J. Hyg., Camb. (1980), 85, 275
Printed in Great Britain

Streptococcal infection in young pigs. V. An immunogenic polysaccharide from *Streptococcus suis* type 2 with particular reference to vaccination against streptococcal meningitis in pigs

By S. D. ELLIOTT and FELICITY CLIFTON-HADLEY
Departments of Pathology and Clinical Veterinary Medicine, University of Cambridge
and JOSEPH TAI
Rockefeller University, New York

(*Received* 20 *February* 1980)

SUMMARY

Of 17 pigs vaccinated with *Streptococcus suis* type-2 capsular polysaccharide plus Freund's Incomplete Adjuvant, all developed opsonizing antibody against *Str. suis* type 2. Of 14 pigs vaccinated with type-2 polysaccharide alone, 4 (possibly 6) developed opsonizing antibody. It is possible that some pigs vaccinated with polysaccharide plus Freund's Incomplete Adjuvant developed opsonizing antibody in response to a 'booster' injection of polysaccharide alone. Of 21 unvaccinated control pigs, late bleeding from 3 showed opsonizing activity against *Str. suis* type 2.

INTRODUCTION

The introduction of intensive methods for the rearing of pigs has been accompanied by an increased incidence of streptococcal meningitis. Pigs are commonly affected between the ages of 4 and 10 weeks. Overcrowding and poor ventilation are probably contributory factors in the spread of infection (Windsor, 1977), but the causal agent is a specific haemolytic streptococcus, *Str. suis* type 2, de Moor's Group R (de Moor, 1963; Windsor & Elliott, 1975). A bacteriological survey extending over the past 5 years showed that in England about 90 % of bacterial meningitis in pigs was caused by this streptococcus; the remaining 10 % of cases was caused by infection with *Str. suis* type 1, de Moor's Groups (S. D. Elliott, unpublished observations). Type 1 is a cause of neonatal septicaemia in pigs and is rarely encountered as a cause of disease after the age of 3–4 weeks (Field, Buntain & Done, 1954; Elliott, Alexander & Thomas, 1966). Because streptococci of a single immunological type are responsible for most of the meningitis in growing pigs, it seemed reasonable to hope that the disease might be controlled by vaccination with *Str. suis* type 2 or with one of its components. The success achieved in the control of human meningococcal meningitis by vaccination with type-specific meningococcal polysaccharides (Gotschlich, Austrian, Cvjetanovic & Robbins, 1978) suggested a similar approach for the control of streptococcal meningitis in pigs.

0022-1724/80/0022-1980 $01.00

In a recent paper we described the isolation and chemical characterization of the capsular polysaccharide of *Str. suis* type 2 (Elliott & Tai, 1978). Here we present the results of an investigation of the immunogenicity of this polysaccharide in pigs.

MATERIALS AND METHODS

Experimental pigs

Large White and Landrace breed pigs were used in these experiments. Those used in Expts 1, 3 and 4 were from the herd maintained at Merton Hall Farm at the School of Veterinary Medicine, Cambridge; those used in Expt 2 were from a neighbouring farm (Holly Lodge Farm). No infection by *Str. suis* had been seen in either herd in recent years.

Streptococci

Str. suis strain R75/L1, alternatively designated D930 in the Rockefeller University collection, is a capsulated representative of *Str. suis* type 2 and has been previously described (Elliott, McCarty & Lancefield, 1977).

Str. suis *type-2 capsular polysaccharide*

This was extracted from strain R75/L1 and purified by a method previously described (Elliott & Tai, 1978). A single batch of purified material was used throughout this investigation.

