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Streptococcal infection in young pigs

III. The immunity of adult pigs investigated by the bactericidal test

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INTRODUCTION

Outbreaks of streptococcal infection in young pigs have been described in England by Field, Buntain & Done (1954) and in the Netherlands by de Moor (1963). The causative organisms are capsulated, haemolytic cocci of specific serological type belonging to *Streptococcus suis*, a subgroup of group D (Elliott, 1966). Piglets susceptible to infection are generally less than 6 weeks of age. In such animals the streptococci gain entrance probably through the respiratory tract, whence they invade the bloodstream, producing a sustained bacteriaemia and infections of the joints and meninges. Some animals die within a few days of infection, others recover completely. Elliott, Alexander & Thomas (1966) showed that the condition can be produced experimentally by spraying broth cultures of *S. suis* into the upper respiratory tract of susceptible piglets but adult animals are normally immune both to the naturally occurring and to the experimentally produced disease.

An analogous condition, joint ill, occurs in lambs and is often caused by a streptococcus of specific type belonging to group C (Blakemore, Elliott & Hart-Mercer, 1941). The resistance of adult sheep to this infection is reflected by the ability of their blood to inhibit the multiplication of joint-ill streptococci *in vitro*; no such inhibition occurs in the blood of susceptible lambs. The present investigation was undertaken to determine whether a similar relationship between susceptibility to infection and bacteriostatic power of the blood exists in *S. suis* infection of pigs, and if so, to characterize the responsible component in the blood.

MATERIALS AND METHODS

The bactericidal test

A modification of the method described by Lancefield (1957) was used throughout this investigation.

Pig blood and serum

Large white and Landrace breed pigs up to 2 years of age were used as the source of blood or serum. One or other of the following methods was used in rearing

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the pigs: (1) reared under conventional conditions; (2) weaned from 2 to 5 days after birth and reared on milk substitute (Amvilac No. 1*); (3) hysterectomy delivered, colostrum-deprived pigs reared under hygienic conditions in isolation. Such gnotobiotic pigs used as a source of serum in some experiments were reared either at the Royal Veterinary College, London, or at Ontario Veterinary College, Guelph, (Yorkshire breed pigs).

Venepuncture

The blood for test was taken from the anterior jugular vein without anaesthetic and delivered into tubes containing heparin (Pularin) 1000 units in 0·2 ml. saline.

Rotation of inoculated blood

After inoculation with streptococci the blood was distributed in 0·4 ml. amounts into glass vials 35 mm × 12 mm. sealed by a screw-on, metal cap lined with a silicone rubber cushion. The vials were placed in a mixing machine in which they rotated end over end at six rev./min. at 37°C.

Streptococcal cultures

Four identical, capsulated strains designed PM1, PM23A, PM32, and C22N were used throughout. A non-capsulated strain, designated A227 and thought to be derived from PM1, was also used. All these strains have been previously described (Elliott, 1966). For use in the bactericidal test the streptococci were grown in 5% horse blood broth for 16 hours at 37°C, and the culture then diluted appropriately in nutrient broth before inoculating into the pig blood.

Streptococcal counts

Two methods were used to enumerate colony forming units (C.F.U.). In early experiments a platinum loop, 3 mm. in diameter, was used to transfer blood samples to the surface of horse blood agar on which they were spread and incubated at 37°C. for 18 hr. before counting the colonies that developed. In later experiments, colony forming units were counted in pour-plates incorporating 0·1 ml. blood samples in 5% horse blood agar; these were incubated at 37°C. for 18 hr. This method was used for counts of broth cultures throughout.

All counts were made in duplicate and expressed as the mean of the C.F.U.

Serum fractionation

Ion exchange chromatography. DEAE cellulose (D.E. 32, Whatman), 800 ml., was packed in a column 3 cm. × 90 cm. The sample was applied in 0·02 M phosphate buffer, pH 7·0, containing 0·01 M sodium azide, and an exclusion peak was collected. Because of difficulty in testing a large number of fractions for bactericidal activity, elution was carried out step-wise using increasing concentrations of NaCl in the starting buffer. The eluate from the column was passed through a Uvicord analyser. The protein-containing material from each elution step was pooled and precipitated

* Amvilac is an antibiotic-free milk substitute, marketed by Glaxo Laboratories, Greenford, Middlesex.

by half saturation with ammonium sulphate. The precipitate was harvested, redissolved in a minimum quantity of water and dialysed against physiological saline.

