FURTHER STUDIES ON BACTERIAL HYPERSUSCEPTIBILITY. II.

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PLATE 14.

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The work reported in this paper is a direct continuation of studies begun several years ago and published in part in a preceding communication by one of the writers (1), in which it was shown that intradermal and presumably, also, general tuberculin reactions could be distinctly differentiated from true anaphylaxis to tuberculoprotein.

As a consequence of the work done at that time, it appeared that in the course of infections with the tubercle bacillus, two distinct varieties of hypersensitiveness may develop. One of these, true anaphylaxis against the protein materials of the bacteria, occurs late in prolonged infections, not sooner than 3 weeks after first lodgment of the living bacteria in the body. This form of hypersusceptibility is analogous to such phenomena as horse serum and other protein anaphylaxis and expresses itself in generalized hypersusceptibility, in the uterine phenomena of Dale, and, as far as the skin is concerned, in the rapid, urticaria-like, evanescent skin reactions so familiar to all physicians who have tested pneumonia patients for horse serum hypersusceptibility by intradermal injections. The other, the tuberculin reaction, occurs much earlier, appearing in tuberculous animals within 8 or 9 days after a massive injection of living bacilli, thus developing several weeks before the anaphylactic reaction appears, and is associated with a skin reaction slower in development and accompanied by deeper pathological injury and slower healing. This reaction, analogous apparently in every way to the so called typhoidin, mallein, abortin, and similar reactions, can be elicited in the infected animal by injection with a residue of tubercle bacillus extract from which all proteins have been removed as far as this can be done by the usual methods of precipitation with acid and boiling in an acid reaction. Moreover, whereas true protein anaphylaxis can be induced in the animals by repeated injections of whole dead bacteria or bacterial extracts, the latter type, or the tuberculin type of reaction, occurs only in animals actually infected with the living organisms, an important point previously observed by Römer (2), Baldwin (3), Krause (4), and others.

We thus have in addition to, and distinct from true protein anaphylaxis, an apparently specific hypersensitiveness associated only with infection, and induced in the sensitive animal with derivatives of the bacteria the chemical structure of which is uncertain but which certainly differ materially from the substances ordinarily spoken of as proteins. Our suspicion that we are dealing with substances of lower molecular structure than proteins is now being investigated by chemical analysis and will be reported in a subsequent paper. Until this work is finished, however, no statement concerning the exact chemical nature of these materials is justified. For possible explanations of the particular association of the tuberculin type of reaction with infection with living bacteria the reader is referred to the discussions in the preceding paper mentioned above. We may reiterate here, however, that the possibility of development of a specific hypersusceptibility on the part of an infected animal to metabolic products of the bacteria produced in quantity and perhaps slightly diffusible, a susceptibility definitely separable from protein anaphylaxis and developing much sooner in the course of infection, would go far to explain many of the injuries and degenerations occurring in bacterial diseases. Moreover, these observations seem to promise further light upon phenomena of hypersusceptibility in general, in that we appear to have been dealing with a form of specific susceptibility in which not only the laws of sensitization but also the physical and chemical properties of the injuring antigen seem to differ materially from those governing protein anaphylaxis.

It seemed more than likely that we were dealing with a general principle. In the first place, this was indicated by the close analogy, in regard to nearly all the circumstances mentioned above, between the tuberculin reaction and such phenomena as the mallein, typhoidin, and abortin reactions in guinea pigs. In these phenomena it seems fairly well established, in view of the recent work of Fleischner, Meyer, and Shaw (5), that infection and not immunization is necessary for the development of skin hypersusceptibility. Again, the substance studied by us is strikingly similar to the substances studied by Dochez and Avery (6) in 1917 with regard to the pneumococcus and other organisms.

Dochez and Avery obtained a substance specifically precipitable in pneumococcus serum in young broth culture filtrates of pneumococci, in the blood and urine of rabbits experimentally infected with pneumococci, and in the blood and urine of pneumonia patients. Fornet (7), moreover, as early as 1906 observed substances precipitable with antityphoid sera, in the blood and urine of typhoid patients, possibly similar to the material of Dochez and Avery. Ascoli (8), too, found specifically precipitable materials in extracts of old and extensively putrefied spleen tissue of anthrax animals, the condition of the material indicating

that he may have worked with similar antigens. Moreover, work of considerable importance along these lines was done by Pick (9) as early as 1902. Pick found that when he filtered salt solution extracts of 3 day old agar cultures of typhoid bacilli, he obtained specifically precipitable materials which gave no biuret reaction and often no Millon reaction, and did not precipitate with tannic acid, picric acid, or uranium acetate. His material was heat-resistant and was not digested by pepsin or trypsin under suitable conditions.

It became necessary, therefore, to extend our observations, first to the problem of whether materials could be obtained analogous to those studied in the tubercle bacillus extracts from similar preparations of other bacteria of biologically varying species; to obtain more light upon the immunological, chemical, and physical properties of these substances; and to determine, if possible, whether animals infected with the respective organisms could be rendered specifically hypersusceptible to these bacterial derivatives.

The present communication represents the beginnings made in these directions.

I.

Specific Serum Reactions with "Residue" Material.

In order to include organisms as widely at variance with each other as possible, from a biological point of view, the experiments were carried out with tubercle bacilli, pneumococci, *Staphylococcus pyogenes aureus*, influenza bacilli, and typhoid bacilli.

Technique of Antigen Preparation.—The remarks in this section will be restricted entirely to the substances spoken of as the "proteose residue" in our last paper, but in designating which we think it wiser now to drop the word "proteose" and to speak of them only as "residue." The technique described below was uniformly followed in producing these substances, and this may be assumed to have been the manner of production throughout, unless special mention is made of modifications. Two distinct procedures were followed, one in which the bacteria were first grown in suitable broth for varying periods, the broth then filtered free of bacteria and subsequently treated exactly like the extracts described below. These broth preparations were used only in order to determine that the living bacteria rapidly secreted this substance into the broth, as had been found to be the case with tubercle bacilli. But since many of the alcohol-precipitable substances in such broth interfered with clean reactions, most of the experiments were done with extracts prepared as follows:

In order to obtain large masses of bacteria for extraction ordinary tin pie plates of two dimensions were employed, approximately 10½ and 11½ inches in diameter, the large one placed over the smaller one in the form of a Petri dish. The lower pie plate was lined with ordinary wrapping paper, simply by pressing the paper into the plate with another plate, putting them together like Petri dishes, and in this way autoclaving.[1] Hormone agar, blood agar, and chocolate agar were poured into these plates as into Petri dishes, incubated over night, and then used. The plates were inoculated with suspensions from agar slants, spread with glass spreaders, and the bacteria were grown, then collected by scraping off with glass spreaders in salt solution, and centrifugalized down. They were then washed, dried over sulfuric acid, and ground with marbles for from 2 to 3 weeks. With tubercle bacilli, staphylococci, and typhoid bacilli, the grinding seemed absolutely necessary for the obtaining of suitable yields of extracted material. With influenza bacilli and pneumococci, grinding was unnecessary. The ground bacteria were placed into shaking bottles in varying quantities of salt solution, alkalinized to a pH of about 9 to 9.4, and shaken for about 4 hours. They were then either immediately used for further preparation, or were neutralized to pH 7 to 7.4, and placed in the ice chest; the neutralization, as will be seen, was necessary in order to avoid deterioration due to possible racemization of the antigenic substances.