Bactericidal tests

The method described by Agarwal, Elliott & Lachmann (1969) was employed. Pig blood, 10 ml, was withdrawn from the anterior vena cava without anaesthetic and mixed with heparin (Pularin) 100 units in 0·1 ml saline. In the Direct Bactericidal Test, to 9 vol. freshly drawn heparinized blood was added 1 vol. young blood broth culture of strain R75/L1 suitably diluted in nutrient broth after incubation for 4 h at 37 °C. The dilution of blood–broth culture employed was such that pour-plates made with 0·2 ml samples of the pig blood immediately after inoculation gave colony counts of approximately 60–600 colony forming units after incubation at 37 °C. Immediately after inoculation 0·4 ml volumes of the blood were dispensed in screw-capped vials (35 × 12 mm) and rotated end over end at 16 rev./min at 37 °C. Pour-plates for colony counts were made from 0·2 ml blood samples taken from duplicate vials after 1 and 3 h rotation. For the Indirect Test, 1 vol. immune pig serum was mixed with 3 vols. freshly drawn heparinized *human* blood; a single donor (S.D.E.) was used throughout this work. The mixture was inoculated with streptococci and pour-plates made for colony counts before and after rotation at 37 °C as described for the Direct Test.

FIRST IMMUNIZATION EXPERIMENT

Our first experiment was to determine whether purified capsular polysaccharide from *Str. suis* type 2 would elicit an immune response in pigs when injected together with an adjuvant. Two different adjuvants were tested, calcium alginate and Freund's Incomplete Adjuvant. The experiment was carried out in three stages and the immunization regimens employed are shown in Fig. 1.

Stage 1. *The effect of calcium alginate on the immunogenicity of type-2 polysaccharide (CHO)*

Each of six pigs from a 19-day-old litter of eight received two subcutaneous injections of type-2 CHO; the remaining two litter-mates were kept as unvaccinated controls. The two injections were separated by an interval of 3 weeks and each injection contained 0·15 mg CHO in 1 ml 2% sodium alginate (Sigma) in saline. In three of the vaccinated pigs the subcutaneous sodium alginate was converted to the calcium salt by an immediate 'follow-up' injection of 1 ml calcium chloride (37·3 mg per ml saline) delivered into the site of the preceding injection (Chase, 1967).

Examined by the indirect bactericidal test, serum taken from the six vaccinated pigs 7 days after their second injection failed to show the presence of opsonizing antibodies for type-2 streptococci. Serum from the two control animals also gave negative results. Pre-immunization serum taken from three of the vaccinated and one of the control pigs showed weak opsonizing activity for the type-2 cocci. This was probably attributable to maternal antibody transferred in the colostrum but eliminated before the first post-vaccination bleeding. Previous work has shown that the blood of many adult sows has opsonizing activity against type-2 cocci (D. Bevin & S. D. Elliott, unpublished results). The source of this age-related immunity of adult pigs will be discussed later in this report.

Stage 2. *The effect of Freund's Incomplete Adjuvant on the immunogenicity of type-2 polysaccharide*

Because of their lack of response to CHO plus alginate, the six vaccinated pigs were given a further course of injections containing type-2 CHO with Freund's Incomplete Adjuvant (F.I.A.). The first of these injections was administered 7 days after the conclusion of stage 1 (Fig. 1). The injection consisted of 0·15 mg CHO in 0·5 ml saline mixed with 0·5 ml F.I.A. and it was repeated 3 weeks later. Serum samples were taken 1 week after the second injection and examined by the indirect bactericidal test. As can be seen from the results set out in Table 1A, opsonizing activity was now present in serum from pigs nos. 1, 2 and 5, but little or none was detected in serum from pigs nos. 3, 4 and 6. Both control pigs gave negative results.

Stage 3. *The immune response to type-2 polysaccharide alone*

Nineteen days after the conclusion of stage 2, pigs 3, 4 and 6 received a final subcutaneous injection of 0·5 mg CHO without adjuvant. Blood samples taken 9