Gel filtration. This was performed on G.200 Sephadex, 2 l., in a column 5 cm. × 100 cm. (Pharmacia) using 0·1 M Tris buffer, pH 7·2, containing 0·5 M-NaCl and 0·015 M sodium azide. Blue dextran 2000 and haemoglobin were used as markers. The column effluent was passed through the Uvicord analyser. Pooled fractions were precipitated by half saturation with ammonium sulphate, the precipitate dissolved in a minimum quantity of water and dialysed against physiological saline.

All fractions were sterilized by filtration before testing.

Mercaptoethanol treatment. Samples were treated with 0·1 M 2-mercaptoethanol for 2 hr. at 37°C. The mercaptoethanol was then removed by dialysis against several changes of saline.

Protein estimations were made by Folin's method and read against a standard curve prepared with human IgG.

EXPERIMENTAL

Effect of capsules on survival of streptococci in porcine blood

Streptococci isolated from the naturally occurring disease are capsulated. Our first experiments, therefore, sought to determine whether the capsule influenced the survival of the cocci in normal piglet blood.

Table 1. *Effect of capsulation on survival of* Streptococcus suis *in blood of piglet no.* 2

	S. suis added to piglet blood (0·4 ml.)	C.F.U. in loopfuls from piglet blood after rotation at 37°C, for stated period		
Designation	C.F.U. in inoculum (0·1 ml.)	0 hr.	3 hr.	6 hr.
Strain A 227 Noncapsulated	192	2	0	0
Strain PM 1 Capsulated	256	2	++	++

In this and succeeding tables C.F.U. signifies colony forming units; ++ signifies innumerable colony forming units.

Two samples of heparinized blood from a 16-day-old piglet (No. 2) were inoculated with 0·1 ml. of a suitably diluted, 16 hr. culture of *S. suis*; one sample was inoculated with a capsulated strain (Strain PM 1), the other with the noncapsulated variant (Strain A 227). The inoculated blood samples were distributed in 0·4 ml. amounts in vials and these were rotated at 37°C. Duplicate loopfuls of blood were taken from duplicate vials before and after rotation for 3 and 6 hr. and the colony forming units (C.F.U.) counted as described under Methods.

The results of this experiment are shown in Table 1. It can be seen that whereas the capsulated cocci grew freely under these conditions, the noncapsulated variant failed to do so. Thirty-five samples of blood from 17 piglets, conventionally reared and less than 6 weeks of age, were tested against the noncapsulated strain A 227.

In none did the cocci show any multiplication. Indeed the blood samples inoculated with this strain usually became sterile within the 6 hr. period of incubation.

Blood samples from 160 conventionally reared piglets of the same age as those tested against the noncapsulated strain were tested against the capsulated cocci. Sixty-eight (42 %) resembled piglet No. 2 (Table 1) in that their blood allowed multiplication. Somewhat unexpectedly, blood from the remaining 92 animals (58 %) was bacteriostatic. This difference between animals of the same age group and, indeed, sometimes from the same litter is difficult to explain and will be discussed later. It is possible that the animals whose blood was bacteriostatic were immune as a result either of colostral antibody or of previous infection with *Streptococcus suis*, for when *colostrum-deprived* piglets have been reared under 'pathogen-free' conditions, with 1 exception (see Table 5) their blood was found to lack bacteriostatic power over the capsulated cocci.

The microscopic examination of 'buffy-coat' preparations from the blood of piglet No. 2 inoculated with capsulated and noncapsulated *S. suis* revealed that after 4 hr. rotation at 37°C. the capsulated cocci were proliferating extra-cellularly whereas the noncapsulated organisms had been phagocytosed by polymorphonuclear leucocytes.

It was concluded from these experiments that the possession of a capsule by strain PM 1 was one factor concerned in the capacity of this strain for multiplication in piglet blood. It seems likely that in protecting the cocci from phagocytosis the capsule functions in a manner analogous to the capsules of pneumococci and the M antigen of group A streptococci.

