ANTIPNEUMOCOCCAL SPECIES IMMUNITY

By ALLAN W. DOWNIE

Senior Freedom Research Fellow

From the Laboratories of the Hospital of the Rockefeller Institute and the Bacteriological Department of the London Hospital Medical College

THERE is some evidence that a certain degree of active immunity to all types of pneumococci may be induced by the use of vaccines prepared from one type, although passive immunity is generally held to be a function of antibodies to the type-specific antigen. Tillett (1928) was able to immunize rabbits against infection with types I and III pneumococci by using a vaccine of rough organisms derived from a type II strain. Mice which were effectively immunized to infection with one type of *Pneumococcus* were found to have a partial active immunity to infection with heterologous types (Yoshioka, 1923). Blake (1932) correlated the natural resistance of normal guinea-pigs to infection with pneumococci of types I, II and III with the presence of group or species agglutinins, and suggested that basic active immunity to pneumococcal infection in man might be similarly dependent on antipneumococcal species antibodies.

Day (1933, 1934) has claimed that under certain conditions it is possible to prepare a species antigen from a single pneumococcal type which is capable of immunizing mice equally well to infection with the homologous and heterologous types, and it is further claimed that such an antigen injected into rabbits produces a species antibody capable of passively protecting mice against all types of pneumococcal infection. These findings have been confirmed by Harley (1935), and both Day and Harley suggest that the species antigen is derived by dissociation from the type-specific antigen of virulent pneumococci.

The practical application of a single vaccine capable of producing immunity to all types of pneumococci prompted this study of the antigenic efficiency of a pneumococcal antigen prepared according to the methods of Day and Harley.

EXPERIMENTAL

Methods. The strains of *Pneumococcus* used in the majority of the experiments were those with which the work recorded in the previous paper was carried out. The methods of testing animals for active immunity and examining sera for antibodies have been described in that communication.

https://doi.org/10.1017/S0022172400011153 Published online by Cambridge University Press

Experiment 1

The antigenic efficiency of the vaccine prepared according to method C (Harley, 1935). Vaccine A, the type-specific antigen, was prepared by adding 50 c.c. of 1·0 % glucose broth to 100 c.c. of culture of type I *Pneumococcus* grown for 16 hr. in broth containing 2·0 % rabbit serum, and incubating for 2 hr. longer. The deposit of bacteria obtained by centrifugation was resuspended in 75 c.c. saline to which $N/10$ HCl was added to make a final concentration of $N/30$ HCl. This was heated at 60° C. for 1 hr., then made up to a volume of 150 c.c. with saline and adjusted to a *p*H of 5·0 with normal NaOH. Half of this suspension was reserved as vaccine A.

Vaccine B, the species antigen, was prepared from the other half by adjusting the reaction to *p*H 8·5–9·0 and incubating for 16 hr. at 37° C. During this period species antigen should appear. The vaccine was finally adjusted to *p*H 5·0 and heated for 20 min. at 60° C.

Stained films from vaccines A and B demonstrated a preponderance of Gram-positive forms.

Mice were injected with these vaccines in two doses of 0·5 c.c. each at 4-day intervals and tested for active immunity 7 days after the second injection. Table I demonstrates that neither vaccine produced an immunity to heterologous types of pneumococci.

Table I. *The immunity produced in mice by antigens A and B*

Infected with culture	Mice immunized with: Antigen A	Mice immunized with: Antigen B	Normal control mice	Colonies from 0·5 c.c.
Type I: 10^{-1}	D D	D D	—	—
10^{-2}	D S	D S	—	—
10^{-3}	S S	S S	—	—
10^{-4}	S S	S S	—	—
10^{-5}	S S	S S	—	—
10^{-6}	S S	S S	D D	—
10^{-7}	—	—	D D	69
10^{-8}	—	—	D D	5
Type II: 10^{-4}	—	D D	—	—
10^{-5}	D D	D D	—	—
10^{-6}	D D	D D	D D	—
10^{-7}	D D	D D	D D	51
10^{-8}	D D	D D	D D	4
Type III: 10^{-4}	—	D D	—	—
10^{-5}	D D	D D	—	—
10^{-6}	D D	D D	D D	—
10^{-7}	D S	D D	D D	50
10^{-8}	S S	D S	D S	4

D = died within 4 days of pneumococcal infection. S = survived 7 days.