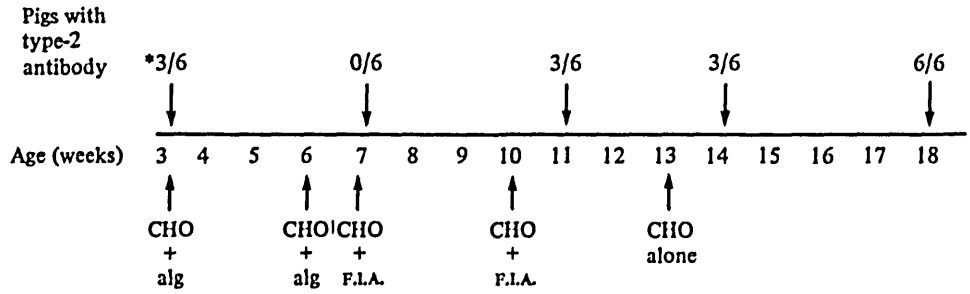

Fig. 1. Immunization regimen and immuno response in six litter-mates vaccinated with type-2 CHO ± adjuvant (Expt 1). * Maternal antibody. ↓, Serial bleedings from each pig. ↑, serial injections into each pig. alg, Na or Ca alginate. F.I.A., Freund's Incomplete Adjuvant.

Table 1A. *Growth of* Str. suis *type 2 in human blood + serum from six pigs vaccinated with type 2 polysaccharide* + F.I.A. (*two injections*)

Source of serum	Result of indirect bactericidal tests: c.f.u. in 0·2 ml human blood + pig serum after rotation for		
Vaccinated pigs (no.)	0 h	1 h	3 h
1	18	0	0
2	439	31	3
3	439	546	++
4	18	31	405
5	18	16	53
6	439	888	++
Unvaccinated pigs			
7	18	65	673
8	439	1600	++

In all tables:
c.f.u. signifies colony-forming units in 0·2 ml blood.
++ signifies confluent growth in pour plates.
F.I.A. signifies Freund's Incomplete Adjuvant.

Table 1B. *Growth of* Str. suis *type 2 in heparinized blood from three pigs vaccinated with type 2 CHO* + F.I.A. (*two injections*) *and final injection of CHO alone*

Source of blood	Result of direct bactericidal tests: c.f.u. in 0·2 ml heparinized pig blood after rotation for		
Vaccinated pigs (no.)	0 h	1 h	3 h
3	193	13	2
4	190	123	326
6	221	27	8
Unvaccinated pig			
8	201	476	++

days later and examined by the *indirect* bactericidal test still showed no significant opsonizing activity but final bleedings taken after a further period of 4 weeks and examined by the *direct* test showed opsonizing activity in all three pigs. No such activity was seen in pig no. 8, one of the unvaccinated controls; the other control pig, no. 7, was not tested (Table 1B).

In this experiment all six vaccinated animals eventually developed opsonizing antibody to the type-2 CHO. Because each pig was subjected to more than one immunization procedure it was hard to say which procedure elicited the immune response. This was especially so in pigs 3, 4 and 6. Although it is possible that in these animals the opsonizing activity resulted from vaccination with CHO alone (stage 3) it may equally well have followed vaccination with CHO + F.I.A. in stage 2 but remained undetected until stage 3, when we changed to the more sensitive *direct* bactericidal test.

From the results of this pilot experiment we concluded, firstly, that the purified type-2 CHO elicited an immune response in pigs when F.I.A. was incorporated in the vaccine; and secondly, that in pigs previously vaccinated with CHO + F.I.A., an immune response may have followed a subsequent 'booster' injection of CHO alone.

SECOND IMMUNIZATION EXPERIMENT

Immune response to type-2 polysaccharide + F.I.A.

This experiment made use of 15 eight-week-old pigs from two litters, C and D. Eight pigs, seven from litter C and one from litter D, were vaccinated with a mixture of type-2 CHO + F.I.A. The remaining five pigs from litter C and two more from litter D were kept as unvaccinated controls. Each vaccinated pig received two subcutaneous injections of CHO (0·15 mg) + F.I.A. at an interval of 2 weeks followed 3 weeks later by a final injection of CHO (0·5 mg) + F.I.A.

Pre-immunization bleedings examined by the direct bactericidal test failed to show opsonizing activity against type-2 streptococci in any of the 15 pigs. Serial bleedings taken during the course of immunization showed an immune response in two vaccinated pigs, C6 and C7, 14 days after their first injection and in another, C2, 17 days after its second injection. All the remaining vaccinated pigs showed opsonizing activity in final bleedings taken 2 weeks after their third injection. The results of the final bactericidal tests are shown in Table 2 from which it can also be seen that bleedings from two of the seven unvaccinated controls, C9 and C12, showed opsonizing activity. This activity first appeared 2 weeks earlier when the pigs were 12–13 weeks old. Neither animal showed signs of overt infection and cultures taken from the nose, throat and submaxillary lymph nodes after slaughter were negative for *Str. suis*.