The further treatment, entirely analogous to the preparation of the tubercle bacillus extracts, was as follows: The material, after shaking, was centrifugalized in order to remove gross particles and then filtered through Berkefeld filters. This opalescent filtrate was precipitated by adding 10 per cent acetic acid, drop by drop, in the cold, until the further addition of acid no longer brought down a precipitate. In all cases a flocculent precipitate appeared which was removed by centrifugalization. This fraction is spoken of as either nucleoprotein or phosphoprotein, or both, and will be made the subject of chemical studies to follow. To the opalescent, still acid, supernatant fluid, a little more acid was added to make sure that nothing further came down, and normal NaOH was then added, drop by drop, and the mixture shaken, after each addition, in order to make sure that we had not, by adding excessive acid, redispersed some of the precipitable nucleoproteins by passing beyond the optimum precipitation point. The procedure was continued up to the neutral point, and as it was approached a precipitate usually came down which was rich in phosphates. When the precipitate appeared it was filtered off and any turbidity appearing during the process was in each case removed by filtration. The preparation was then acidified and boiled at the acid reaction for from 3 to 5 minutes. In many instances hardly any coagulable proteins came down. In some, especially with typhoid bacilli, appreciable turbidity developed; this was then removed by filtration through a Berkefeld filter. The filtration was carried out while the material was hot and the filtrate

[1] Lately large glass Pyrex pie plates with tin covers have been found to be more convenient.

was allowed to cool after filtration. As stated in the previous paper, occasionally a very slight haze perhaps due to Bence-Jones protein appeared. Whenever this appeared, it was again removed by filtration.

The resultant material was water-clear and was now neutralized before use, to approximately pH 7; a moderate precipitate again developed which was shown to consist largely of phosphates. This again was removed.

This final material is the so called residue with which we are dealing.

Chemical tests were done with sufficient frequency to show that experiments such as those outlined below could be obtained with every organism used with materials which reacted negatively to the biuret, Hopkins-Cole, Millon, and sulfosalicylic acid protein tests.

All these antigens gave precipitation turbidity with ten volumes of absolute alcohol, which varied in intensity in different preparations. It is not impossible that the alcohol-precipitable material is merely incidental, carrying down the antigen material with it. We say this because antigenic potency did not quantitatively parallel alcohol precipitation.

One of the great difficulties in characterizing the substances studied is the possibility that we were dealing with minute quantities of true protein, too slight to be detected chemically, but still sufficient to give biological reactions. This can be conclusively determined only by actual chemical analysis, a task which has so far been rendered difficult by the very small actual quantities available. It will be interesting to note in this connection that, in several cases, we compared the biuret reaction and alcohol precipitation of antigens which gave powerful specific reactions, with dilutions of horse serum in which the protein concentration was about 1:1,000. In such cases the horse serum solution, which still gave a strong biuret reaction, gave a much fainter alcohol precipitation than the residue antigen, which no longer gave a biuret, a fact which indicates, at least indirectly, that most of the material that comes down with the alcohol is not biuret-reacting protein. Protocols are given below of experiments by which the antigenic properties and the specificity of residue antigens prepared as above, from various bacteria, were investigated.

Experiment 1. Specific Precipitation of Residue Antigens with Specific Antisera.—

Serum.	Residue.	Precipitation.	
Influenza Serum 103	Influenza.	+++ in 3 min.	++++ in 15 min.
	Pneumococcus.	0	
	Staphylococcus.	0	
	Typhoid.	0	
	Tubercle.	0	
Pneumococcus serum (horse).	Influenza.		0
	Pneumococcus.	+++ in 3 min.	++++ in 15 min.
	Staphylococcus.		0
	Typhoid.		0
	Tubercle.		0
Staphylococcus Serum 55	Influenza.		0
	Pneumococcus.		0
	Staphylococcus.	++ in 5 min.	++++
	Typhoid.		0
	Tubercle.		±
Normal rabbit "	Influenza.	0	
	Pneumococcus.	0	
	Staphylococcus.	0	
	Typhoid.	0	
	Tubercle.	0	
" horse "	Influenza.	0	
	Pneumococcus.	0	
	Staphylococcus.	0	
	Typhoid.	0	
	Tubercle.	0	

Typhoid serum and tubercle serum were not included in this series because of the weakness of the sera available at the time. It is unnecessary to duplicate experiments of this kind since they were done again and again with results entirely alike in significance, though with some sera a very faint non-specific reaction was occasionally obtained, always so faint that it did not interfere with correct interpretations.

Experiments with broth filtrates similarly treated are not given since they were essentially the same, except that specificity was not so sharp. The broth filtrate method for general work was abandoned after the appearance of these substances in the broth had been definitely shown.

Experiment 2. Experiment to Determine Whether the Alcoholic Precipitate Really Carries the Specific Precipitable Material.—This experiment was performed because of the occasional heavy serum reaction with material giving a relatively faint alcoholic precipitate.

2 cc. each of pneumococcus, influenza, and staphylococcus residue antigens were precipitated with ten volumes of absolute alcohol, centrifugalized, dried, and taken up in one-half isotonic salt solution—isotonicity controlled on washed sheep cells.

Antigen.	Serum.		Precipitation.
Pneumococcus antigen precipitated with alcohol and redissolved.	+	pneumococcus serum.	++++ immediate.
		influenza "	±
		staphylococcus "	−
Staphylococcus antigen precipitated with alcohol and redissolved.	+	pneumococcus "	±
		influenza "	±
		staphylococcus "	++++
Influenza antigen precipitated with alcohol and redissolved.	+	pneumococcus "	−
		influenza "	++++
		staphylococcus "	−

It is noticeable in this experiment that after alcohol precipitation and resolution the reactions are, if anything, more sharp and specific than before.

Having determined the precipitability of these residue antigens, we next investigated the question of their complement-fixing powers. In regard to the tubercle bacillus, complement fixation by these residues is being studied in our laboratory and at Saranac by Petroff who will report this phase of the work in a subsequent publication.

Experiment 3. Experiment to Determine the Complement-Fixing Properties of the Residue Antigen.—The quantities given in this experiment are based upon a considerable number of titrations, these being necessary since we had, of course, no significant quantitative measure for the amounts of antigen present in the final residues and since the antibody contents of the various sera must have varied enormously. The obvious technical description of complement fixation is omitted. Complement was always titrated in the usual way and about 1.5 to 2 units employed. Preliminary incubation was always for 1 hour, and readings were made by constant observation after the addition of the sensitized cells. ++++, as usual, signifies complete fixation.