Three rabbits were injected with each vaccine. Doses of 1·0 c.c. were injected by the intravenous route on three successive days, and at 4-day intervals three subsequent series of injections were given. Seven days after the last injection the animals were bled from an ear vein and tested for active immunity 6 days later by the intradermal injection of 0·1 c.c. of the virulent

Table II. *Antibodies in the sera of rabbits immunized with antigens A and B*

Rabbit no.	Immunized with	Titre of agglutinins against		Precipitation with polysaccharides		Mouse protective antibodies		Test for active immunity
		Type I	Type II	Type I	Type III	Type I	Type II	type I
449	Antigen A	1 : 80	−*	+ + +	−	not tested		S
450		1 : 160	−	+ + +	−			S
451		1 : 10	−	+ + +	−			S
452	Antigen B	1 : 10	−	+ + +	−			S
453		1 : 10	−	+ + +	−	10^{-1}†	‡	S
454		1 : 10	−	+ + +	−			S
479	Control							Dead 46 hr.

* = negative in a serum dilution of 1 : 2.

† = 0·2 c.c. of serum protected against 10^{-1} dilution of culture.

‡ = no protection against 10^{-7} dilution of culture (31 colonies).

type I culture. Table II shows that vaccines A and B both produced antibodies which were purely type-specific in character.

Experiment 2

Day (1933) concluded from his experiments that species antigen was formed in the bodies of pneumococci in ageing cultures prior to obvious autolysis. It seemed possible therefore that, if antigen were prepared from cultures at different stages of growth by the method already outlined, evidence in support of his findings might be forthcoming. Accordingly, after the addition of the glucose broth, portions of a culture of type I *Pneumococcus* were removed at the end of 1, 2, 3 and 4 hr. In these successive samples the reaction was found to be increasingly acid from *p*H 6·4 to 5·0. The four samples were submitted to the procedure used for preparation B in the first experiment. Microscopic examination of the four preparations made before and after the final incubation showed that, whereas before incubation all contained chiefly Gram-positive bacteria after incubation the first two showed a preponderance of Gram-negative cocci. These first two preparations had cleared somewhat during incubation while the other two cleared only slightly if at all, and after incubation the majority of the bacteria seen in stained films were still Gram-positive.

Although the four preparations had undergone varying degrees of autolysis all gave similar results when used for the immunization of mice. Two injections protected mice against 0·5 c.c. of 10^{-2} to 10^{-4} dilutions of young cultures of virulent type I pneumococci but failed entirely to give any protection against 0·5 c.c. of 10^{-7} or 10^{-8} dilution of type II culture—amounts which in plate cultures showed 44 and 3 colonies respectively.

Experiment 3

Antigens prepared by Day's alcohol method—(method A, Harley, 1935). In this method the pneumococci, obtained from cultures prepared as before, are treated with 50 % alcohol for 10 min. After removal of the alcohol by centrifugation, the pneumococci are suspended in saline, the reaction is adjusted to

*p*H 7·6 or made slightly more alkaline, *p*H 8·5–9·0, and the suspension incubated for some hours before the final heating at 60° C. with *p*H 5·0. Day states that storage of the suspensions at 5° C. for a week before the final incubation seemed to give more active preparations.

In the present experiment the cultures of type I pneumococci were grown under the same conditions as in Exp. 1. After treatment of the deposit of organisms by alcohol some preparations were incubated immediately, others were kept in the refrigerator at a temperature of approximately 4° C. for 8 days before incubation. In some instances the *p*H was adjusted to 7·4, in others to 8·5–9·0 before the final incubation. Observations on morphology revealed that many of the pneumococci were rendered Gram-negative by the alcohol treatment. After further incubation the suspensions usually showed some clearing, and microscopical examination at this time showed only Gram-negative organisms, many of them shadowy forms.