The results of this experiment confirmed our previous finding that type-2 polysaccharide was immunogenic in pigs when injected with F.I.A. The presence of opsonizing activity in blood from two unvaccinated control pigs will be discussed later in this report.

Table 2. *Growth of* Str. suis *type* 2 *in heparinized blood from eight pigs vaccinated with type* 2 *CHO*+F.I.A. (*three injections*)

Source of blood	Result of direct bactericidal tests: C.F.U. in 0·2 ml pig blood after rotation for		
Vaccinated pigs (no.)	0 h	1 h	3 h
C1	156	1	0
C2	158	0	0
C3	159	1	25
C4	195	16	97
C5	52	3	11
C6	54	1	0
C7	35	0	0
D1	56	0	0
Unvaccinated pigs			
C8	178	36	409
C9	134	2	33
C10	260	214	++
C11	127	186	++
C12	63	7	0
D2	65	39	926
D3	59	41	390

THIRD IMMUNIZATION EXPERIMENT

The third immunization experiment made use of ten litter-mates, seven of which were injected with type-2 polysaccharide and three kept as unvaccinated controls. The pigs were 5 weeks old when first injected. The experiment was carried out in two stages and the immunization regimen employed is shown in Fig. 2.

Stage 1

Four pigs received 0·15 mg CHO in saline by the subcutaneous route and three pigs the same dose in F.I.A. The injections were repeated after an interval of 14 days. Seven days later blood samples were taken for examination by direct bactericidal tests the results of which are shown in Table 3A.

From Table 3A it can be seen that opsonizing activity against type-2 cocci was present in post-vaccination samples from pig 19 (one of the three injected with CHO+F.I.A.) and pig 13 (one of four injected with CHO alone). No opsonizing activity was found in blood from the remaining five vaccinated and three control pigs.

Stage 2

Stage 2 of this experiment began 2 weeks after the conclusion of stage 1 (Fig. 2). In stage 2, each of the seven previously vaccinated pigs received two further injections of polysaccharide. None of the injections in stage 2 contained F.I.A. The third injections were of 0·15 mg CHO administered subcutaneously in saline and the fourth, 2 weeks later, were of 0·5 mg in saline by the intradermal route. Blood samples were taken 7 days after each injection and examined in direct bactericidal tests. The results of the final tests only are shown in Table 3B.

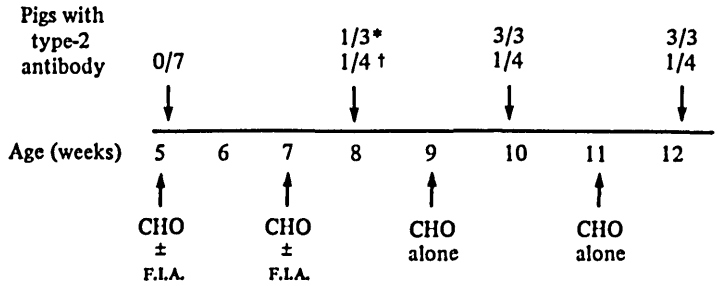

Fig. 2. Immunization regimen and immune response in seven litter-mates vaccinated with type-2 CHO ± F.I.A. (Expt 3). * Pigs vaccinated with CHO + F.I.A. † Pigs vaccinated with CHO alone.