Serum.	Antigen.	Amount of antigen.	Complement fixation. Residue antigens.	
		cc.	*20 min.*	
Influenza Serum 520, 0.025 cc.	Influenza.	0.01	++++	
	Staphylococcus.	0.05	0	
	"	0.01	0	0.3
	Typhoid.	0.1	0	1 to 10 compl.
	"	0.2	0	± 2 units.
	Tubercle.	0.1	0	
				1 hr.
Staphylococcus serum (rabbit) 0.025 cc.	Influenza.	0.01	0	0
	Staphylococcus.	0.05	++++	++++
	"	0.01	+++	+++
	Typhoid.	0.1	+	+
	"	0.2	+++	+++
	Tubercle.	0.1	++++	++++
Typhoid Serum 1043 (rabbit) 0.05 cc.	Influenza.	0.01	0	0
	Staphylococcus.	0.05	0	0
	"	0.01	0	0
	Typhoid.	0.1	++++	+++
	"	0.2	++++	++++
	Tubercle.	0.1	++++	++++
Tubercle bacillus serum (sheep) 0.05 cc.	Influenza.	0.01	0	0
	Staphylococcus.	0.05	0	0
	"	0.01	0	0
	Typhoid.	0.1	±	0
	"	0.2	±	0
	Tubercle.	0.1	++++	++++
Normal rabbit serum 0.025 cc.	Influenza.	0.01	0	
	Staphylococcus.	0.05	0	
	"	0.01	0	
	Typhoid.	0.1	0	
	"	0.2	+	
	Tubercle.	0.1	+	Complete hemolysis in
Normal sheep " 0.05 cc.	Influenza.	0.01	0	12 min.
	Staphylococcus.	0.05	0	
	"	0.01	0	
	Typhoid.	0.1	0	
	"	0.2	0	
	Tubercle.	0.1	0	

In this experiment some non-specificity is apparent. The staphylococcus serum, while it fixed with staphylococcus in quantities as low as 0.01 cc., fixed with larger quantities of typhoid and tubercle antigen. It also gave a ++++ fixation with tubercle antigen. However, there was a + fixation only of this serum with as much as 0.1 cc. of typhoid antigen. The typhoid serum also fixed with the tubercle antigen. It is noteworthy that the tubercle bacillus serum did not fix with either the typhoid or the staphylococcus antigen.

It is not at all unlikely, as subsequent modifications of technique have shown, that these non-specific reactions may be eliminated by more careful quantitative determinations. With the influenza antigen we never had the slightest difficulty, since it was always absolutely specific, in some experiments fixing in quantities as low as 0.001 cc. of the residue which gave only a slight turbidity on alcohol precipitation. This experiment with all its defects is cited since it illustrates the kind of irregularity to be expected, and yet definitely indicates that the principle of the presence of specific antigenic substances in the residues may be maintained.

A number of similar experiments were carried out with redissolved alcoholic precipitates of the residue materials as antigen. In order to save space the experiments are not cited in protocol, but we may state that in an experiment carried out on May 24, 1922, in which the same sera were used and the redissolved alcoholic precipitates of the same antigens employed in the above experiment were used, results entirely analogous to those above cited were obtained, except that the non-specific fixation of the tubercle antigen with the staphylococcus rabbit serum was to a large extent eliminated.

It is important to mention in passing that we have noticed in a number of cases that, in spite of uniform lack of either antihemolytic or hemolytic properties on the part of the antigen, the smaller amounts of antigen, that is amounts of 0.05 and 0.01 cc., gave better fixation than the larger amounts. This cannot be explained at present and must be subjected to further investigation.[2]

[2] It is also of the utmost importance that we should mention our uniform failure to obtain fixation with the pneumococcus residue and pneumococcus horse or bovine serum, in spite of many attempts and in spite of the fact that the pneumococcus antigen was heavily precipitated by both these sera. This might seem to contradict the opinions expressed by one of the writers in a recent publication as to the identity of the complement-fixing and precipitating antibodies and, of course, will be investigated. But it has been found in all these experiments that both the horse and bovine antipneumococcus sera used, possessed to a powerful degree the auxilytic effect described in former years by Manwaring (Manwaring,

II.

Experiments on the Stability of the Residue Antigen.

Early in the experiments the fact that boiling in acid did not destroy the skin-reactive capacity of the residue antigen from tubercle extracts led to the further investigation of the stability of these antigenic substances as obtained from other bacteria. Residue materials were collected from the various organisms which were being studied, and were subjected to serological test to determine their specific reaction capacities. A number of experiments were then carried out to ascertain whether or not they were destroyed by the application of heat at various hydrogen ion concentrations. The following experiment illustrates the first method carried out for this purpose.

Experiment 4. Stability of the Antigenic Material on Boiling in Alkaline and Acid Reactions.—All the antigens described above were precipitated against specific and heterologous sera and found to give specific precipitates. In every case, two 5 cc. fractions of each of the antigens were placed into small 50 cc. Erlenmeyer flasks and brought, respectively, to a pH of 5.4 and 9.4. Each specimen was then boiled in a reflux condenser for 1 hour and at the end of this time they were titrated to ascertain that the pH had not changed and precipitates, which developed in almost every case and will be noted specifically below, were then removed by rapid centrifugalization until the supernatant fluid was clear. These were then brought to pH 7 to 7.2 by careful titration, and precipitation tests were set up. The above description applies to each of the experiments below.

W. H., *Centr. Bakt., 1te Abt., Orig.*, 1906, xlii, 75), an effect like the conglutinin phenomenon of Bordet and Gay (Bordet, J., and Gay, F. P., *Ann. Inst. Pasteur*, 1906, xx, 467), and this is most likely to account for the lack of parallelism between complement fixation and precipitation in these cases. The larger the amount of serum used, the more prompt was the hemolysis, although the serum itself had no sensitizing effect upon red blood cells. This point seems to be of sufficient theoretical importance to be investigated as soon as we can produce a sufficiently strong antipneumococcus rabbit serum and analyze the apparent conglutinin effect of several specimens of antipneumococcus horse serum which we have noticed and which is particularly curious in face of the fact that, so far as we know, conglutinins have not been described in horse serum.

Comparative Precipitation Tests on Staphylococcus Antigens, Original and Boiled for 1 Hour at pH 9.4 and 5.4.

Material.	Precipitation.
Staphylococcus antigen (original) + staphylococcus serum.....	++++
" " " + influenza "	−
" " " + pneumococcus "	+ to ++
" " (boiled 1 hr. at pH 9.4) + staphylococcus serum..	−*
" " (" 1 " " pH 9.4) + influenza "	−*
" " (" 1 " " pH 9.4) + pneumococcus "	+ to ++*
" " (" 1 " " pH 5.4) + staphylococcus "	+++
" " (" 1 " " pH 5.4) + influenza "	−
" " (" 1 " " pH 5.4) + pneumococcus "	+

* This was repeated with like result.

