessful attempts at the same procedure were made. The following statement, quoted from this communication, summarizes the condition in which the problem was left by us at that time.

"It must be stated, however, that the nucleoprotein fraction, precipitated from the original extracts with acid in the cold, always retained tuberculin activity. In spite of repeated reprecipitation and resolution, we have never succeeded in entirely removing the capacity of inducing skin reactions of the tuberculin type from these [acetic] acid-precipitable substances. This may be due to the adsorption of [residue] material by the heavy flakes of the precipitate. On the other hand, subsequent experiments suggested the possibility that these nucleoproteins (?) which constitute the bulk of the soluble material of the bac-

terial cell might represent the mother substance from which the other materials [residue] are derived."[4]

Thus the earlier work showed that the residue material was precipitable by homologous serum more sharply than was the nucleoprotein, at least in the cases of sera prepared in the usual manner of injection of whole bacteria, but always retained a definite capacity to induce skin reaction in tuberculous animals. We were led to entertain renewed prospects of experimentally accomplishing a functional separation of these two materials by an observation made in the course of attempts to purify tuberculin residue by an advanced student, Dr. Tomacek, working upon this subject under the direction of one of us (Mueller). In these experiments it seemed that trypsin and pepsin digestion of crude tuberculin tended to remove the skin-reactive properties without proportionately destroying the precipitability of the tuberculin by immune sera. Subsequent pursuit of this clue showed that similar results could be easily obtained by a still further removal of protein left in the residue after the first precipitation with acetic acid in the cold and after boiling in acid. This was accomplished by following these procedures by precipitation with tannic acid as follows:

Experiment on the Separation of the Allergy and Precipitable Fractions of Tuberculin.

Crude tuberculin is first precipitated with alcohol. This brings down both the skin-reactive and the specific precipitable materials. The filtrate is discarded and the precipitate redissolved. This solution is now treated with acetic acid in the cold in the same manner in which we first separated nucleoprotein and residue from extracts of pulverized tubercle bacilli. The acetic acid precipitate representing the so called nucleoprotein fraction can be redissolved in weak alkali for subsequent test. We may state in passing that this so called nucleoprotein never seems to go into complete solution, always remaining opalescent, and if filtered through Berkefeld filters shows considerable loss, indicating that such so called nucleoprotein solutions are really suspensions; and while we retain the name for convenience and believe the material to be of a protein nature, we are by no means sure that these characteristic bacterial proteins are true nucleoproteins.

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[4] We have omitted in this quotation the words "proteose materials," since our original assumption that these materials might be of a split protein nature has been corrected by subsequent work.

After removal of the acetic acid precipitate, the filtrate remaining is the crude residue which, in our older work, precipitates with immune serum and still retains a definite capacity for inducing skin reactions in tuberculous animals. This is now further treated in the hope of removing the remnants of skin-reactive substances. After neutralization, this filtrate is precipitated with a concentrated solution of tannic acid until no further precipitate appears. The precipitate is then filtered off and the tannic acid removed with barium hydroxide and with lead hydroxide in the usual manner.

Tests of materials so treated in a recent experiment were as follows:

Precipitation.

Precipitate. {Concentrated.	+
{1:10.	0
{1:10	++++
Final filtrate. {1:100	+++
{1:600	+

Skin Reactions Performed with the Same Materials on Four Tuberculous Guinea Pigs.

	Guinea Pig A.	Guinea Pig B.	Guinea Pig C.	Guinea Pig D.
Precipitate (1:10)	++	++	+++	+++
Filtrate (1:10)	±	—	+ strong.	.+

This experiment and others like it indicated that, while an absolutely clean separation had not yet been obtained, it was nevertheless possible, by methods of protein precipitation, to obtain two fractions, one of which, the residue, contains the materials precipitated by antiserum and almost free from allergy-inducing substances; the other, the so called nucleoprotein fraction, containing very little material precipitable by the immune sera, but representing the bulk of the allergy-inducing substances. The experiments have been sufficiently consistent to convince us of the separate nature of the two substances.

GENERAL CONCLUSIONS.

1. There seems no further possibility of questioning the opinion that the development of tuberculin sensitiveness is definitely associated with the development of tissue reactions in the form of in-

flammatory processes; the mere development of antibodies on the injection of dissolved bacterial materials does not induce allergy.