Table 3A. *Growth of* Str. suis *type 2 in heparinized blood from pigs vaccinated with type-2 CHO* ± F.I.A. *(two injections)*

Source of blood		Result of direct bacterial tests: c.f.u. in pig blood after rotation for		
(Pig no.)	Vaccinated with	0 h	1 h	3 h
11	CHO alone	438	++	++
12	CHO alone	431	++	++
13	CHO alone	422	215	274
17	CHO alone	418	++	++
14	CHO + F.I.A.	508	354	++
18	CHO + F.I.A.	524	++	++
19	CHO + F.I.A.	395	0	0
15	Unvaccinated	462	++	++
16	Unvaccinated	508	++	++
20	Unvaccinated	513	++	++

Table 3B. *Growth of* Str. suis *type 2 in heparinized blood from pigs vaccinated with type-2 CHO alone after previous vaccination with CHO* ± F.I.A. *(see Table* 3A)

Source of blood	Result of direct bacterial tests: c.f.u. in 0·2 ml blood after rotation for		
(pig no.)	0 h	1 h	3 h
11*	47	344	++
12*	41	256	++
13*	46	90	505
17*	50	300	++
14†	44	0	0
18†	48	9	22
19†	32	0	0
15 (unvaccinated)	50	82	707
16 (unvaccinated)	52	358	++
20 (unvaccinated)	45	369	++

* Previously injected with CHO alone (see Table 3A).
† Previously injected with CHO + F.I.A. (see Table 3A).

https://doi.org/10.1017/S0022172400063312 Published online by Cambridge University Press

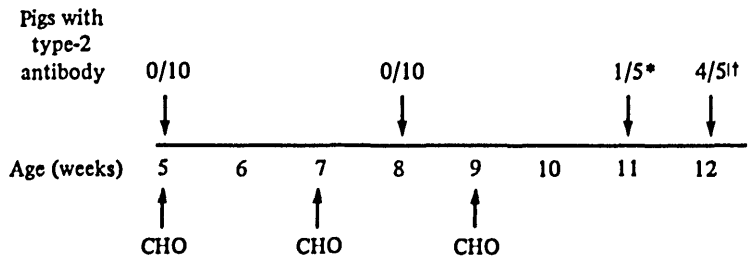

Fig. 3. Immunization regimen and immune response in ten pigs (litters A and B) vaccinated with type-2 CHO alone (Expt 4). * Litter A. † Litter B.

From Table 3B it can be seen that 7 days after their fourth injection all three pigs vaccinated in stage 1 with CHO plus F.I.A. showed marked opsonizing activity. The same result had been seen 2 weeks earlier when these pigs were bled and tested 7 days after their third injection (see Fig. 2). In two of these pigs (nos. 14 and 18) the activity found in stage 2 may have resulted from the injection of the polysaccharide alone. Of the four pigs injected throughout with CHO alone, only one (no. 13) developed opsonizing activity. This activity first appeared during stage 1 and showed no significant increase during stage 2.

In our examination of the final blood samples we found that one of the three unvaccinated controls (pig no. 15) had developed slight opsonizing activity against type-2 cocci. No such activity had been found previously in the three control animals.

FOURTH IMMUNIZATION EXPERIMENT

The fourth experiment made use of 19 five-week-old pigs from two litters (A and B). Nine pigs were kept as unvaccinated controls. The remaining ten animals each received three intradermal injections of type-2 polysaccharide in saline; no adjuvant was used in this experiment. The first two injections were of 0·15 mg and the third of 0·5 mg CHO with an interval of 2 weeks between each injection.

The regimen of injections and bactericidal tests employed in this experiment is shown in Fig. 3. Direct bactericidal tests on blood samples taken 1 week after the second injection showed no opsonizing activity against type-2 cocci in either the vaccinated or control animals. The tests were repeated on the five pigs from litter A 2 weeks after their third injection and on the five pigs from litter B 1 week later. The results of these tests are set out in Tables 4A and B.

From Table 4A it can be seen that 2 weeks after its third injection of polysaccharide, pig no. 48 had weak opsonizing activity against type-2 cocci. Blood from four vaccinated litter-mates gave no convincing evidence of opsonizing activity nor was any found in the unvaccinated controls.