This experiment shows that the antigenic properties of the staphylococcus substance are destroyed by prolonged boiling (perhaps racemized) at pH 9.4, but remain intact for 1 hour at an acid reaction of 5.4. The slight non-specific reactions are not effected by this treatment.

Comparative Precipitation Tests on Influenza Antigens, Original and Boiled for 1 Hour at pH 9.4 and 5.4.

Material.	Precipitation.
Influenza antigen (original) + influenza serum.....	++++
" " " + pneumococcus serum.....	+ to ++
" " " + staphylococcus "	−
" " (boiled 1 hr. at pH 9.4) + influenza serum.....	−
" " (" 1 " " pH 9.4) + pneumococcus serum.....	+
" " (" 1 " " pH 5.4) + influenza "	++++
" " (" 1 " " pH 5.4) + pneumococcus "	± to +

This series shows that, as with the staphylococcus antigen, boiling in an alkaline reaction destroys antigenic properties of the influenza substance, while similar treatment with acid has no effect.

Comparative Precipitation Tests on Pneumococcus Antigens, Original and Boiled for 1 Hour at pH 9.4 and 5.4.

Material.	Precipitation.
Pneumococcus serum + original pneumococcus antigen.....	++++
" " + pneumococcus antigen at pH 9.4 over night.....	++++
" " + " " " " pH 9.4 in reflux condenser boiled 1 hr.....	++++
" " + pneumococcus antigen at pH 5.4 in reflux condenser boiled 1 hr.....	++++

This series shows that the pneumococcus substance, like the tubercle bacillus material, is more resistant than either the staphylococcus or the influenza residue.

Having thus shown that boiling in a reflux condenser[3] for 1 hour at an acid reaction does not materially change any of the antigens employed, but that boiling at pH 9.4 destroyed the specific precipitability of the influenza and staphylococcus antigens, we carried out similar experiments in which the severity of the procedure was increased by subjecting the residue antigens to autoclave digestion. The two antigens were used which were apparently the most susceptible to boiling with alkali to see whether in an acid reaction which did not affect them after boiling in a reflux condenser, they could be destroyed under conditions of increased pressure.

[3] During the process of boiling in a reflux condenser both in the acid and alkaline specimens, but chiefly in the acid, rather heavy precipitates came down. These were removed by centrifugalization and proved by the molybdate test to contain copious phosphates. Removal of these precipitates did not seem to detract in any way from the specific precipitability of the material, and it suggests the possibility of, in this way, further purifying the antigen. In the experiment with the staphylococcus antigen boiled 1 hour at pH 9.4 the suspicion arose that possibly the antigenic properties which had been removed from the solution might have come down with the precipitate. For this reason, the precipitate was redissolved in salt solution and tested against serum, but with negative results, showing that this was not merely a precipitation of antigenic properties. It was also to be noted that, although alkaline hydrolysis removed antigenic properties from the influenza and staphylococcus antigens, it did not change the slight non-specific reactions which these antigens gave with the pneumococcus horse serum.

Experiment 5. Effect of Autoclave Digestion on the Precipitin and Complement-Fixing Reactions of the Influenza and Staphylococcus Antigens.—Antigens used on the previous day, Influenza Residue A and staphylococcus residue, were set up for precipitin and complement-fixing reactions in the morning. The residues of these antigens, plus pneumococcus antigen, were brought to the following reactions, the divergence in pH being due to the necessity of working with small quantities and letting well enough alone: influenza 5.4, pneumococcus 5.8, and staphylococcus 5.2. These materials were then autoclaved at three atmospheres for 1 hour, the actual three atmospheres being at 145°C. for 40 minutes. They were removed from the autoclave, neutralized from pH 7.2 to 7.4, and precipitation tests were again set up, also complement fixation tests, as follows:

Precipitation Tests.

Material.	Precipitation.	
	Before autoclaving.	After autoclaving.
Normal Serum 1 + Influenza Residue A	0	0
" " 1 + staphylococcus residue	+	0
" " 2 + Influenza Residue A	0	0
" " 2 + staphylococcus residue	±	0
Influenza " 520 + Influenza Residue A	++++	0
" " 520 + staphylococcus residue	+	0
Staphylococcus Serum 55 + Influenza Residue A	0	0
" " 55 + staphylococcus residue	+++	++

Complement Fixation Tests.

Residue antigens.	Influenza Serum 520, 0.025 cc.	Staphylococcus Serum 55, 0.025 cc.	Normal Rabbit Serum 1, 0.025 cc.	Normal Rabbit Serum 2, 0.025 cc.
Before autoclaving.				
0.01 cc. of Influenza Antigen A	++++	0	0	0
0.001 " " " " "	++++	0	0	0
0.1 " " staphylococcus antigen	0	++++	±	0
0.01 " " " "	0	+	0	0
After autoclaving.				
0.01 cc. of Influenza Antigen A	0	0	0	0
0.001 " " " " "	0	0	0	0
0.1 " " staphylococcus antigen	0	++++	0	0
0.01 " " " "	0	++	0	0

This experiment, then, illustrates the fact that apparently the more delicate antigen, the influenza one, is altered under a pressure of three atmospheres in an acid reaction, whereas the staphylococcus antigen, being more resistant, is slightly diminished in antigenic potency but not destroyed.

A similar experiment, in which the pneumococcus and the staphylococcus residues were used, is the following.

Experiment 6. Effect of Autoclave Digestion on the Precipitin Reaction of the Staphylococcus and Pneumococcus Antigens.—Staphylococcus and pneumococcus residues prepared 5 days previously were precipitated against the respective sera as follows:

Staphylococcus serum +	staphylococcus residue ++++ within a few minutes; strong ring formation.
	pneumococcus residue negative after 2 hours.
Pneumococcus serum +	staphylococcus residue negative after 2 hours.
	pneumococcus residue ++++ almost immediately; powerful ring formation.

These antigens, about 2.5 cc. of each, were acidified with a drop of 10 per cent acetic, and now turned litmus strongly red. Not titrated because available volume too small. Autoclaved at four atmospheres for 40 minutes.

Neutralized by careful addition of weak soda till both about pH 7.4.

Again set up as above with like result except that the precipitin with the homologous staphylococcus tube comes out much more slowly and weakly than before autoclaving; takes about 10 minutes to be distinct, but then gradually deepens. That in the pneumococcus tube comes out as rapidly and distinctly as before. Specificity apparently not impaired by the autoclave treatment.

This experiment shows that in an acid reaction the pneumococcus residue is not altered to the slightest degree by subjection to a pressure of four atmospheres for 40 minutes in an autoclave.

Similar experiments were done with these antigens on repeated occasions and in general it was shown that the precipitability and specificity of the pneumococcus and tubercle bacillus residues were not affected by acid and autoclaving; the staphylococcus residue, however, was definitely altered and the influenza residue was completely deprived of its antigenic properties by similar treatment.