Table 2. *Survival of capsulated* Streptococcus suis *in sow and piglet blood*

Source of blood	C.F.U. in inoculum (0·1 ml.) added to blood (0·4 ml.)	C.F.U. in 0·1 ml. of inoculated blood after rotation at 37°C. for stated period				
		0 hr.	2 hr.	4 hr.	6 hr.	24 hr.
Sow	50	12	2	9	18	28
Sow	20	2	2	1	7	0
Piglet (8 days old)	50	6	8	+ +	+ +	+ +
Piglet (8 days old)	20	4	3	144	+ +	+ +

Effect of age of the pig on the bacteriostatic power of its blood

In the next experiment the growth of capsulated *S. suis* was compared in blood taken from a piglet and from its dam. A sample of each was inoculated and rotated with *S. suis* as in the previous experiment. Duplicate pour-plates for colony counts were made from 0·1 ml. samples taken before and at intervals during rotation.

Table 2 records the number of colonies developing in the pour-plates from a typical experiment. It can be seen that the capsulated cocci failed to multiply in adult blood but, as in the previous experiment, they grew freely in the piglet blood. Blood samples from four different sows, reared conventionally, were tested in this manner, but in no case did they permit growth of capsulated *S. suis*.

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'Buffy-coat' preparations made from adult sow blood and rotated with capsulated organisms showed mainly intracellular cocci. It was concluded that phagocytosis was probably responsible for preventing the growth of these streptococci in adult blood.

The next experiment sought to determine at what age the bacteriostatic property against capsulated *S. suis* appeared in the blood of pigs reared under conventional conditions.

Serial blood samples were taken from two piglets at intervals of from 4 to 28 days up to the age of 53 days (piglet No. 2) and 68 days (piglet No. 228). The samples were tested immediately after venepuncture for their capacity to inhibit the growth of capsulated *S. suis*. The bactericidal tests were performed as already described and although the samples could not be tested simultaneously an attempt was made to keep standard the conditions of the individual tests.

Table 3. *Effect of piglet age on bacteriostatic activity of its blood against capsulated* Streptococcus suis

Serial number of piglet	Age of piglet when tested (days)	C.F.U. in inoculum (0·1 ml.) added to piglet blood (0·4 ml.)	C.F.U. in inoculated blood after rotation at 37°C for stated period	
			0 hr.	6 hr.
2	16	256	1	+ +
	22	330	4	+ +
	32	272	2	+ +
	38	290	3	0
	44	142	1	2
	53	338	6	0
				3 hrs.
228	6	24	6	112
	10	45	11	+ +
	11	50	12	+ +
	20	124	31	+ +
	47	71	18	52
	55	55	14	60
	68	67	17	0

Piglet 2: C.F.U. were estimated from loopfuls of inoculated blood (surface streak).
Piglet 228: C.F.U. were estimated from 0·1 ml. samples of inoculated blood (pour-plates).

The results of several different tests on each piglet are set out in Table 3. It can be seen that the blood of both animals became bacteriostatic between 5 and 7 weeks after birth. Thirteen other piglets, conventionally reared, were tested in the same way and in all the blood became bacteriostatic in from 5 to 8 weeks.

From these experiments it was concluded that during the first weeks of life the blood of about 40% of conventionally reared and a higher proportion of gnotobiotic piglets permits the growth of capsulated *S. suis* when tested *in vitro*. At this age the polymorphonuclear leucocytes appear unable to phagocytose capsulated cocci although they are able to engulf noncapsulated cocci. During the first 5 to 8 weeks of life in a normal environment the piglets become resistant to infection with *S. suis* In the same period their blood becomes bacteriostatic for capsulated

S. suis and their polymorphonuclear leucocytes capable of disposing of these organisms by phagocytosis. It should be mentioned here that, in the case of two piglets delivered by hysterectomy and reared in 'pathogen-free' conditions, the blood had not become bacteriostatic when the final tests were made 156 days after birth. It seemed possible that in conventionally reared animals the bacteriostatic power of the blood resulted from the presence of antibody induced by previous infection with and specifically directed against *S. suis*. Experiments were therefore designed to show whether bacteriostatic activity could be transferred by serum from sow to piglet blood and, if so, whether such activity could be specifically 'blocked' by extracts of *S. suis*.

