STUDIES IN IMMUNIZATION BY A SPECIES ANTIGEN

I. THE PRESENCE OF 'SPECIES ANTIGEN' AND OF 'OPPOSITION FACTOR' IN BACTERIA OTHER THAN PNEUMOCOCCI

BY H. B. DAY, M.D., F.R.C.P., *From the Institute of Pathology and Research, St Mary's Hospital, London*

INTRODUCTION

The first article in this series (Day, 1942) described the preparation from pneumococci of a 'species antigen', so called because it can excite an immunity against all types of pneumococci. Species antigen is a natural constituent of the *Pneumococcus*, but its action is usually counterbalanced by the presence of another pneumococcal constituent that has the property of opposing immunization; this latter substance may be termed 'opposition factor' until its real nature is determined.

While species antigen is a non-protein substance, apparently of carbohydrate nature, the opposition factor is contained in the protein material of *Pneumococcus*, but it is resistant to agents which denature protein.

To obtain a rapid and satisfactory degree of species immunization it is necessary to employ special extracts of pneumococci which contain sufficient species antigen with but little or no opposition factor to cause interference.

PRESENT WORK

In previous work some experiments had indicated that injections of species antigen obtained from pneumococci protected mice against streptococci, provided the latter organisms were not of great virulence (Day, 1934).

If these observations were correct, it was probable that streptococci contained species antigen, since the corresponding antibody conferred immunity in mice against streptococci. Hence suitable preparations made from streptococci should provoke immunity to pneumococci. Since the pneumococci to be used were of standard maximum virulence, the tests could be performed with greater exactitude than in the converse experiments.

For the extraction of these bacteria the various methods found suitable for pneumococci were employed. These have been described in the first article of this series and are summarized in the protocols to follow.

Streptococcus haemolyticus

Harley's method applied to fresh organisms from a serum-broth culture gave an extract which on injection immunized mice against pneumococci. This method also succeeded when the streptococci had been killed by alcohol and kept resuspended in saline for some days, as in the following experiment:

Protocol

(*a*) Streptococci resuspended in saline after centrifuging down an 18 hr. serum-broth culture. An equal volume of alcohol added and mixed. Centrifuged.

(*b*) Cocci resuspended in saline, *p*H 6·5, the volume being half that of the original culture.

(*c*) Suspension kept 10 days at room temperature.

(*d*) Half its volume of $N/10$ HCl added to suspension. Heated to 60° C. for 1 hr. Centrifuged apparently clear.

(*e*) Extract neutralized with $N/10$ NaOH, then made alkaline (*p*H 10–11) and kept at 37° C. for 75 min.

(*f*) Acidified and recentrifuged to remove any protein.

(*g*) Heated to 60° C. for 25 min.

This preparation was injected into mice (dose 0·3 c.c.) which were tested a week later against serial dilutions of a culture of virulent type I *Pneumococcus*.

Pneumococcus test doses	Controls	Treated mice
5 cocci	Died	Survived
50 ,,	Died	Survived
500 ,,	Died	Died
5000 ,,	—	Survived

Several different strains of haemolytic streptococci were investigated with similar results. The species antigen was best obtained by treatment of whole suspensions; extraction of the bodies of streptococci that had been reduced by prolonged autolysis or by chemical treatment was unsuccessful.

Streptococcus (*indifferent*)

This strain was obtained from a cerebro-spinal fluid. It was avirulent to mice.

Harley's method applied to fresh organisms gave a successful immunizing preparation. Still better results were obtained by the use of a taurocholate mixture on the cocci after storage in dilute HCl, as described in the following experiment:

Protocol

(*a*) The streptococci were treated with 50% alcohol and then resuspended in $N/40$ HCl. Kept 14 days at room temperature and a further 12 days in the refrigerator.

(*b*) Centrifuged. Supernatant fluid kept; it was heated to 60° C. for 30 min. Then overneutralized and kept at *p*H 10·5 for 75 min. This formed *Preparation A*.

(*c*) The cocci were resuspended in 10% sodium taurocholate. To this suspension half its volume of $N/10$ HCl and 1½ vol. of alcohol were added. Mixture contained 50% alcohol.

(*d*) The suspension was kept at 37° C. for 20 hr., then heated to 60° C. for half an hour.

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press

(e) Cooled to room temperature and an equal volume of alcohol added. Left 3 hr., then centrifuged. Deposit taken up in saline.