Three pigs from litter B (nos. 31, 35 and 39) developed an unrelated, intercurrent, intestinal infection following their third injection of polysaccharide. Antibiotic therapy in these three pigs interfered with the bactericidal tests carried out at this time. The tests on all the pigs of litter B therefore were repeated 1 week later, 3

Table 4A. *Growth of* Str. suis *type-2 in blood from five pigs (litter A) vaccinated with type-2 CHO alone and from four unvaccinated litter-mates*

Source of blood		Result of direct bactericidal tests: c.f.u. in pig blood after rotation for		
(pig no.)	Vaccinated with	0 h	1 h	3 h
41	CHO alone	614	872	++
43		619	++	++
44		636	++	++
47		667	++	++
48		610	259	227
40	Unvaccinated	588	++	++
42		670	++	++
45		650	++	++
46		633	++	++

Table 4B. *Growth of* Str. suis *type-2 in blood from five pigs (litter B) vaccinated with type-2 CHO alone and from five unvaccinated litter-mates*

Source of blood		Result of direct bactericidal tests: c.f.u. in pig blood after rotation for		
(pig no.)	*Vaccinated with*	0 h	1 h	3 h
30	CHO alone	188	49	1100
33		192	58	2000
34		211	64	768
35		175	++	++
37		218	47	682
31	Unvaccinated	193	209	++
32		195	520	++
36		219	351	++
38		255	1146	++
39		222	176	++

weeks after their third injection with CHO (see Fig. 3). The results of these tests are shown in Table 4B. It can be seen that the blood from two vaccinated pigs (nos. 34 and 37) showed weak opsonizing activity against type-2 cocci, confirming the results obtained a week earlier but not shown here. The blood from pigs 30 and 33 showed doubtful activity – none had been apparent 1 week earlier – and blood from pig no. 35 (which suffered the intercurrent infection) showed no activity at any time.

In summary, the results of this experiment showed that of ten pigs vaccinated with CHO alone, three developed weak opsonizing activity against type-2 streptococci. Blood samples from two others showed doubtful activity. No opsonizing activity was detected in blood from the remaining five vaccinated animals or from nine unvaccinated controls.

DISCUSSION

Previous work has shown that the capsular polysaccharide of *Str. suis* type 2 has a molecular weight of at least 100000 and contains five different sugars, including sialic acid. In these respects it resembles the type polysaccharides of group-B streptococci implicated in human neonatal meningitis (Elliott & Tai, 1978). The results of our present experiments indicate that vaccination with the purified capsular polysaccharide elicits in pigs antibody with opsonizing activity against type-2 cocci. This immune response was enhanced when Freund's Incomplete Adjuvant was incorporated in the vaccine but, injected alone, the polysaccharide evoked a weak antibody response in at least 4 and possibly in 6 out of 14 pigs. Furthermore our results suggest that a 'booster' injection of polysaccharide given to pigs previously vaccinated with polysaccharide plus adjuvant may have led to an increase in the opsonizing activity of the pigs' serum.

The presence of opsonizing activity against type-2 cocci in late bleedings from 3 of 21 unvaccinated control pigs requires comment. No case of overt *Str. suis* infection had been seen on the farms in which these animals were reared and our attempts to find evidence of latent infection by post-mortem cultures were unsuccessful. Two other possible explanations for the presence of type-2 antibody in these control pigs merit consideration. First, these animals may have harboured atypical type-2 streptococci. We have occasionally encountered attenuated strains deficient in capsular polysaccharide and liable to be overlooked in primary cultures but able to elicit type-specific antibody when injected into rabbits. Secondly, the opsonizing activity against type-2 cocci in the control pigs may have been elicited by an immunologically cross-reacting component in some otherwise unrelated micro-organism present in these animals. Such an explanation has been invoked by Robbins and his colleagues to account for age-related natural immunity to human meningitis. In support of this hypothesis they have described cross-reacting capsular polysaccharides in *E. coli*, *N. meningitidis*, *H. influenzae* and *Str. pneumoniae* (Robbins *et al.* 1973).