Capacity of serum from normal and gnotobiotic pigs to produce bacteriostatic activity in piglet blood

An experiment was designed to show whether bacteriostatic activity against capsulated *S. suis* could be transferred from sow to piglet blood by the addition of normal sow serum (Indirect Bactericidal Test).

Table 4. *Bacteriostatic effect of sow serum added to piglet blood inoculated with* Streptococcus suis *and rotated at* 37° *C*

Addition (0·1 ml.) to inoculated piglet blood (0·4 ml.)	C.F.U. in 0·1 ml.* piglet blood after rotation at 37° C. for stated periods	
	3 hr.	4 hr.
Sow serum diluted 1:1	1	0
Sow serum diluted 1:2	0	0
Sow serum diluted 1:5	6	1
Sow serum diluted 1:10	20	76
Sow serum diluted 1:20	380	+ +
Saline	400	+ +
Nil	260	+ +

* The inoculated piglet blood contained 14 C.F.U. in 0·1 ml. before rotation.

A heparinized sample of piglet blood was inoculated with *S. suis* and dispensed in vials in 0·4 ml. amounts. To these were then added 0·1 ml. amounts of normal sow serum serially diluted in normal saline. Control vials received piglet blood either alone or with saline instead of sow serum. The vials were then rotated at 37° C. and sampled at intervals for colony counts in pour-plates.

The results of this experiment are shown in Table 4. It can be seen that the cocci failed to grow freely in blood to which sow serum had been added in dilutions up to 1/10; higher dilutions permitted growth. Serum from five normal sows tested in this way was found to confer bacteriostatic activity when added to piglet blood in dilutions up to 1/10. The same result was achieved with serum from three piglets, 6 to 8 weeks old, whose blood had become bacteriostatic for *S. suis* at that age. On the other hand, serum from seven out of eight gnotobiotic pigs, aged from 11 to 22 weeks, failed to promote bacteriostatic activity when added to normal piglet blood. These results are set out in Table 5.

Table 5. *Effect of serum from conventionally reared and gnotobiotic pigs on growth of* Streptococcus suis *in piglet blood*

Pigs providing serum for test			
Method of Rearing	Age	Number tested	Number of pigs whose serum caused bacteriostasis when added to piglet blood
Conventional	2 years	5	5
Conventional	6–10 weeks	3	3*
Gnotobiotic	22 weeks	2	0
Gnotobiotic	11 weeks	6	1†

* Blood from these pigs did not show bacteriostatic activity against *S. suis* when tested before 5 weeks of age. † Three samples of serum taken from this animal at 1 to 8 weeks of age produced bacteriostasis against capsulated *S. suis* in normal piglet blood.

Table 6. *Effect of* Streptococcus suis *polysaccharides on bacteriostatic effect of normal sow serum added to piglet blood*

Addition made to piglet blood inoculated with *S. suis*	C.F.U. in inoculated blood* after rotation at 37°C. for stated period	
	3 hr.	4 hr.
Sow serum + capsular polysaccharide	138	++
Sow serum + cell wall polysaccharide	3	0
Sow serum + broth	15	1
Piglet serum	++	++

* The inoculated piglet blood contained seven C.F.U. in 0·1 ml. before rotation. The polysaccharides were tested in a final concentration of 0·08 mg/ml. of sow serum.

Specific inhibition of bacteriostatic activity by capsular polysaccharide from Streptococcus suis

It was obviously desirable to know whether the bacteriostatic effect of normal sow serum was specifically directed against *S. suis*. A serum sample was therefore treated with capsular polysaccharide from this micro-organism to see whether the bacteriostatic power was thereby reduced.

A partially purified preparation of capsular polysaccharide from *S. suis* (Elliott, 1966) was dissolved in saline to give a concentration of 0·1 mg./ml. To 0·4 ml. of this solution was added 0·1 ml. of normal sow serum. In control tubes the sow serum was diluted either in nutrient broth or in a saline solution of cell-wall polysaccharide (0·1 mg./ml.) extracted from the noncapsulated strain of *S. suis*, strain A 227. Indirect bactericidal tests were set up as in the previous experiment; piglet blood containing capsulated *S. suis* (0·4 ml.) was mixed with 0·1 ml. amounts of serum plus polysaccharide or serum plus broth, rotated at 37°C. and colony counts made in the usual manner.

