THE ANTIPNEUMOCOCCUS PROPERTIES OF NORMAL SWINE SERUM

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In 1921, Bull and McKee (1) reported that the serum of normal chickens when injected into mice and guinea pigs confers upon them a notable degree of specific immunity to infection with pneumococci of various types. They also showed that the factor responsible for this immunity is associated with the serum globulin. Robertson and Sia (2), 1924 to 1927, by using serum-leucocyte mixtures, demonstrated the presence of naturally occurring antipneumococcus opsonins in the blood of certain normal animals which are resistant to pneumococcus infection. Evidence was presented which indicated that the degree of resistance of these animals is proportional to the concentration of the opsonins in their blood. These opsonins were described as thermolabile, being destroyed on heating at 65°C., and appeared to be type-specific in action. During the course of the experiments it was noted that the serum of the more resistant animals agglutinated type-specific, virulent pneumococci. Of the animal sera examined, that of the pig showed the greatest opsonic action.

In 1929, Sia (3) found that the serum of normal pigs injected intraperitoneally into white mice confers upon them a marked degree of resistance to pneumococcus infection and showed by the method of absorption with virulent pneumococci that the protective action of the pig serum is of a type-specific character. In a personal communication he has stated that the principles of the serum upon which the protective action depends are thermolabile, and are associated with the serum globulin.

The following study is a repetition of certain of Sia's experiments, and a further analysis of the antipneumococcus immune reactions of normal swine serum.

Material and Technique

Normal Animal Sera.—The swine serum was obtained from blood of full grown, normal animals. After several hours in the ice box the serum was separated by centrifugation, passed through a Berkefeld filter, and stored in the ice box at a temperature of approximately 5°C. Other normal animal sera were obtained in the same way, either from the abbatoir, or from cardiopuncture, and were treated in the same manner.

Normal Serum Globulin.—This was obtained from the swine serum by the addition of an equal volume of a saturated solution of ammonium sulfate. The precipitate was washed and dialyzed against distilled water. The dialysate was taken up in a slightly alkaline solution of physiological saline.

Immune Sera.—The antipneumococcus horse serum was furnished by the New York State Board of Health. The antipneumococcus rabbit serum was prepared by injecting normal rabbits intravenously with heat-killed suspensions of type-specific strains of Pneumococcus, after the method of Cole and Moore.

Cultures.—The stock, type-specific, virulent pneumococcus cultures, Types I, II, and III, were kept in blood broth and passed through mice often enough to maintain a degree of virulence such that 0.000,000,1 cc. of an 8 hour subculture would regularly kill 18 gm. mice within 36 hours.

The stock cultures of non-type-specific, avirulent, R pneumococci used were derived from strains of the virulent S stock cultures.

Bacterial Suspensions.—10 hour broth cultures of pneumococci were heated at 65°C. for 30 minutes. The bacteria were removed by centrifugation and resuspended in one-tenth the original volume of salt solution. Suitable dilutions were made just before use.

Sheep Corpuscles.—These were obtained from defibrinated sheep blood collected at the abbatoir. They were washed and diluted to 5 per cent of blood concentration with normal saline.

Hemolytic Amboceptor.—This serum was prepared by injecting intravenously into normal rabbits at 3 to 5 day intervals gradually increasing numbers of washed sheep erythrocytes.

Complement.—Serum was removed from blood obtained by cardiopuncture of large normal guinea pigs.

Protection Tests.—These were performed in duplicate, employing white mice weighing from 16 to 18 gm. Unless otherwise indicated, the serum was injected intraperitoneally 4 hours before the culture was given. Surviving animals were kept under observation for 7 days after injections.

Agglutination Tests.—With type-specific, virulent pneumococci, 0.1 cc. of the concentrated bacterial suspension was added to varying amounts of the serum (0.5 to 4.0 cc.) and sufficient saline was added to make the final volume in each tube equal 4.1 cc. These suspensions were placed at 37°C. for 2 hours, and in the ice box overnight. They were examined for agglutination before and after centrifugation at 1200 R.P.M. for 10 minutes.