(f) Made alkaline (pH 10·5) and kept at 37° C. for 75 min. This formed *Preparation B*.

The two preparations were neutralized; the volume of each was equal to that of the original culture used. Injected into two sets of mice (dose 0·3 c.c.) which were tested a week later against type I *Pneumococcus*.

Pneumococci test doses	Controls	Inoculated mice (2 sets) Prep. A	Prep. B
4 cocci	Died	Survived	Survived
40 „	Died	Died	Survived
400 „	Died	Survived	Survived
4,000 „	—	Survived	Survived
40,000 „	—	—	Survived
400,000 „	—	—	Survived

Note. Similar results were obtained with type II *Pneumococcus* as test organism.

The success of this method was due to certain procedures, as was ascertained by other experiments. The acid-alcohol-taurocholate mixture gave poor results when applied to fresh streptococci; as a preliminary measure storage in HCl was much superior to storage in weakly acidified saline.

An important detail was the high proportion of alcohol (50%) used in the taurocholate mixture, sufficient to prevent solution of the species antigen and protein. As a result of this treatment of the cocci the subsequent application of alkali was able to extract a considerable amount of species antigen with but little solution of the protein.

When a smaller proportion of alcohol was employed, the protein was less affected and more soluble, so that interference from opposition factor nullified the effect of the antigen liberated, as shown in the following experiment:

Protocol

(a) The streptococci were treated with 50% alcohol and then kept in N/40 HCl for 12 days at room temperature.

(b) Centrifuged. The cocci were resuspended in 10% taurocholate. To this half its volume of N/10 HCl and a similar amount of alcohol were added. The strength of alcohol in the mixture was therefore 25%.

(c) Suspension kept 23 hr. at 37° C., then heated to 60° C. for 1 hr.

(d) Cooled; 2 vol. of alcohol added to 1 vol. of suspension. Left 3 hr., then centrifuged. Deposit taken up in saline.

(e) Made alkaline (pH 11) and kept at 37° C. for 75 min. Neutralized.

(f) Preparation divided into two parts; one part centrifuged clear of undissolved material.

These two parts were injected into two sets of mice which were tested a week later against serial dilutions of a culture of virulent type I *Pneumococcus*.

In the experiment above, the preparation cleared of suspended matter gave much better but still imperfect protection; much opposition factor had probably been dissolved.

Pneumococci test doses	Controls	Mice inoculated with Original prep.	Centrifuged prep.
14 cocci	Died	Survived	Survived
140 „	Died	Died	Survived
1,400 „	Died	Died	Died
14,000 „	—	Died	Died
140,000 „	—	Died	Survived

Investigations had now shown that a considerable degree of mutual species protection could be secured by injection of suitable preparations of pneumococci and streptococci. Both classes of organism contained an immunizing 'species' antigen associated with an opposing factor. The existence of these constituents could be ascribed to the close family relationship of the cocci; indeed their nucleo-protein is known to be similar in its serological reaction.

Staphylococcus aureus

It was a natural step to extend the investigation to the *Staphylococcus aureus*, also a Gram-positive coccus.

Staphylococci in culture soon exhibit individual differences as they age; some undergo autolysis earlier than others. To obtain organisms in a more uniform state it was found advisable to use the reinforced cultures as described in the previous article on pneumococci.

Harley's method gave a preparation which on injection protected mice against only a minimum dose of virulent pneumococci. The extraction by solvents of staphylococci reduced by ordinary autolysis gave poor results; this appeared due to a large amount of opposition factor liberated. As with pneumococci, the employment of dilute acid or of carbolic as a preliminary process was superior, as used in the following experiment:

Protocol

(a) Staphylococci were collected from a reinforced culture and treated with 50% alcohol. Centrifuged down.

(b) Cocci resuspended in saline and one-third volume of N/10 HCl added (=N/40). Kept 12 days at room temperature.

(c) Acid suspension neutralized. Kept 17 days at room temperature.

(d) Centrifuged. The supernatant fluid was removed, made acid (pH 5) and heated to 60° C. for half an hour. This formed *Preparation A*.

(e) The deposit of cocci was resuspended in N/30 HCl. Heated to 80° C. for half an hour.

(f) Suspension made well alkaline, then incubated (37° C.) for 75 min. at pH 9·5.