The various preparations were used for the immunization of mice, two intraperitoneal injections being made before testing 7 or 8 days later with living organisms. The results only differed from those recorded above in that the protection afforded against the homologous type I culture was less than before. In no instance was there any evidence of protection of significant degree against pneumococci of types II and III. The lower degree of protection against infection with the type I culture afforded by these alcohol-treated preparations seemed to be associated with the degree of autolysis observed.

Experiment 4

It seemed possible that the failure to obtain preparations capable of inducing active resistance in mice against heterologous types might have been due to the strain of *Pneumococcus* used. The strain of type I used throughout Day's work was kindly furnished by Dr Harley, and further experiments were made with this strain.

Several preparations were made from a culture grown under the same conditions as before. Vaccines *a*, *b* and *c* were made from portions of the culture removed 1, $2\frac{1}{2}$ and $3\frac{1}{2}$ hr. respectively after the addition of the glucose broth by the technique outlined in the first experiment. Vaccine *d* was prepared by the alcohol method from a portion of the culture removed $2\frac{1}{2}$ hr. after the addition of glucose broth. This preparation, adjusted to *p*H 5·0 after the alcohol treatment, was kept in the refrigerator for 8 days. The reaction was then adjusted to *p*H 8·5 and the preparation incubated at 37° C., for 3 hr. before finally adjusting the reaction to *p*H 5·0 and heating. For the purpose of control two vaccines were made from a culture of a haemolytic *Streptococcus* grown under the same conditions. These were treated by the methods used for *Pneumococcus* preparations *b* and *d* and are referred to as vaccines 1 and 2. Morphological examination of the vaccines made when they were ready for use showed that the streptococci in vaccines 1 and 2 were practically all Gram-positive. The

majority of the pneumococci in vaccines *a*, *b* and *c* were Gram-positive, while in vaccine *d*, although the organisms still stained sharply, only a few retained the Gram stain.

The results of the test of active immunity in mice made 7 days after the last of two immunizing injections are shown in Table III.

Table III. *Test for active immunity in mice which had been treated with vaccines of type I pneumococci* (*a–d*) *and haemolytic streptococci* (1 *and* 2)

Infected with culture	Mice immunized with vaccines						Control normal mice	Colonies from 0·5 c.c.
	a	*b*	*c*	*d*	1	2		
Type I: 10^{-1}	D D	D D	D S	D S	—	—	—	—
10^{-2}	D S	D D	D S	S S	—	—	—	—
10^{-3}	S S	S S	D S	S S	—	—	—	—
10^{-4}	S S	S S	S S	S S	D D	D D	—	—
10^{-5}	S S	S S	S S	S S	D D	D D	—	—
10^{-6}	S S	S S	S S	S S	D D	D D	D D	—
10^{-7}	—	—	—	—	D D	D D	D D	54
10^{-8}	—	—	—	—	D D	D D	D D	6
Type II: 10^{-4}	D D	D D	D D	D D	D D	D D	—	—
10^{-5}	D D	D D	D D	D D	D D	D D	—	—
10^{-6}	D S	D D	D D	D D	D D	D D	D D	—
10^{-7}	D S	D D	D D	D S	D D	D D	D D	33
10^{-8}	D S	D S	D S	D S	D S	S S	D S	4

Here again there is little indication that the preparations had evoked resistance to infection with the heterologous type of *Pneumococcus*. With vaccine *a* there is some slight suggestion of this, and a further experiment was made with this preparation. Mice given three doses of the vaccine at intervals of 3 days all succumbed to the test dose of type II *Pneumococcus*, but in this case the culture of type II used for infection seemed to be slightly more virulent as all the control mice died. Serum obtained from mice of this immunized group 7 days after the last immunizing injection showed agglutination of type I suspension but no agglutinins against type II organisms in a serum dilution of 1 : 3. Slight precipitation occurred when the serum was mixed with a 1 : 50,000 dilution of type I polysaccharide, but no precipitation with a similar dilution of type III polysaccharide.