These experiments, as well as observations upon the preservation of the antigens in the ice box, indicated that, whereas the residues were all extremely resistant to treatment by heat in acid reactions, they were to some degree susceptible to similar treatment when the reaction was alkaline. The suggestion that racemization might account for this is, of course, an obvious one, and for this reason

an experiment was done in which the antigens were rendered alkaline to a pH of 9.4, and preserved in this condition in the ice chest and tested from day to day.

Experiment 7. Rapidity of Deterioration of the Residue Antigens at pH 9.4 at Low Temperatures.—Antigens of influenza, pneumococcus, staphylococcus, and tubercle bacilli prepared as usual by alkaline extraction, subsequent precipitation of acid-precipitable material, filtration, boiling in acid state, and refiltration. All of these brought to pH 7 to 7.2, and precipitated as follows:

Material.	Precipitation.
Influenza serum + influenza antigen.....	++++
" " + pneumococcus antigen.....	−
" " + staphylococcus "	−
" " + tubercle "	−
Pneumococcus serum + influenza "	+ to ++ slow.
" " + pneumococcus antigen.....	++++ immediate.
" " + staphylococcus "	+ to ++ slow.
" " + tubercle "	Not done.
Staphylococcus " + influenza "	−
" " + pneumococcus "	−
" " + staphylococcus "	++ to +++ slow.
" " + tubercle "	Not done.

Tubercle bacillus serum not done with other antigens because too little serum available. This controlled in another experiment done on same day.

Tubes of each antigen brought to pH 9.4, and set away in ice chest. Specimens removed at 24 hour intervals on May 17, 18, and 19, 1922, 12, 24, 48, and 72 hours, respectively; such specimens in each case brought to pH 7 and 7.2 and precipitations done.

May 17. After 24 hours all precipitated.

May 18. After 48 hours influenza antigen no longer reacted, but all others still active as before.

May 19. After 72 hours, staphylococcus, pneumococcus, and tubercle antigens still precipitable and specificity tests done and found as above.

May 22. After 6 days tubercle and pneumococcus antigens again tested and are still precipitable by their respective sera, not noticeably less than at first. (No staphylococcus antigen left.)

This experiment, then, shows that the influenza antigen deteriorates within 48 hours at a reaction of pH 9.4, whereas neither pneumococcus, tubercle bacillus, nor staphylococcus is affected within 74 hours by such treatment, and the tubercle antigen, as well as the pneumococcus,

as was to be expected, retained activity for as long as 6 days. It is apparent that except for the influenza antigen, alkali has no very powerful deteriorating effect upon the residues. Whether or not the powerful effect of alkali upon the influenza, and its definite but less powerful effect upon the staphylococcus residue are due to a possible racemization, we cannot say until we can work with larger quantities of these materials.

Does the Residue Antigen Incite Antibody Formation in Animals?

It is thus plain that the residue of bacterial extracts from which all coagulable proteins have been removed as completely as this can be done, is still capable of reacting specifically with antisera and that this is true of a considerable number of different bacteria of widely varying biological properties. As far as the pneumococcus is concerned, we believe that the substance described by Dochez and Avery is the same which we have isolated by extraction of the bacterial bodies and that it is analogous to the tuberculin substance that we obtained by this method last year and with which we carried out the work on skin reactions then reported.

These residue substances, then, are antigens at least in the sense that they will react with specific antibodies. Are they antigens also in the sense that they may induce the formation of antibodies? This, of course, was a very obvious line of inquiry and we can briefly summarize our knowledge, in this regard, by stating that up to the present time no one in our laboratory has succeeded in producing specific antibodies by the injection of the residue antigens. From this we know definitely that the residue antigens do not induce antibody formation when injected into animals with the same ease with which this can be accomplished with whole bacteria or bacterial extracts. Until more extensive animal immunization with these materials has been attempted, we would not venture to claim that antibodies are not induced by them at all. It may be, of course, that up to the present time we have not been able to inject sufficiently large quantities. On the other hand, our failure may be due to the rapid elimination of these substances in the urine, as with the pneumococcus substances described by Dochez and Avery. Attempts to induce antibody for-

mation are being continued. Again, it is at least worth speculating upon the possibility that these substances may represent the so called "*Haptenes*" foretold by Landsteiner (10) and characterized as capable of reacting with antibodies without being able to induce antibody formation. In a recent paper on the chemical modification of proteins and the antigenic alterations so obtained, Landsteiner speaks of such substances, and suggests in his final reasoning that tuberculin may fall into this category. It is at least possible that in the materials described above we are dealing with substances of this kind.

It is not impossible that the work of Meyer (11), who observed that certain tapeworm lipoids were incapable of inducing complement-fixing antibodies on injection into animals, although they furnished a good antigen for specific complement fixation, is pertinent in this connection. This, however, is not certain because in reactions in which lipoidal materials were used we may be dealing with phenomena comparable to the Wassermann reaction. However this may be, there seems to be very little doubt about the complete correspondence of our results with those of Pick (9). Pick mentions the fact that the biuret-free bodies obtained by trypsin digestion of a typhoid extract, which were still precipitable by specific serum, seemed to have lost their property of antibody formation upon injection into the animal, and he (9) states in unqualified terms that he believes that the properties of inducing antibody formation in the body and the phenomena of specific precipitability *in vitro* may not be entirely parallel attributes.

III.

Experiments on Animals.

Since, from experiments like those cited above, it was plain that the residue antigens of bacteria could be specifically precipitated and were specifically capable of fixing complement with antibodies, it was necessary to see whether such specificity extended to their reactions with the infected animal body. The experiments in this direction are not complete and are being confirmed, but it has seemed wise to add to this paper information along these lines as far as we have obtained it up to the present time.

The first question we attempted to answer was whether specific residue antigens prepared from bacteria other than the tubercle bacillus would give reactions with tuberculous animals which were sensitive to the tubercle bacillus residue. The following experiments are examples of a considerable number done in order to elucidate this point.

Experiment 8. Specifity of Skin Reactions Tested with Tubercle Bacillus Residue.—The antigens used in this experiment had all been previously tested by precipitation and complement fixation and had been found to be specific. They all consisted of residues prepared by the general method described at the beginning. They were intracutaneously injected in quantities of approximately 0.1 cc. into each of four guinea pigs, two tuberculous animals and two normals. The results were as follows:

Animal No.	Antigens.			
	Tubercle.	Staphylococcus.	Influenza.	Pneumococcus.
Tuberculous guinea pigs.				
1	+++	−	−	±
2	+++	−	−	−
Normal controls.				
3	−	++	−	++
4	±	+	±	−

A similar experiment performed under conditions identical with the above gave the following results.