(g) Centrifuged. The supernatant fluid was removed, acidified and heated to 60° C. for half an hour. This formed *Preparation B*.

(h) The residual cocci were heated in formamide at 120° C. for 15 min. Cooled. Two volumes of acetone added.

(i) Centrifuged. The deposit was extracted with N/100 NaOH at 37° C. for half an hour.

(j) Centrifuged clear and neutralized. This formed *Preparation C*.

The volume of each preparation was made equal with

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press

saline to that of the reinforced culture used. Each injected into a set of mice, dose 0·3 c.c. These were tested a week later against virulent type I pneumococci.

Pneumococci test doses	Controls	Mice inoculated with Prep. A	Prep. B	Prep. C
10 cocci	Died	Survived	Survived	Survived
100 ,,	Died	Survived	Survived	Survived
1,000 ,,	—	Died	Survived	Died
10,000 ,,	—	Died	Died	Survived
100,000 ,,	—	—	Died	Died

Similar preparations made from a similar suspension of staphylococci taken direct from $N/40$ HCl after 12 days' storage yielded very little species antigen, owing apparently to the relative insolubility of the cocci.

Neisseria. Since species antigen had been extracted from reduced pneumococci and staphylococci that had entirely lost their original Gram-positive character, it was probable that the *Neisseria* also contained this antigen. Although comparatively few experiments were performed with these organisms they were sufficient to establish species antigen as a constituent.

Meningococcus

Protocol

(*a*) Meningococci were treated with 50% alcohol, centrifuged and the cocci resuspended in $N/40$ HCl. Kept at room temperature for 12 days.

Suspension divided into two portions:

(1) To one portion extra HCl added to bring strength to $N/30$ HCl. Heated to 60° C. for 90 min.

Centrifuged clear. Overneutralized and kept at *p*H 10·5 for 1 hr. Then neutralized—*Preparation A*.

(2) To the other portion an equal volume of 10% taurocholate and a similar amount of alcohol added. (Strength of alcohol in mixture—33%.) Kept at 37° C. for 23 hr.

2 vol. of alcohol added to 1 vol. of the mixture. Centrifuged after 2 hr.

Deposit heated in $N/30$ HCl at 60° C. for 45 min.

Overneutralized and kept at *p*H 10·5 for 1 hr. Then neutralized—*Preparation B*.

Mice inoculated with these preparations were tested a week later against type I *Pneumococcus*.

Pneumococcus	Controls	Mice inoculated with Prep. A	Prep. B
2 cocci	Died	Survived	Died
24 ,,	Died	Survived	Died
240 ,,	Died	Survived	Died
2400 ,,	—	Died	Survived

This result illustrates the effect of interference by opposition factor. Preparation B had not been centrifuged nor finally heated, and contained so much opposition factor that it could only produce a paradoxical immunity.

Gonococcus

Protocol

(*a*) Gonococci were washed off in saline from a serum-agar culture. Centrifuged down and resuspended in a small volume of 1 in 20 carbolic. After half an hour 7 vol. of saline added to lower the concentration of carbolic to 1 in 160.

(*b*) Kept at 37° C. for 18 hr., then for 6 days in refrigerator.

(*c*) Centrifuged. Cocci resuspended in saline (*p*H 6·5) and kept at 37° C. for 18 hr.

(*d*) To suspension half its volume of $N/10$ HCl added. Heated to 60° C. for 2 hr.

(*e*) Overneutralized to *p*H 10–11 and kept at 37° C. for 90 min.

(*f*) Acidified and heated to 60° C. for half an hour.

The preparation was injected into mice which were tested against type II pneumococci a week later.

Pneumococci test doses	Controls	Inoculated mice
8 cocci	Died	Survived
80 ,,	Died	Survived
800 ,,	Died	Died
8000 ,,	—	Survived

The following experiment showed the presence of 'opposition factor' in gonococci:

Protocol

(*a*) Gonococci collected as before and treated with 50% alcohol. Centrifuged down and resuspended in saline.

(*b*) Kept 6 weeks in refrigerator.

(*c*) A little $N/10$ NaOH added to suspension; considerable solution. Neutralized after 2 min. Alkali added till *p*H = 10. Kept at 37° C. for 45 min.

(*d*) Centrifuged (not quite clear). Fluid divided into two portions:

(1) To one part an equal volume of alcohol added. Precipitate collected after 3 days.