The results of this experiment are shown in Table 6. It can be seen that capsular polysaccharide from *S. suis* in a final concentration of 0·08 mg. per ml. 'blocked' the bacteriostatic activity of sow serum added to piglet blood. Cell-wall polysaccharide from the noncapsulated variant failed to do so.

The results of this experiment strongly suggested that inhibition of the growth of *S. suis* in the blood of conventionally reared pigs was promoted by antibody specifically directed towards the capsules of these micro-organisms. Efforts to demonstrate such antibody by *in vitro* methods were unsuccessful; the methods employed included precipitation, agglutination, complement fixation and anti-globulin tests. An experiment was therefore designed to show whether antibody could be demonstrated in normal sow serum by its specific protection of susceptible piglets against experimental infection with *S. suis*.

Protective effect of normal sow serum against Streptococcus suis *infection in piglets*

Elliott *et al.* (1966) showed that bacteriaemia can be produced in susceptible piglets by spraying broth cultures of *S. suis* into the nasopharynx and that such infection can be prevented by the prior administration of serum from piglets convalescent from experimental infection. In the protection experiment now to be described 28 piglets were divided equally into two groups. The animals in one group had received subcutaneously 20 ml. of normal sow serum. Twenty-four hours later 5 ml. of an overnight broth culture of *S. suis* was sprayed into the nasopharynx of each of the 28 animals comprising both groups. Blood cultures were made from all the animals 2 days later and thereafter periodically up to 7 days.

Of the 14 animals that had received normal sow serum only one developed a bacteriaemia within 7 days of spraying. Of the 14 that received no serum, ten developed a *S. suis* bacteriaemia within 7 days of experimental infection.

Separation of bacteriostatic component from normal sow serum

The experimental evidence here presented strongly suggested that the resistance of adult pigs to *S. suis* infection resulted from active immunity following subclinical infection contracted in early life. The circulating antibody responsible appeared to be directed specifically against the capsular polysaccharide of *S. suis*. It was present in the blood in concentrations sufficient for detection by protection tests or by the bactericidal test but too small for recognition by the other *in vitro* serological tests employed. We therefore attempted to separate the responsible component in pig serum by fractionation procedures such as would enable us to compare its physical properties with those of the known immunoglobulins.

With this object in view 550 ml. of normal sow serum was treated in the manner shown in Fig. 1. Details of the procedures used are given below and under Materials and Methods.

Step 1. *Euglobulin precipitation.* Serum from a 2-year-old sow was separated into euglobin and pseudoglobulin fractions by dialysis against distilled water. Both fractions were made to the original volume of serum and tested at a dilution of 1/25 for bacteriostatic activity (Table 7). Only the pseudoglobulin fraction showed the activity at this dilution. The euglobulin showed activity undiluted and at a 1/10 dilution.

Step 2. Ammonium sulphate precipitation. The pseudoglobulin fraction was brought to 0·3 saturation with solid ammonium sulphate in the cold. The precipi-

tate (0·3 fraction) was centrifuged, re-dissolved in water and dialysed against physiological saline. The supernatant was brought to 0·5 saturation by the addition of further ammonium sulphate and a 0·3–0·5 fraction collected. The second supernatant was brought to 0·7 saturation and a 0·5–0·7 fraction obtained. The final supernatant was discarded.