Experiment 9. Specificity of Skin Reactions Tested with Tubercle Bacillus Residue.—In this experiment the residue antigens used for complement fixation on the preceding day were used as prepared for tubercle bacilli, influenza bacilli, pneumococci, and staphylococci, respectively. They were intracutaneously injected in quantities of approximately 0.1 cc. into each of five guinea pigs, two injections being given under either flank. The results were as follows:

Animal No.	Antigens.			
	Staphylococcus.	Tubercle.	Influenza.	Pneumococcus.
Tuberculous guinea pigs.				
5	−	++	−	++
6	−	++	−	+
Normal controls.				
7	−	−	−	++
8	−	−	−	+++
9	−	−	−	++++

It is apparent from these experiments and others like them that the general specificity of the antigens holds good in the animal body as well as *in vitro*. The tuberculous animals, while distinctly susceptible by intradermal test to the tubercle bacillus residue, do not react specifically to antigenic residues from other bacteria. It is interesting to note also that the pneumococcus residue antigen possessed, in these experiments as in some others, a not inconsiderable degree of non-specific toxicity for normal animals, and that to a very slight degree this was also true of the staphylococcus antigen in a few animals. This point will be referred to later.

The next point of importance was to find out by actual experiment whether the converse of the above conditions was true; namely, whether specific skin hypersusceptibility to residue antigens could be induced in guinea pigs infected with the other bacteria mentioned above.

To a considerable extent an affirmative answer to this question has been furnished by the work of previous investigators, particularly of recent years by the important contributions of Fleischner and Meyer (12), and of Fleischner, Meyer, and Shaw (5). It has, of course, been known for a long time that there is a striking analogy between the tuberculin and the mallein reactions, mallein being produced in a manner analogous to that of old tuberculin and very probably containing physically and chemically comparable active substances. They found that the laws governing such reactions in *abortus*-infected animals were completely analogous to those revealed for tuberculous guinea pigs, especially by Baldwin and Krause; namely, that the reaction is associated with actual infection and that even very intensive immunization with dead bacilli or the proteins derived from them never induced typical skin reactions. In some of their tests the material used for intradermal injection consisted of 8 week glucose broth

cultures of the organisms heated to 100°C. for 1 hour, a procedure which makes it likely that their materials were analogous to our own. In a later communication, Fleischner, Meyer, and Shaw (5) add a considerable number of their own experiments to a valuable review of skin hypersusceptibility in which they again note this difference in regard to skin hypersusceptibility between infected and immunized animals, not only for *B. abortus bovinus*, but for the typhoid bacillus, in the case of which they sometimes obtained temporary cutaneous hypersensitiveness after the intravenous injection of living bacilli.

This universal agreement in regard to the differentiation between cutaneous hypersensitiveness and true anaphylaxis, with our own analysis of the conditions in tuberculosis, would seem to indicate that a generalization of our ideas concerning the specific susceptibility of infected animals to the residue materials might almost be justified. In spite of this close analogy, however, we determined to continue the experiments in the hope of obtaining specific skin hypersensitiveness to the residue antigens in animals infected with the various organisms employed in the general investigation.

The great difficulty in such experiments is the fact that, while it is quite easy to obtain subacute and chronic infections in guinea pigs with tubercle bacilli, it is a difficult task to produce similar reasonably prolonged infections in these animals with the other bacteria. The animals in most cases either recover rapidly, or die acutely within a few days, and if repeatedly injected with small doses they are apt to become immune. Moreover, when a noticeable degree of skin hypersusceptibility in such infections is attained, it is apt to be more short lived than the state of anaphylactic protein hypersusceptibility, a point that Meyer mentions, that appears from Krause's work concerning the parallelism between the activity of a focus and skin sensitiveness, and which also was apparent in some of our own work. We may mention in passing that in our first paper, recognizing the apparent dependence of skin hypersusceptibility to actual infection, we attempted to imitate the conditions of absorption from an active focus of bacteria by treating guinea pigs with large injections of nucleoproteins, hoping that the gradual breaking down of these materials might sensitize in a manner similar to that obtained in the infected animal. More recently, with Petroff, we have carried on such studies, both with the nucleoprotein fraction and with killed tubercle bacilli,

with degrees of success which will be published later. In these experiments, too, the transitory nature of the typical skin reactiveness has been noticed. The reactions, therefore, must be done at a very definite time in order to yield positive results.[4]

We tried a great many different methods of producing infections in guinea pigs. None of these methods, however, have been uniformly successful and it would, therefore, needlessly prolong our communication to go into details about all of them. The following experiment is perhaps the best so far obtained in which specific intracutaneous reactions observed to staphylococcus residue material were obtained in guinea pigs infected with *Staphylococcus pyogenes aureus*. In these staphylococcus experiments the method employed was a modification of that described by one of the writers with Raymond some time ago (13). Small celloidin capsules were filled with staphylococcus-infected agar; small holes were left in the capsules which were then dropped into the peritoneal cavity of the animal.

The following is one of the experiments done with animals prepared in this way.

Experiment 10. Specificity of Skin Reactions Tested with Staphylococcus Residue. —June 29, 1922. Six guinea pigs were operated upon under ether anesthesia and capsules filled with agar, into which staphylococcus had been inoculated and in which a considerable number of holes had been left, were tucked into the peritoneal cavity. Three of these guinea pigs died between June 29 and July 10. July 10. Skin reactions were done on survivors, as well as on tuberculous and normal controls. These tests were repeated on July 13, readings in each case being made after 24 hours.

The staphylococcus material used in this experiment was made from staphylococcus powder that was quite old and apparently had begun to putrefy. It had

[4] We would like to call attention here to a curious fact which has caused us a great deal of difficulty and may be of interest to others working with skin reactions in guinea pigs. We have found that when very young guinea pigs were used, animals under 250 gm., intracutaneous reactions of all kinds were apt to give irregular results. Whether this is due to the difficulty of applying the dose entirely into the superficial layers of the skin in thin skinned animals, or whether there is a physiological reason for these inconsistencies, we cannot say. Bacteriologists engaged in the titration of diphtheria antitoxin by the Römer method have told us that they have noticed similar irregularities in young guinea pigs and are using, habitually, animals of 350 to 400 gm. or over for intracutaneous work.

an unpleasant odor and in solution was alkaline. However, prepared in the usual manner, it precipitated powerfully with staphylococcus sera.

Animal No.	Tests of July 10, 1922.		Tests of July 13, 1922.	
	Staphylococcus antigen.	Tubercle antigen.	Staphylococcus antigen.	Tubercle antigen.
Staphylococcus guinea pigs.				
10	+ strong.	−	++	−
11	+++	−	+ strong.	−
12	+ to ++	−	July 11. Died.	
Control tuberculous guinea pigs.				
13	+	++	−	+++
14	+	++ to +++	+	+
Normal controls.				
15	−	−	±	−
16	++ (no redness; diffuse area of edema).	−	−	−

In these experiments, then, a distinct but relatively feeble reaction to the staphylococcus residue antigen was obtained in some staphylococcus-infected guinea pigs about 11 days after injection. The animals were very sick at this time and our general impression was that the reactions were done too late. On July 14, the first two staphylococcus guinea pigs listed in this series were each given 2 cc. of residue antigen intraperitoneally, 4 cc. being given at the same time to two normal controls somewhat lighter in weight than the staphylococcus guinea pigs. The staphylococcus animals were quite sick, but did not die.