2. Passive transfer of tuberculin allergy to guinea pigs may be accomplished by injections not only of the tissue extracts of the tuberculous foci of rabbits, but also of the sera of such rabbits, provided multiple, well developed, and not too advanced lesions are present in the rabbits furnishing them. The exact criteria by which such results can be regularly obtained have not yet been ascertained; our results in this respect, though definite, have been irregular and occasional. It is clear, moreover, that the capacity to convey such allergic hypersensitiveness has no relationship to the precipitating capacity of the serum for residue materials.

3. Just as the allergy-conveying power of the serum and its precipitating powers for residue are separable, so, also, the bacterial extracts representing the antigens for these reactions are separable, the residue material being particularly concerned in the specific precipitations with immune serum, the so called nucleoprotein being associated with allergic reactions in the tuberculous animals.

DISCUSSION.

The tuberculin reaction thus depends on something associated with the inflammatory reactions taking place about the lodgment of living or dead tubercle bacilli. That this, while mainly local, is not purely local, follows from the fact that animals are allergic over the entire skin, while the tuberculous foci may be limited to a lymph node or an organ. Two possible mechanisms may be considered to explain this. On the one hand, we can assume that a definite tissue reaction is necessary to liberate from the bacteria the materials which then are absorbed for general active sensitization of the body as a whole. On the other hand, it may be possible that the reacting antisubstance is produced locally in the course of the tissue reaction and passes out from this, passively sensitizing the rest of the body. That this latter process is probably an important part of the phenomenon is indicated by the facts that tissue extracts of the lesions as well as occasional sera will transmit hypersensitiveness. This appears not only from our own work, but from the earlier work of

Bail and Lange. And that this certain something that passes out from the lesions is not an ordinary precipitating antibody, as found in the sera of animals immunized with bacillary extracts and whole bacteria, is apparent from our own work.

Incidentally this work indicates a fundamental difference between the results of immunization with coagulable proteins like horse serum, egg albumin, etc., and those observed with bacteria. In the former case, as far as we know, the test-tube antibody reactions take place between the proteins and the circulating antibodies; and reactions of protein hypersusceptibility in the animal body take place between the whole proteins and the same antibodies now presumably sessile upon the cells. In the case of bacterial immunization, on the other hand, injection of the whole bacteria and the bacterial extracts, as usually employed for this purpose, leads to the formation of antibodies which react in test-tube precipitations with the non-protein bacterial residues, although these residue fractions are neither capable of inducing antibody formation when injected by themselves, nor, when sufficiently purified, are they capable of inducing reactions in the allergic animal. Just how these relations are altered when purified nucleoprotein material from bacterial extracts is used for immunization remains for further study. Interesting in this connection is the recent publication of Lancefield (16).

Our own interest in these observations is considerably enhanced by the belief that they may have no inconsiderable bearing upon the processes of bacterial immunity in general. As we have pointed out above, and in other papers, the tuberculin reaction in all probability represents a principle widely applicable to all bacterial infections. Its analogy with the mallein, typhoidin, and abortin reactions is indisputably close, and our own work has indicated that similar allergy may develop whenever an infection has lasted more than 10 days, or is repeated in the same individual at frequent intervals. For the development of such allergies, however, it is not sufficient that dissolved or soluble bacterial materials be administered, but there seems to be required an actual tissue reaction in contact with the invading microorganisms. The mechanism by which such a reaction leads to the development of allergy is as yet obscure, but is certainly not the ordinary antibody-antigen reaction.

If we consider these facts in connection with the opinions of many thoughtful students of tuberculosis (Baldwin and Krause especially), opinions which are being corroborated in work now going on conjointly both in this laboratory and in the Saranac Laboratory in the hands of Petroff, that the allergic state and increased resistance are parallel and perhaps causally related phenomena, the obvious inference arises that these substances upon which allergy depends may possess protective functions different from and based on a different mechanism from those possessed by antibodies.

Such a conception, it seems to us, might aid in understanding the difficulties encountered in attempts at producing curative sera by the injection of dead bacteria, and again emphasizes the fundamental differences between sera obtained in this way and those obtained by the treatment of animals with living and virulent bacteria, or convalescent sera—subjects which are very much in the foreground of bacteriological investigation at the present time.

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