Discussion

Immunity to the *Pneumococcus* as a species, in so far as it has been shown to exist, may be dependent on two antigens, or partial antigens, which are common to all types of pneumococci—the C carbohydrate of Tillett *et al.* (1930) and the heterophile antigens described by Bailey & Shorb (1931). The C carbohydrate isolated by Tillett *et al.* was found to be non-antigenic in mice and rabbits, but the recent work of Enders *et al.* (1936) suggests that the relative resistance of certain animals and human beings to pneumococcal infection may be due to the presence of an antibody or antibody-like substance reacting with this carbohydrate. This view might explain the correlation of natural resistance to pneumococcal infection and the presence of species

agglutinins observed in certain guinea-pigs by Blake (1932) and the active immunity to heterologous types of pneumococcus noted in vaccinated rabbits by Tillett (1927, 1928) and in mice by Yoshioka (1923). Further, the C carbohydrate may be an important part of the heterophile antigen common to different types of pneumococci, although it would appear that this antigen is not identical with that present in sheep red cells or guinea-pig tissues (Bailey & Shorb, 1933). The serum of rabbits in Tillett's experiments did not protect mice against heterologous types of pneumococci, while heterophile antibody sera? although possessing some protective power in rabbits, were found by Powell *et al.* (1933) to have no protective action against pneumococcal infection in mice. The value of heterophile antibody in the treatment of pneumococcal pneumonia in man would seem to be doubtful (Finland *et al.* 1935), and it would appear that for the production of effective passive immunity to the *Pneumococcus* type-specific antibody is essential.

Whether the C carbohydrate is responsible for the active resistance to infection with heterologous types observed in vaccinated animals or not, it should be emphasized that the degree of such resistance is less than that which is determined by the type-specific carbohydrate and effective only against the homologous type of *Pneumococcus*. The protocols in the papers of Day and Harley do not indicate a high degree of immunity to heterologous types. The dose of type I organisms which failed to kill mice previously treated with "species antigen" from type II culture varied from 100 to 10,000 cocci. In the course of the present work it has frequently been observed that a single intraperitoneal injection of a suitable vaccine of type I *Pneumococcus* would protect against one million lethal doses of the homologous organism. On the other hand, the results of the experiments recorded in this paper failed to give evidence that effective immunization of mice or rabbits against heterologous types could be produced by the methods recommended by Day and Harley. It is evident from their work that the results varied with different preparations, and it may be that the experiments recorded above were unsuccessful because of the omission of some necessary precautions not sufficiently appreciated.

There seems little doubt from the results of various workers that in certain animal species some degree of active resistance to infection may be induced against the *Pneumococcus* as a species and independent of type-specific antibodies, but there is little evidence that effective passive immunity can be conferred by antibodies to *Pneumococcus* "species antigen".

SUMMARY

In the experiments recorded the various antigens prepared from type I pneumococci failed to produce effective resistance in mice to heterologous types or to produce demonstrable antibodies against these types in mice or rabbits.

ACKNOWLEDGEMENT. I wish to thank Dr Cole and Dr Avery for laboratory facilities while part of this work was being carried out and for their interest and advice.

REFERENCES

BAILEY, G. H. & SHORB, M. S. (1931). *Amer. J. Hyg.* **13**, 831.
—— —— (1933). *Ibid.* **17**, 329, 358.
BLAKE, F. G. (1932). *Trans. Ass. Amer. Physicians*, **47**, 152.
DAY, H. B. (1933). *J. Path. Bact.* **36**, 77.
—— (1934). *Ibid.* **38**, 171.
ENDERS, J. F., WU, CHAO-JEN & SHAFFER, M. F. (1936). *J. Exp. Med.* **64**, 425.
FINLAND, M., RUEGSEGGER, J. M. & FELTON, L. D. (1935). *J. Amer. Med. Ass.* **105**, 1180.
HARLEY, D. (1935). *Brit. J. Exp. Path.* **16**, 14.
POWELL, H. M., JAMIESON, W. A., BAILEY, G. H. & HYDE, R. (1933). *Amer. J. Hyg.* **17**, 102.
TILLETT, W. S. (1927). *J. Exp. Med.* **46**, 343.
—— (1928). *Ibid.* **48**, 791.
TILLETT, W. S., GOEBEL, W. F. & AVERY, O. T. (1930). *Ibid.* **52**, 895.
YOSHIOKA, M. (1923). *Z. Hyg. InfektKr.* **97**, 386.

(*MS. received for publication* 25. IX. 37.—Ed.)