The next experiment is similar in significance to the above, except that because of the feebleness of the reactions above indicated, we determined to test not only with staphylococcus residue antigen, but with the nucleoprotein fraction as well. In the work with tuberculosis although the residue antigen gave excellent skin reactions, somewhat stronger reactions had always been obtained by using the nucleoprotein fraction and it had never been possible to free this nucleoprotein fraction of its skin-reactive properties, however frequently it was precipitated and redissolved. The relationship between this nucleoprotein fraction and the residue is not clear. We

believe from certain experiments which will be reported separately that one possible explanation may be the fact that the nucleoprotein fraction represents the mother substance of the residue. This is a question which we cannot enter into in the present connection. Thinking, however, that perhaps the animals were but feebly sensitized and that a more powerful reaction substance might bring out a more definite result, we used both redissolved staphylococcus nucleoprotein and residue antigen in the next experiment, as follows:

Experiment 11. Specificity of Skin Reactions Tested with Staphylococcus Residue and Staphylococcus Nucleoprotein.—

Animal No.	Tests of July 17, 1922.			Tests of July 24, 1922.	
	Staphylococcus residue.	Staphylococcus nucleoprotein.	Tubercle residue.	Staphylococcus residue.	Staphylococcus nucleoprotein.
Staphylococcus guinea pigs; capsule inserted June 29, 1922.					
17	−	+	−	July 22. Died.	
18	−	+++	−	−	−
Capsules inserted July 12, 1922.					
19	+	+++	−	−	−
20	+	+++	+	+++	+++
21	−	+	−	July 20. Died.	
Tuberculous guinea pigs.					
22	+	+	++		
6	−	+	++++		
Normal controls.					
23	±	±	+		
24	−	+	+		
No. 25. Staphylococcus guinea pig; capsule inserted July 20, 1922.				−	−
No. 26. Normal guinea pig.				−	−

Whatever may be the relationship between the nucleoprotein fraction and the residue, a problem for which we have not yet a definite answer, this experiment shows that, just as in tuberculous

guinea pigs, the nucleoprotein fraction gives a more powerful response than does the residue antigen, but in one staphylococcus guinea pig (No. 20) a definite residue antigen response was obtained 12 days after the capsule was inserted. This same guinea pig gave a staphylococcus nucleoprotein reaction a week earlier than this when the residue reaction was very faint. This, too, would indicate that the nucleoprotein reaction was due to its possible contents of the residue material rather than representing a true nucleoprotein reaction, since, in that case, it would mean active sensitization in 5 days. However, this question needs further investigation. Fig. 1 represents a drawing made of Guinea Pig 20 on July 24, showing what we consider to be +++ reactions with the staphylococcus residue and nucleoprotein antigens.

We add one other experiment in which apparently specific reactions were obtained in guinea pigs sensitized by injection of living typhoid bacilli. It is necessary to state that this is the only frankly positive experiment of three made with the typhoid bacillus; but it seems worth citing because the result was definite, and until we find a better method of rendering the skin of guinea pigs reactive to typhoid bacilli, it will not be easy to multiply these results.

Experiment 12. Specificity of Skin Reactions Tested with Typhoid Residue.— Four guinea pigs ranging from 300 to 450 gm. were injected intracutaneously with one-twentieth of a slant of the Rawlings typhoid strain on May 11, 15, 19, 23, and 29, 1922. June 6. These guinea pigs, together with two normal controls, were intracutaneously injected with the residue antigen of tubercle bacilli frequently described in these communications and a similar residue antigen of typhoid bacilli made in the identical manner.

Animal No.	Tests on June 7, 1922, a.m.	
	Typhoid residue antigen.	Tubercle residue anti gen.
Typhoid guinea pigs.		
27	+ to ++	−
28	±	−
29	++	−
30	+++ to ++++	−
Normal controls.		
31	+	−
32	±	−

This experiment seemed to show definitely that it is possible by repeatedly inoculating guinea pigs with living typhoid bacilli to produce a condition in which they will react by specific skin reactions to typhoid residue antigen and remain normal to similar injections of the tuberculin antigen. Fig. 2 is a drawing made from the tests on Guinea Pig 30 of this experiment.

As stated above, the work on the production of specific skin hypersusceptibility to residue antigen in guinea pigs is still going on and it will be reported more extensively at a later date. The above experiments have been added to this paper, however, in order to indicate the general trend of the work and to bring the subject up to date, as far as we have been able to investigate it.

SUMMARY.

When filtered alkaline extracts of pulverized bacteria of several varieties are precipitated with acid in the cold, boiled with acid, and all materials thrown down by these procedures removed, there remains a small amount of an alcohol-precipitable material which no longer gives any of the ordinary chemical reactions for proteins, such as the biuret, Hopkins-Cole, Millon, and sulfosalicylic acid reactions. The only protein reaction usually given by this material is a very weak xanthoproteic reaction. Nevertheless, the material, which is, as far as we can determine at present, free from coagulable protein, is specifically precipitable by homologous antiserum and gives specific complement fixation reactions.

Such material can also be obtained from organisms like the influenza bacillus, pneumococcus, and meningococcus by extraction without preliminary grinding of the bacteria, and is present in filtrates of young and old broth cultures of the organisms.

We believe that these acid- and heat-resistant antigenic materials are analogous to tuberculin and to the pneumococcus substances with which Dochez and Avery (6) made their observations some years ago.

The stability of these substances is considerable and was investigated particularly because we thought this represented an indirect method of eliminating the possibility of their protein nature. In all cases boiling in a reflux condenser at an acid reaction ranging from pH 5 to 6 for 1 hour failed to destroy the antigenic specificity of the

residue antigens. After such treatment satisfactory and specific precipitation reactions could be obtained. Similar boiling in alkaline reactions, however, destroyed the precipitability of staphylococcus and influenza residues. Subjected to autoclave digestion at an acid reaction of pH 5.4 for 1 hour at from three to four atmospheres, none of the antigenic residues investigated, except that obtained from the influenza bacillus, were destroyed. The pneumococcus and tubercle bacillus residue antigens were resistant to boiling for 1 hour, both in acid and alkaline reactions (pH 5.4 and 9.4). In fact, none of the procedures resorted to made any difference with these two last mentioned substances. It would seem that these facts would add considerable weight to the assumption that the materials dealt with were not ordinary whole proteins.

On preservation in the ice box at an alkaline reaction of pH 9.4, the influenza residue deteriorated within 48 hours, but the other antigens withstood similar treatment for 6 days.

In spite of the fact that these residue antigens were precipitable by homologous sera produced by immunization with the whole bacteria or their unfractionated extracts, we have so far failed to produce antibodies in animals by injecting these residues. While this may be due to inability to inject sufficient amounts of the material it still suggests strongly the possibility that we may be dealing with substances that are antigenic only in the sense that they are able to react with antibodies, but are themselves incapable of inciting antibody production. We suggest, in this connection, the possibility of the relationship between the power of antibody production and molecular size. This phase of the work is being continued on a more extensive scale.