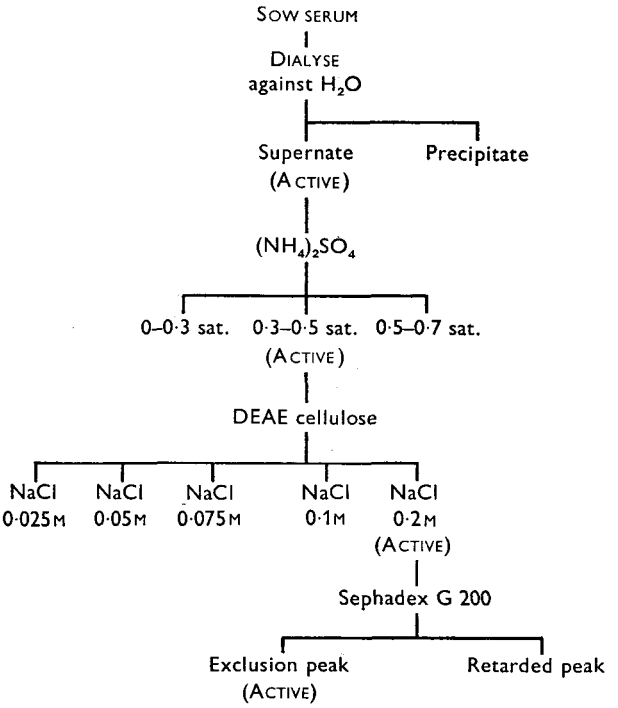

Fig. 1. Separation of bacteriostatic component from sow serum.

The three fractions were tested for bacteriostatic activity at a dilution corresponding to 1/10 of the initial pseudoglobulin concentration (Table 7). Only the 0·3–0·5 saturation fraction showed activity. Roughly 6·5 g. of this fraction were recovered from 550 ml. serum (approximately 12 mg./ml.)

Step 3. *Ion exchange chromatography on DEAE cellulose.* The 0·3–0·5 saturation ammonium sulphate fraction from pig pseudoglobulin was further fractionated on DEAE cellulose as described under Materials and Methods. Roughly 4 g. protein was applied to a column containing approximately 800 ml. ion exchanger. After the exclusion peak had been collected, step-wise elution was performed with starting buffer containing NaCl to a final concentration of 0·025 M, 0·05 M, 0·075 M, 0·1 M and 0·2 M, successively.

The eluted fractions were pooled, precipitated by half saturation with ammonium sulphate, dissolved in water and dialysed against physiological saline. The final volume of each fraction was approximately 20 ml., corresponding roughly to a 25-fold concentration of the serum. The fractions were tested for bactericidal activity diluted 1/20. As may be seen in Table 7, only the 0·2 M NaCl fraction showed inhibitory activity in these conditions. The protein concentration of this fraction

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was approximately 30 mg./ml. Roughly 600 mg. of this fraction was recovered from 550 ml. serum (approximately 1 mg./ml.).

Step 4. Gel filtration on G200 Sephadex. Ten ml. of the 0·2 M-NaCl fraction from DEAE fractionation were applied to a 2 l. G200 Sephadex column. Blue dextran and haemoglobulin were added as markers. The elution pattern is shown in Fig. 2. Fractions were pooled as shown and concentrated by ammonium sulphate precipitation as before. It will be seen from Table 7 that only the exclusion peak showed bacteriostatic activity.

Table 7. *Bacteriostatic effect of sow serum fractions added to piglet blood inoculated with* Streptococcus suis

Fractionation of sow serum	Serum fraction (0·1 ml.) added to inoculated piglet blood (0·4 ml.)	C.F.U. in 0·1 ml. piglet blood after rotation at 37°C. for stated period		
		0 hr.	3 hr.	4 hr.
Step 1				
Dialysis V.H_2O	Supernate dil. 1 = 25 (pseudoglobulin)	11	10	6
	Precipitate dil. 1 = 25 (euglobulin)		117	++
Step 2				
$(NH_4)_2SO_4$ added to supernate from step 1	Precip. from 0·3 sat. (Fract. 1)	10	9	155
	Precip. from 0·3 to 0·5 sat. (Fract. 2)		0	0
	Precip. from 0·5 to 0·7 sat. (Fract. 3)		35	230
Step 3				
DEAE cellulose treatment of fract. 2 from step 2	Peak 1 eluted by buffer	16	40	136
	Peak 2 eluted by NaCl 0·025 M		12	155
	Peak 3 eluted by NaCl 0·05 M		12	++
	Peak 4 eluted by NaCl 0·075 M		46	75
	Peak 5 eluted by NaCl 0·1 M		10	173
	Peak 6 eluted by NaCl 0·2 M		1	3
Step 4				
Gel filtration* of peak 6 from step 3	Exclusion peak	8	4	5
	Retarded peak		23	275

Appropriate controls were included in all tests but have been omitted from Table 7 because inactive fractions also served as 'negative' controls. * Gel filtration was carried out using Sephadex G200.