(2) To other part ether added and shaken up. Ether removed and replaced by alcohol. Precipitate collected after 3 days.

(*e*) The two precipitates were taken up in alkaline saline (*p*H 11) which was then made slightly acid (*p*H 6). Kept at 4° C. overnight.

(*f*) The two preparations were centrifuged clear and heated (acid) to 60° C. for half an hour.

The two preparations were injected in mice which were tested a week later against type I *Pneumococcus*.

Pneumococci test doses	Controls	Alcohol ppt. Prep. A	Ether-alcohol ppt. Prep. B
10 cocci	Died	Died	Survived
100 ,,	Died	Died	Survived
1,000 ,,	—	Died	Died
10,000 ,,	—	Died	Died

In this experiment the use of ether caused a firm precipitate of coagulated bacterial protein, from which some species antigen was extracted. When antigen and protein were precipitated by 50% alcohol, firm coagulation was absent and enough opposition factor dissolved out to annul the action of species antigen. Experiments on pneumococci showed that washing with ether did not remove the opposition factor.

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press

BACILLI

Since different classes of cocci alike contained species antigen and opposition factor, it was reasonable to expect that these constituents were to be found in most if not in all bacteria. Investigations on a comprehensive scale were hardly practicable, but a few experiments on different classes of bacilli were sufficient to support this hypothesis.

Escherichia

Protocol

(*a*) Coliform bacilli collected from a broth culture were suspended in a small volume of saline. An equal volume of alcohol added and mixed.

(*b*) After 10 min. the suspension was diluted with four times its volume of saline, thus reducing the alcohol percentage to 10.

(*c*) Kept at room temperature 6 days. Centrifuged.

(*d*) Bacilli resuspended in saline (*p*H 6·5). Kept 2 days at room temperature.

(*e*) Half its volume of $N/10$ HCl added. Heated to 80° C. for 30 min.

(*f*) Overneutralized and kept at *p*H 10·5 for 20 hr. in a refrigerator.

(*g*) Acidified and centrifuged. Supernatant fluid kept. Deposit heated in formamide at 120° C. for 10 min. Cooled. 2 vol. of acetone added; this caused a flocculent precipitate.

(*h*) Precipitate collected by centrifuge and taken up in the first fluid extract. Kept at *p*H 9·5 overnight in refrigerator.

(*i*) Acidified and centrifuged clear.

This preparation was injected into mice which were tested a week later against type I *Pneumococcus*.

Pneumococci test doses	Controls	Inoculated mice
5 cocci	Died	Died
50 ,,	Died	Died
500 ,,	Died	Died
5,000 ,,	—	Survived
50,000 ,,	—	Died

The paradoxical immunity secured indicates an excessive amount of opposition factor left in the preparation. Acid in the cold does not precipitate all the protein dissolved by fairly strong alkali.

Klebsiella pneumoniae

Protocol

(*a*) Friedlander's bacilli collected and resuspended in formamide.

(*b*) Kept 3 days at room temperature; then heated to 125° C. for 10 min.

(*c*) Cooled; an equal volume of 10% taurocholate added. This caused some precipitate: centrifuged clear.

(*d*) To the fluid an equal volume of alcohol added. Precipitate collected and taken up in saline.

(*e*) Made alkaline (*p*H 9–9·5) and kept at 37° C. for 2 hr.

(*f*) Acidified and centrifuged clear.

(*g*) Heated to 60° C. for half an hour. Recentrifuged clear of more protein precipitated by the heating.

The preparation was neutralized with a little alkali and injected into mice which were tested a week later against type I *Pneumococcus*.

Pneumococci test doses	Controls	Inoculated mice
13 cocci	Died	Survived
130 ,,	Died	Survived
1,300 ,,	—	Survived
13,000 ,,	—	Died

In contrast to the result obtained in the preceding experiment with coliform bacilli, this preparation of Friedlander's bacillus gave a moderate degree of solid immunity. This was attributed to removal of protein precipitated by hot acid, as a final procedure. As shown by other experiments, this removal does entail a loss of species antigen by adsorption to the precipitate.

Haemophilus pertussis

(*a*) *Pertussis* bacilli washed off a blood agar culture with saline. Suspension centrifuged. Bacilli resuspended in saline and an equal volume of alcohol added and mixed. Centrifuged.

(*b*) Bacilli resuspended in saline at *p*H 6·5. Kept 6 days at room temperature.