The bactericidal test as used here was not designed to give a quantitative estimate of the antibody response. Pig no. 19, injected with polysaccharide plus adjuvant, showed the best response in terms of opsonizing activity, but this was not demonstrable in serum diluted more than 1 in 6. By contrast, precipitating antisera raised in rabbits against formalized suspensions of *Str. suis* have shown opsonizing activity at dilutions greater than 1/1000 (unpublished results). It must be concluded that in our vaccinated pigs the immune response to purified polysaccharide was small. More relevant to our objective is whether this response would protect against infection with *Str. suis* type 2. Passive protection tests with immune sera have not been practicable because we are unable to produce experimental type-2 infection in pigs with regularity. This is in marked contrast to our experience with *Str. suis* type 1. Sprayed into the upper respiratory tract, type 1 caused a high incidence of septicaemia and meningitis in piglets (Elliott, *et al.* 1966). Protection against such infection was achieved by prior injection of bactericidal antisera from convalescent piglets. It seems reasonable to hope that

antiserum with opsonizing activity against *Str. suis* type 2 might protect against type-2 infection and that vaccination with type-2 polysaccharide would achieve this end.

We thank Drs D. W. B. Sainsbury and T. J. L. Alexander for access to pigs at Merton Hall Farm and Holly Lodge Farm, respectively. We thank especially Mr Eric Barnard and Mr Martin O'Dell for their skilled handling of the experimental animals.

This work was supported by U.S. Public Health Service Grants HL-03919 and AI-15230 and by a grant from the Jowett Fund in the University of Cambridge. F. C.-H. holds an Agricultural Research Council Veterinary Fellowship and receives additional support from the Dalgety Research Fund; her part in this work will be incorporated in her dissertation for the Ph.D. degree of the University of Cambridge.

REFERENCES

Agarwal, K. K., Elliott, S. D. & Lachmann, P. J. (1969). Streptococcal infection in young pigs. III. The immunity of adult pigs investigated by the bactericidal test. *Journal of Hygiene* **67**, 491.

Chase, M. W. (1967). *Methods in Immunology and Immunochemistry*, vol. 1 (ed. C. A. Williams and M. W. Chase), p. 202. New York: Academic Press.

Elliott, S. D., Alexander, T. J. L. & Thomas, J. H. (1966). Streptococcal infection in young pigs. II. Epidemiology and experimental production of the disease. *Journal of Hygiene* **64**, 213.

Elliott, S. D., McCarty, M. & Lancefield, R. C. (1977). Teichoic acids of group D streptococci with special reference to strains from pig meningitis. *Journal of Experimental Medicine* **145**, 490.

Elliott, S. D. & Tai, J. Y. (1978). The type-specific polysaccharides of Streptococcus suis. *Journal of Experimental Medicine* **148**, 1699.

Field, H. I., Buntain, D. & Done, J. T. (1954). Studies on pig mortality I. Streptococcal meningitis and arthritis. *Veterinary Record* **66**, 653.

Gotschlich, E. C., Austrian, R., Cvjetanovic, B. & Robbins, J. B. (1978). Prospects for the prevention of bacterial meningitis with polysaccharide vaccines. *Bulletin of the World Health Organization* **56**, 509.

Moor, C. E. de (1963). Septicaemic infection in pigs caused by haemolytic streptococci of new Lancefield groups designated R, S and T. *Antonie van Leewenhoek* **29**, 272.

Robbins, J. B., Gotschlich, E. C., Liu, T. Y., Schneerson, R., Handzel, Z. T., Argaman, M., Parke, J. C. & Myerowitz, R. L. (1973). Bacterial antigens cross-reactive with the capsular polysaccharides of *Haemophilus influenzae* type b, *Neisseria meningitidis* Groups A and C and *Diplococcus pneumoniae* types I and III. *Proceedings of the Symposium on New Approaches for Inducing Natural Immunity to Pyogenic Organisms* (ed. J. B. Robbins, R. E. Horton and R. M. Krause), pp. 45–56. U.S. Department of Health, Education and Welfare, Publication no. (N.I.H.) 74-553.

Windsor, R. S. (1977). Meningitis in pigs caused by *Streptococcus suis* Type II. *Veterinary Record* **101**, 378.

Windsor, R. S. & Elliott, S. D. (1975). Streptococcal infection in young pigs. IV. An outbreak of streptococcal infection in weaned pigs. *Journal of Hygiene* **75**, 69.