Effect of 2-mercaptoethanol on bacteriostatic activity of sow serum

Unfractionated sow serum and the bacteriostatic component obtained by Sephadex filtration of the active fraction above described were mixed each with an equal volume of 0·2 M 2-mercaptoethanol and incubated at 37°C. for 2 hr. The mercaptoethanol was then removed by prolonged dialysis against normal saline. The nondialysable residue was sterilized by filtration and tested for bacteriostatic activity against capsulated *S. suis* in piglet blood.

The results of this experiment are shown in Table 8, from which it can be seen that the bacteriostatic activity of both the unfractionated sow serum and the

active component obtained by gel-filtration were destroyed by the mercaptoethanol treatment, thus confirming the provisional identification of an IgM immunoglobulin as the active component.

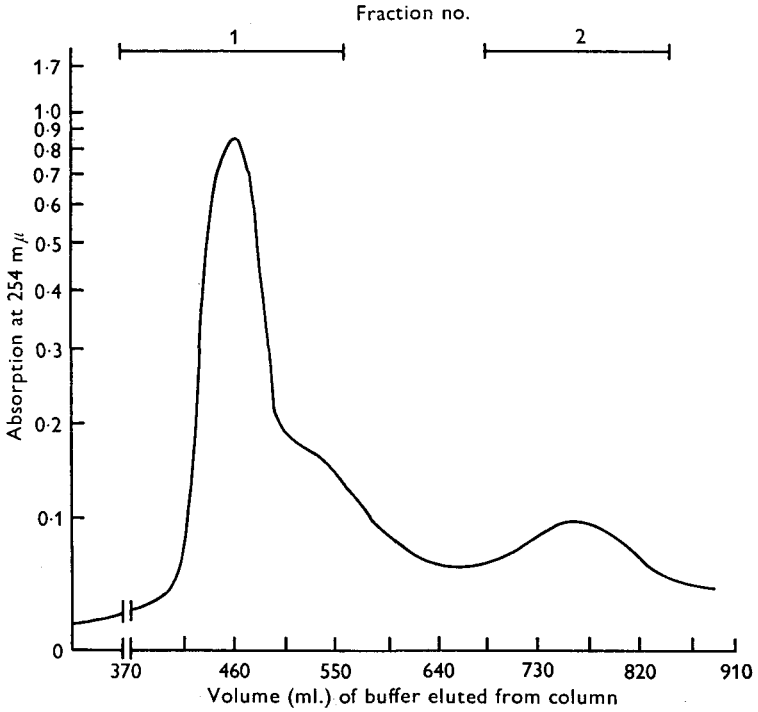

Fig. 2. G 200 Sephadex gel filtration of active fraction from DEAE cellulose column.

Table 8. *Effect of 2-mercaptoethanol on bacteriostatic activity of sow serum and its active component separated by gel filtration**

Addition (0·1 ml.) to piglet blood inoculated with *S. suis* (0·4 ml.)	C.F.U. in 0·1 ml. of inoculated blood after rotation at 37°C. for stated period	
	3 hr.	4 hr.
Sow serum before SH treatment	7	4
Sow serum after SH treatment	74	+ +
* Gel filtration exclus. peak before SH treatment	1	5
* Gel filtration exclus. peak after SH treatment	42	151
Saline	130	+ +

* See step 4, Table 7. SH = 2 Mercaptoethanol (0·2 M). The inoculated piglet blood contained 14 C.F.U. in 0·1 ml. before inoculation.

DISCUSSION

Streptococci cause neonatal infections in a variety of domesticated animals including man, (Eickhoff *et al.* 1964), horses (Gunning, 1947), sheep and pigs. The micro-organisms responsible for these infections of the newborn rarely cause

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disease in adults of the same species and they are usually without virulence for laboratory animals. Evidence presented here suggests that in pigs specific immunity is a factor in adult insusceptibility and that such immunity may result from inapparent infections of early life.