(*c*) To suspension half its volume of $N/10$ HCl added. Heated to 60° C. for 1 hr. Centrifuged.

Fluid. The acid extract was made alkaline (*p*H 10–11) and kept at 37° C. for 3 hr. Then made just acid (*p*H 6·5).

Kept just acid 1 day. Made more acid (*p*H 5) and heated to 60° C. for half an hour. Centrifuged clear. Neutralized—*Preparation A*.

Deposit. The deposit of bacilli dissolved in formamide at room temperature. Heated to 100° C. for 1 min. Cooled; an equal volume of alcohol added.

Precipitate collected and taken up in saline. Made well alkaline (*p*H 12) for 1 min., then neutralized and kept overnight at *p*H 6·5.

Made more acid (*p*H 5) and heated to 60° C. for half an hour. Centrifuged clear. Neutralized—*Preparation B*.

The two preparations were injected into two sets of mice which were tested a week later against type I *Pneumococcus*.

Pneumococci test doses	Controls	Prep. A Acid extract	Prep. B Formamide prep.
11 cocci	Died	Died	Survived
110 ,,	Died	Died	Survived
1,100 ,,	—	Died	Survived
11,000 ,,	—	Died	Died

Similar preparations made from fresh bacilli failed to induce immunity. On such bacilli formamide had much less effect.

Pseudomonas pyocyanea

Protocol

(*a*) Bacilli washed off an agar slope with saline. Centrifuged.

(*b*) Resuspended in fresh saline. Half its volume of $N/10$ HCl added. Heated to 60° C. for 1 hr.

(*c*) Centrifuged; did not come clear.

(1) *Supernatant fluid* (cloudy). Overneutralized and kept at *p*H 9·7 for 90 min. at 37° C.

Kept slightly acid (*p*H 6·5) for 2 days in refrigerator.

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press

Acidified to pH 5 and heated to 60° C. for 35 min. Centrifuged clear. Neutralized—*Preparation A*.

(2) *Deposit*. Heated in formamide at 122° C. for 15 min. Cooled and centrifuged clear of undissolved residue.

To extract 2 vol. of acid alcohol added.

Precipitate collected and kept in alkaline saline (pH 8·7) for 2 days in the refrigerator.

Centrifuged clear. To extract half its volume of $N/10$ HCl added; heated to 60° for 45 min. Neutralized—*Preparation B*.

The two preparations were injected in two sets of mice which were tested a week later against type I pneumococci.

Pneumococci test doses	Controls	Mice inoculated with	
		Prep. A	Prep. B
23 cocci	Died	Survived	Survived
230 „	Died	Survived	Died
2,300 „	—	Died	Survived
23,000 „	—	Died	Died

Mycobacterium tuberculosis

The following method was tried on this very resistant organism, but it is doubtful if any species antigen was obtained in solution:

Protocol

(*a*) Tubercle bacilli were washed off the culture medium with 50% alcohol. Left in this medium.

(*b*) A fortnight later the 50% alcohol was replaced by absolute alcohol, and this was renewed the next day.

(*c*) Alcohol removed and the deposit of bacilli boiled in xylol at 115° C. for 20 min. Xylol removed; bacilli washed in two changes of alcohol by the centrifuge.

(*d*) Bacilli suspended in saline. Half its volume of $N/30$ HCl added; heated to 60° C. for 10 min., then to 80° C. for 30 min.

(*e*) Centrifuged, not quite clear. Fluid was neutralized, made alkaline (pH 10·3) and kept at 37° C. for 3 hr.

(*f*) Kept overnight slightly acid (pH 6·5), made more acid (pH 5·5) and centrifuged. Not clear. Heated for half an hour to 60° C.

The acid preparation was neutralized and injected in mice which were tested a week later against type I pneumococci.

Pneumococci test doses	Controls	Inoculated mice
51 cocci	Died	Survived
510 „	Died	Died
5100 „	—	Died

No immunity followed the injection of a clear formamide extract made from the residue of bacilli heated in HCl. It is quite possible that the slight degree of protection afforded by the acid 'extract' was due to some absorption of antigen *in vivo* from the treated bacilli left suspended in the preparation.