Agglutination tests with avirulent R pneumococci were performed in the usual way with 0.5 cc. of suitable bacterial suspension, and 0.5 cc. of diluted serum, kept at 37°C. 2 hours, and in the ice box overnight.

Precipitin Tests.—0.2 cc. of serum, and 0.5 cc. of the diluted substances used as antigen were made up with normal saline to a final volume of 1.0 cc. The mixtures were incubated at 37°C. for 2 hours, and kept in the ice box overnight.

Antipneumococcus Protective Action

In accord with Sia's results, it was found that the serum of normal swine injected intraperitoneally confers upon white mice a marked degree of temporary immunity to pneumococcus infection. Under the same conditions the serum of other normal animals, including that of chickens, showed no such action (Table I).

The maximum protective action of swine serum is obtained when 1 cc. of the serum is injected intraperitoneally into mice 4 to 8 hours before inoculation with pneumococcus culture. The degree of the protective action of the serum against pneumococcus infection varies with the type of the pneumococci employed. The protective power is greatest against pneumococci of Type II, somewhat less against those of Type I, and much less, though definite, against pneumococci of Type III.

The concentration of the protective principles in normal swine serum, as indicated by the optimal protective dose for mice, is relatively low in comparison with the high concentration of protective antibody in the serum of animals artificially immunized by injections of pneumococci. The approximate amounts of antipneumococcus horse and rabbit serum needed to give the same relative degree of protection that is afforded by the optimal dose of the swine serum, are given in Table II.

The antipneumococcus protective power of normal swine serum is greatest when the serum is fresh. On standing, the protective power gradually falls off, and after 3 or 4 months in the ice box this capacity is usually entirely lost. The protective action of the serum against infection with Type I pneumococci is usually lost more rapidly than that against infection with Type II pneumococci. A specimen of the serum which when fresh affords average protection against both Types I and II pneumococci infection may, after standing 8 weeks, show no protective action against pneumococci of Type I, and still

TABLE I

Tests for Antipneumococcus Protective Action with the Serum of Normal Animals

	Pneumococcus culture	Mouse protection tests with normal sera													
		Swine		Ox		Sheep		Guinea pig		Rabbit		Chicken		Pigeon	
	Type.....	I	II	I	II	I	II	I	II	I	II	I	II	I	II
	cc.														
1.0 cc. serum—intraperitoneally. 4 hr. interval	0.1	DD	DD	—	—	—	—	—	—	—	—	—	—	—	—
	0.01	DD	SS	—	—	—	—	—	—	—	—	—	—	—	—
	0.001	SS	SS	—	—	—	—	—	—	—	—	—	—	—	—
	0.000,1	SS	SS	—	—	—	—	—	—	—	—	—	—	—	—
	0.000,01	SS	SS	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD
	0.000,001	SS	SS	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD
1.0 cc. serum alone.....		S		S		S		S		S		S		S	

0.000,000,1 cc., Type I culture, without serum, D
0.000,000,1 cc., Type II culture, without serum, D

D = died. S = survived. — = not done.

protect mice against a thousand lethal doses of Type II culture. The serum appears to retain its protective capacity undiminished for a longer time if kept frozen.

The protective principles of normal swine serum are thermolabile. They are destroyed completely by heating the serum to 65°C. for 30 minutes. Moreover, the protective power which has been lost by heating or thorough aging is not restored by the addition of 10 per cent of fresh serum.