Our work on the reactions of the residue materials in infected animals indicates, as far as we have gone, that complete analogy exists in this respect between the conditions prevailing in guinea pigs infected with these organisms and those previously elucidated for tuberculous animals. This is in keeping with previous knowledge concerning the analogies between the mallein and tuberculin reactions and the studies on skin hypersusceptibility in *Bacillus abortus*- and typhoid-infected guinea pigs reported by Meyer and his coworkers.

It would seem from all these facts that, in guinea pigs infected with bacteria capable of forming foci in the body, infection is followed within a variable, but relatively short time (5 days to 2 weeks) by a type of hypersusceptibility which is distinct from protein anaphylaxis and which may be determined by intradermal skin reaction. It appears likely that the growing bacteria elaborate in the animal body a metabolic product, possibly not a whole protein, which, though practically non-toxic to normal animals, may become highly and specifically injurious to the infected ones.

Such a conception, if further confirmed, would lead to greater clearness in our comprehension of the toxic effects occurring in infections with organisms not true exotoxin producers and, judging by the cellular injuries observed in severe skin reactions, may easily explain focal necrosis and the deeper cellular degenerations observed in the course of many bacterial diseases.

The general bearing of this work upon conceptions of hypersusceptibility is obvious and has been briefly discussed in another paper. Its chief significance is in holding out the hope that we may be able to elucidate the mechanism of a type of specific hypersusceptibility in which the antigen concerned is not a coagulable protein and in which the laws of sensitization in regard to time and quantity differ from those recognized in true protein anaphylaxis.

It seems likely that a recognition of the fact that physical and chemical differences in the substances leading to various forms of specific hypersusceptibilities in the animal body must necessarily influence the mechanism of sensitization, may furnish a clue to further investigations. As such materials become simpler in structure, they fail to induce typical antibody production and by gradually increased diffusibility transfer the reactions from the cell surface to the interior of the cell. The extremes of the scale of differences would be represented by protein anaphylaxis, on the one hand, and drug idiosyncrasies, on the other. Although this suggestion is largely speculative, it has seemed worth mentioning as a line of reasoning suggested by our work.

Incidentally, these studies may indicate the usefulness of the residue antigens for specific precipitation and complement fixation reactions for routine purposes in laboratory investigations.

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EXPLANATION OF PLATE 14.

FIG. 1. Guinea Pig 20. July 24, 1922. Experiment of July 23 to 24. This animal was one of two out of six left alive, into the peritoneal cavities of which perforated celloidin capsules filled with staphylococcus-infected agar had been placed on July 12. For previous records of this animal see the other experiment in the text. The point marked 1 is the reaction appearing after 24 hours at the site of the intracutaneous injection of 0.1 cc. of the residue antigen prepared as usual. At the point marked 2 there was a similar reaction with the so called nucleoprotein fraction.

FIG. 2. Guinea Pig 30. Tested on June 7, 1922. This animal had received five intracutaneous injections of living typhoid bacilli and was tested with residue antigens from the tubercle bacillus and from the typhoid bacillus on the 8th day after the last injection. The spot marked 1 represents the reaction, after 24 hours, to the injection of tubercle bacillus residue antigen. The spot marked 2 represents the point at which the typhoid bacillus residue antigen was injected.

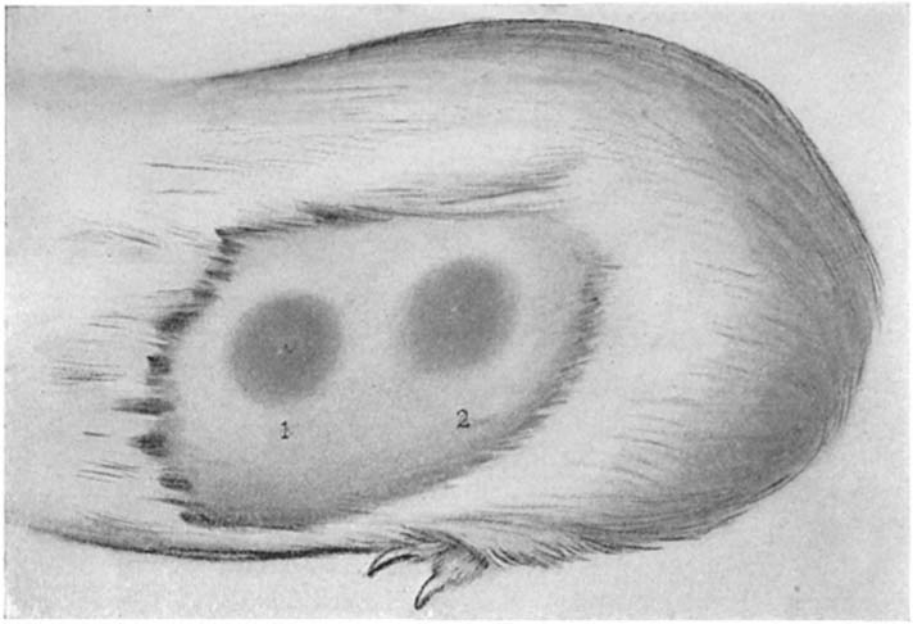

FIG. 1.

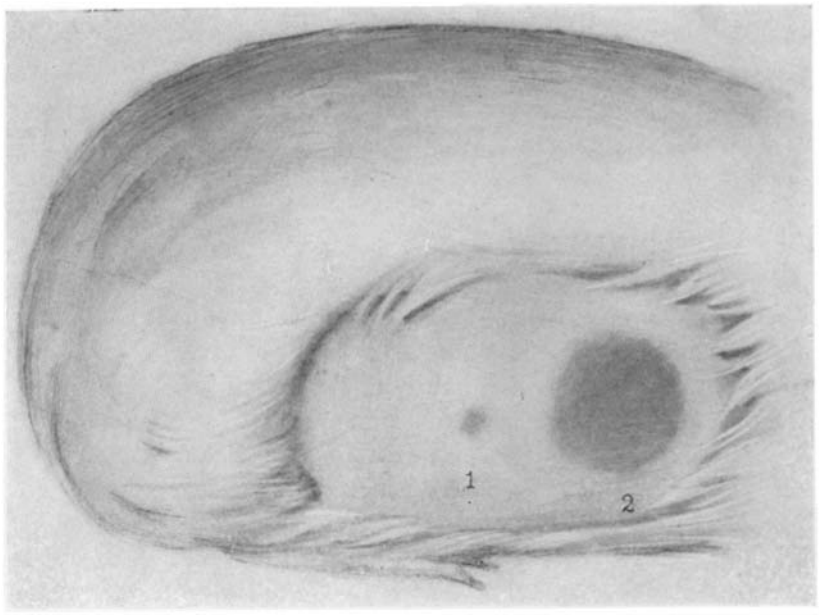

FIG. 2.

(Zinsser and Parker: Bacterial hypersusceptibility. II.)