NON-PATHOGENIC BACTERIUM

Species antigen or a substance of similar action had been found in such widely different classes of bacteria that this constituent might bear no relation to the actual or potential virulence of microbes. For investigation of a non-pathogenic bacterium *M. lysodeikticus* was a convenient choice. This coccus has been cultivated in this laboratory for years and is noted for its ready solubility by lysosyme.

Mycobacterium lysodeikticus

This organism proved resistant to chemical action and no species antigen was obtained by Harley's method nor by the use of formamide. In the following experiment lysosyme was employed as a solvent:

Protocol

(*a*) A suspension of the cocci in saline was dissolved by the addition of a few drops of egg albumin solution.

(*b*) The solution was treated with 1½ vol. of acetone. This gave a flocculent precipitate.

(*c*) The precipitate was taken up in saline, made alkaline (pH 9) and kept for 18 hr. in the refrigerator.

(*d*) Centrifuged clear. Fluid kept at neutral pH for 1 day.

(*e*) Acidified to pH 6 and heated to 60° C. for 25 min.

The preparation was neutralized and injected into mice which were tested a week later against type I *Pneumococcus*.

Pneumococci test doses	Controls	Inoculated mice
15 cocci	Died	Survived
150 „	Died	Survived
1,500 „	Died	Died
15,000 „	—	Died*

* This mouse lived twice as long as the controls.

REMARKS

The different methods detailed in the protocols are given as examples only, because far more study would be required to select the most suitable for the extraction of each bacterium. As described in the preceding article on pneumococci, none of the methods tried are really satisfactory. Failures to obtain an immunizing preparation were not infrequent and were due to two main causes—resistance of the bacterium to the solvent so that no antigen was obtained, or to excessive solution of the protein containing the opposition factor. But a more or less successful preparation was obtained from all the bacteria investigated.

No comparison is valid between the results given by preparations made from the different bacteria. Not only does their solubility vary, but the presence of other antigens and possibly heat-resistant toxic substances may influence the result.

A note may be added on the use of formamide. The formamide used with success had a decided acid reaction (pH 4), but other samples may be quite alkaline. It is unsafe to use a hot alkaline solvent, since this is destructive of antigenic activity. But if the formamide be heated alone first, an alkaline reaction is changed to acid, or a trace of HCl may be added to give an acid reaction. The natural acidity of some samples is due to the liberation of formic acid and ammonium formate.

SUMMARY AND DISCUSSION

The experiments here described proved that preparations made from widely different classes of bacteria are

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press

capable of inducing immunity in mice against pneumococci. The success of these preparations was found to depend on the differential separation of an active immunizing antigen from another bacterial constituent —an 'opposition factor'—which interferes with immunization. Further investigation and comparison depend largely on the discovery of a really effective method for the isolation and purification of the active antigen. Until its chemical nature is determined, the identity of the pneumococcal species antigen with similarly acting substances found in other bacteria remains unproved.

Pathogenic bacteria depend for their virulence and invasiveness on certain products individual to the species or type, and specific immunity is due to the development of the appropriate antibodies. But chemotherapy, as in the case of the sulphonamides, interferes with a less specialized metabolic process of the microbes and is neither species nor type specific in action. It is reasonable to infer that bacteria may possess in common a vital process associated with the presence of a common constituent which may be antigenic. 'Species antigen' may be a substance of this nature.

In view of the wide distribution of 'species antigen' among bacteria generally, it is probable that this constituent has already been separated and named in accordance with its presence in a particular species, but irrespective of its potential immunizing capacity.

So far as pneumococci are concerned, there is good evidence that species antigen may be identical with the species C carbohydrate. Tillett (1927) found that rabbits injected with successive courses of heat-killed pneumococci, whether of types I, II, III or of avirulent rough strains, developed an equal increase of resistance to infection by type III *Pneumococcus*. In 1936 Enders, Wu & Shaffer varied the procédure, and by injections of rough pneumococci prepared an antiserum which gave passive protection to rabbits against infection by type III *Pneumococcus*. This antiserum lost its protective power after absorption with the species C carbohydrate.

Meanwhile the reactivity of the skin and serum of pneumonia patients to the different constituents of pneumococci had been studied. Tillett & Francis (1930) found that a non-protein fraction of *Pneumococcus*—apparently C carbohydrate—gave a positive intradermal response and a precipitin reaction with the sera of pneumonia patients, irrespective of the type of pneumococcus causing the infection. These reactions occur early in the active phase of the disease and soon disappear in convalescence. Similar reactions to the type-specific carbohydrate develop only at the time of crisis and persist during convalescence.