TABLE II

A Quantitative Comparison of the Protective Action of Normal Swine Serum with That of Antipneumococcus Horse and Rabbit Sera against Pneumococcus Infection

	Type II pneumococcus culture	Results of mouse protection tests with					
		Normal (swine)		Type II (horse)		Type II (rabbit)	
		1.0 cc.	0.5 cc.	0.002 cc.	0.001 cc.	0.005 cc.	0.002 cc.
	cc.						
Serum in 1.0 cc. volume. 4 hr. interval	0.1	DD	—	DD	—	DD	—
	0.01	SS	—	SS	DD	SS	—
	0.001	SS	DD	SS	DD	SS	DD
	0.000,1	SS	DD	SS	SS	SS	DD
	0.000,01	SS	SS	SS	SS	SS	SS
	0.000,001	SS	SS	SS	SS	SS	SS

0.000,000,1 cc. culture, without serum, D

D = died. S = survived. — = not done.

The antipneumococcus protective principles of normal swine serum can be specifically absorbed with type-specific, virulent, S pneumococci. When the serum is treated for a short time with relatively small numbers of pneumococci, the capacity to protect mice against infection with pneumococci of the homologous type is completely lost, while the protective action against pneumococci of heterologous types remains unchanged. The minimal number of pneumococci specifically absorbing the protective principles within 30 minutes was found to be in the ratio of two volumes of a 10 hour pneumococcus broth culture to one volume of serum.

The protective action against Type I and Type II pneumococcus infection was also found to be markedly reduced by absorption of the serum with non-type-specific avirulent R pneumococci. While with the numbers of bacteria employed the protective principles of the serum were not always completely absorbed by these degraded pneumococci, they were always reduced in titre.

TABLE III

The Loss in Antipneumococcus Protective Power of Normal Swine Serum by Aging and by Heating

	Pneumococcus culture	Results of mouse protection tests with swine serum									
		When fresh		Kept below 5°C. for				Heated at 65°C. 30 min.		Inactivated serum plus 10 per cent of fresh serum	
				8 wks.		16 wks.					
	Type.....	I	II	I	II	I	II	I	II	I	II
	cc.										
1.0 cc. serum. 4 hr. interval	0.1	—	DD	—	—	—	—	—	—	—	—
	0.01	DD	SS	—	DD	—	—	—	—	—	—
	0.001	SS	SS	—	DD	—	—	—	—	—	—
	0.000,1	SS	SS	—	SS	—	—	—	—	—	—
	0.000,01	SS	SS	DD	SS	DD	DD	DD	DD	DD	DD
	0.000,001	SS	SS	DD	SS	DD	DD	DD	DD	DD	DD
1.0 cc. serum only.	S	S		S		S		S		S	

0.000,000,1 cc. Type I culture, without serum, D
0.000,000,1 cc. Type II culture, without serum, D

D = died. S = survived. — = not done.

Absorption with bacteria other than Pneumococcus, or with non-specific adsorbents, for example charcoal and kieselguhr, did not, under the conditions of the experiments, affect the antipneumococcus protective action of swine serum.

The protective action was not affected by the addition of the specific capsular polysaccharide of Pneumococcus, either when added to the serum before injection or when given with the culture. On the other hand, under similar conditions, when antipneumococcus horse se-

rum was so diluted that the protective power per volume was equivalent to that of swine serum, and to this diluted serum there was added the homologous pneumococcus polysaccharide, the protective power of the horse serum was entirely lost.

That the protective action of swine serum does not depend on the presence of complement is shown by the effects of heating the serum to 56°C. for 10 minutes. This was found to inactivate completely

TABLE IV

The Antipneumococcus Protective Action of Normal Swine Serum after Absorption with S and R Pneumococci, and Non-Specific Agents (Charcoal and Kieselguhr)

	Pneumococcus culture	Results of mouse protection tests with swine serum							
		Unabsorbed—normal serum		Absorbed by Type II S pneumococci		Absorbed by R pneumococci		Absorbed by non-specific adsorbents	
	Type.....	I	II	I	II	I	II	I	II
	cc.								
1.0 cc. serum. 4 hr. interval	0.1	—	DD	—	—	—	—	—	DD
	0.01	DD	SS	DD	—	—	—	DD	SS
	0.001	SS	SS	SS	—	—	—	SS	SS
	0.000,1	SS	SS	SS	—	DD	DD	SS	SS
	0.000,01	SS	SS	SS	DD	DS	SS	SS	SS
	0.000,001	SS	SS	SS	DD	SS	SS	SS	SS

0.000,000,1 cc. Type I culture, without serum, D
0.000,000,1 cc. Type II culture, without serum, D

D = died. S = survived. — = not done.

the complement not removed by filtration, but the antipneumococcus protective action of filtered serum was not changed by this treatment.