S. suis is the usual cause of streptococcal infection in piglets. Adult pigs are insusceptible to experimental infection with these micro-organisms, whereas piglets within the first 4 weeks of life can be infected by spraying broth cultures into the nasopharynx. We have shown that with increasing age the blood of piglets becomes inhibitory to the growth of *S. suis* and it seems reasonable to suppose that this is associated with decreasing susceptibility to infection. The bacteriostatic action of the blood results from phagocytic activity mediated by a serum component directed specifically against the capsular polysaccharide of the streptococci.

Such evidence as we have been able to gather from the behaviour of this component under the fractionation procedure applied to pig serum suggests that it belongs to the IgM class of macroglobulins. Its presence in greater part in the pseudoglobulin fraction of the pig serum was an unexpected finding but its behaviour on Sephadex and DEAE cellulose and the destruction of its bactericidal activity by mercaptoethanol confirms its identification as IgM. We cannot say with certainty that this antibody results from previous infection with *S. suis*, but three facts support such a possiblity: first, there is evidence that of normal sows at least 7% carry *S. suis* in the upper respiratory tract (Elliott *et al.* 1966); secondly, the same investigators found that in naturally occurring outbreaks of *S. suis* infection, throat cultures reveal all piglets of a litter to be infected although a minority may show signs of overt disease; and finally, in the present investigation three out of four pigs reared under pathogen-free conditions failed by the age of 6 months to develop bacteriostatic activity in their blood against *S. suis*; in our experience pigs reared under conventional conditions develop such activity within the first 8 weeks of life. From these considerations it seems likely that, where *S. suis* is concerned, inapparent infections may greatly exceed in number those causing overt diseases and may be the source of the specific immunity of adult pigs.

We have no explanation to offer for our observation that, of the normal piglets examined within 3 weeks of birth, the blood of nearly 60% was bacteriostatic for *S. suis*. Presumably these were immune to infection. Sometimes piglets with and without bacteriostatic activity were found in the same litter. We have not examined colostrum for bacteriostatic antibody although this might be one source of passive immunity during the first weeks of life. It seems likely that with respect to the amount of colostrum received all piglets in a litter are not equal.

SUMMARY

Phagocytosis prevents the multiplication of *Streptococcus suis* in blood samples from conventionally reared adult pigs. It is mediated by antibody, probably IgM, present in the serum of adults and specifically directed against the capsular polysaccharide of *S. suis*. The blood of young pigs reared in a 'pathogen-free' environ-

ment allows multiplication of encapsulated *S. suis*. Of 160 piglets conventionally reared, the blood of 92 (58 %) did not permit multiplication of *S. suis*. Blood from the remaining 68 (42 %) allowed multiplication but became bacteriostatic when the animals reached the age of 6 to 8 weeks. Serum from adult pigs confers bacteriostatic activity on blood from susceptible piglets *in vitro* and passively protects such piglets against experimental *S. suis* infection *in vivo*.

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REFERENCES

Blakemore, F., Elliott, S. D. & Hart-Mercer, J. (1941). Studies on suppurative polyarthritis (joint-ill) in lambs. *J. Path. Bact.* **52**, 57.

Eickhoff, T. C., Klein, J. O., Daly, A. K., Ingall, D. & Finland, M. (1964). Neonatal sepsis and other infections due to group B beta-haemolytic streptococci. *New Engl. J. Med.* **271**, 1221.

Elliott, S. D., (1966). Streptoccocal infection in young pigs. I. An immunochemical study of the causative agent (*PM* streptococcus). *J. Hyg., Camb.* **64**, 205.

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

Field, H. I., Buntain, D. & Done, J. I. (1954). Studies on piglet mortality. I. Streptococcal meningitis and arthritis. *Vet. Rec.* **66**, 653.

Gunning, O. V. (1947). Joint-ill in foals (pyosepticaemia) with special reference to the prophylactic treatment of the foal at birth. *Vet. J.* **103**, 47.

Lancefield, R. C. (1957). Differentiation of group A streptococci with a common R antigen into 3 serological types with special reference to the bactericidal test. *J. exp. Med.* **106**, 525.

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