Control observations on other patients showed that this reactivity to the species carbohydrate or non-protein constituent of pneumococci was not confined to pneumonia, but was also found in rheumatic fever, in severe streptococcal infections and in staphylococcal osteomyelitis. The reaction was absent in healthy persons and in cases of malaria, measles and varicella.

These findings and the identity of the pneumococcal constituent with C carbohydrate were confirmed by later work. Ash (1933) discovered a positive reaction to C carbohydrate in some cases of *Bact. coli* infection. Abernethy & Francis (1937), in a reinvestigation, obtained the same positive results in pneumonia and other cases of coccal infection as before. They found the reaction present during the acute phase and complications, but absent in nine fatal cases of pneumonia.

If we admit the probable identification of 'species antigen' with C carbohydrate, these observations are of special significance. They point to the liberation of a common antigenic substance not only in pneumonia but in other bacterial infections. Since a similar antigen is contained in these bacteria, the non-specific character of the reaction is readily explained.

It was evident to the Rockefeller workers that the C carbohydrate reaction in infections differed from other immunological phenomena. Not only was it non-specific, but it appeared early in the active phase of the disease before immunity developed. Abernethy (1937) further discovered that the reaction developed during experimental pneumococcal infection in monkeys but not in rabbits.

Recently (1941) Abernethy & Avery have found that the substance in the sera of patients that reacts with C carbohydrate does not conform to the usual type of antibody. It belongs to the albumin, not to the globulin fraction of the serum protein, and is insoluble in water containing a trace of calcium. MacLeod & Avery (1941) were able to isolate this reactive protein and to differentiate it by means of a precipitin test with a specific antiserum made by injecting rabbits with the separated fraction of human reactive serum.

In the present article the subject of non-specific reaction has been approached from a different angle and the large gap in our knowledge leaves little common ground for discussion. But if species antigen be closely related to C carbohydrate, there is a possible explanation of much that is at present obscure in the peculiar and almost unexplored action of the 'opposition factor' with which it is associated in the bodies of bacteria.

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press

REFERENCES

ABERNETHY, T. J. (1937). Studies on the somatic C polysaccharide of *Pneumococcus*. II. The precipitin reaction in animals with experimentally induced pneumococcic infection. *J. Exp. Med.* **65**, 75–89.

ABERNETHY, T. J. & AVERY, O. T. (1941). The occurrence during acute infections of a protein not normally present in the blood. I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C polysaccharide of *Pneumococcus*. *J. Exp. Med.* **73**, 173–82.

ABERNETHY, T. J. & FRANCIS, T., JR. (1937). Studies on the somatic C polysaccharide of *Pneumococcus*. I. Cutaneous and serological reactions in pneumonia. *J. Exp. Med.* **65**, 59–74.

ASH, R. (1933). Nonspecific precipitins for pneumococcic fraction C in acute infections. *J. Infect. Dis.* **53**, 89.

DAY, H. B. (1934). The preparation of pneumococcal species antigen. *J. Path. Bact.* **38**, 171–3.

DAY, H. B. (1942). Studies in immunization by a species antigen. I. Preparation of species antigen from pneumococci. *J. Hyg., Camb.*, **42**, 532–46.

ENDERS, J. F., WU, C. J. & SHAFFER, M. F. (1936). Observations on a non-type specific humoral factor involved in resistance to *Pneumococcus* type III. *J. Exp. Med.* **64**, 425–38.

MACLEOD, C. M. & AVERY, O. T. (1941). The occurrence during acute infections of a protein not normally present in the blood. II. Isolation and properties of the reactive protein. III. Immunological properties of the C reactive protein and its differentiation from normal blood proteins. *J. Exp. Med.* **73**, 183–90, 191–200.

TILLETT, W. S. (1927). Increased resistance to type III infection induced in rabbits by immunization with R and S forms of *Pneumococcus*. *J. Exp. Med.* **46**, 343–56.

TILLETT, W. S. & FRANCIS, T., JR. (1930). Serological reactions in pneumonia with a non-protein somatic fraction of *Pneumococcus*. *J. Exp. Med.* **52**, 561–71.

(*MS. received for publication* 12. III. 43.—Ed.)

https://doi.org/10.1017/S0022172400013036 Published online by Cambridge University Press