There is in normal swine serum a natural hemolysin for sheep erythrocytes that requires complement for the completion of its action. 0.5 cc. of the serum will usually completely hemolyze 1.0 cc. of 5 per cent sheep corpuscles in the presence of 0.05 cc. of guinea pig complement within 1 hour at 37°C. The antipneumococcus protective action of previously inactivated normal swine serum is not altered by absorption of this heterophile antibody with sheep corpuscles.

The principles in the serum responsible for the specific protective action are contained in the globulin fraction. These facts are illustrated by a typical experiment recorded in Table V.

The Antipneumococcus Agglutinating Action

As Sia observed, type-specific virulent pneumococci are agglutinated by the undiluted or slightly diluted serum of normal swine. This is best demonstrated when the amounts of serum employed are relatively large in proportion to the number of pneumococci present, and when the suspension is slowly centrifugalized after it has been

TABLE V

Antipneumococcus Protective Action of the Globulin Fraction of Normal Swine Serum and of the Whole Serum after Inactivation of the Complement and Absorption of the Hemolysin

	Type II pneumococcus culture	Results of mouse protection tests with			
		Normal serum	Globulin (in serum volume)	Serum, heated, 56°C., 10 min.	Heated serum, absorbed by sheep r.b.c.
	cc.				
1.0 cc. serum, 4 hr. interval	0.1	DD	DD	DD	DD
	0.01	SS	SS	SS	SS
	0.001*	SS	SS	SS	SS
0.000,000,1 cc. culture, without serum, D					

D = died. S = survived.

* Mice receiving smaller doses of culture also survived.

kept for 2 hours at 37°C., and overnight in the ice box. The agglutinated pneumococci form a firm disc, similar to that seen following specific agglutination with homologous antipneumococcus serum. The agglutinating action of swine serum is greatest with Type III, and least marked with Type I Pneumococcus. This action is lost after heating the serum at the same temperature at which the antipneumococcus protective principle is destroyed. It is also lost after absorption with homologous S organisms. The serum of other animals tested did not agglutinate virulent S pneumococci to any considerable degree.

In addition, it has been found that avirulent R pneumococci are

TABLE VI

The Agglutination of R Pneumococci by Normal Swine Serum and the Serum of Other Normal Animals

Serum dilutions	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
(*a*) Swine serum									
When fresh	+++	++++	++++	++++	++++	++++	++++	++	−
After 16 wks., 5°C.	+++	++++	++++	++++	++++	++++	++	+	−
Heated, 65°C., 30 min.	+++	++++	++++	++++	+++	+++	+++	+	−
Absorbed by Type II Pneumococcus	+++	++++	++++	++++	+++	+++	+++	±	−
(*b*) Other animal sera									
Ox	++++	++++	++++	+++	++	−	−	−	−
Sheep	++++	++++	++	±	−	−	−	−	−
Rabbit	++++	+++	±	−	−	−	−	−	−
Chicken	++++	++	±	−	−	−	−	−	−
Pigeon	++++	++++	++++	++++	++++	++	−	−	−

Separate controls of the bacterial suspension and sera in saline—negative.

++++ = complete agglutination; − = no agglutination.

also agglutinated in normal swine serum, and this occurs even when the serum is diluted as high as 1:640. The agglutinated R pneumococci form a flocculent sediment, in no way distinguishable from that produced by the use of the sera of animals artificially immunized to R pneumococci. This property is not destroyed by heating the serum to 65°C., and even after the serum has been kept for a long time it still retains this property, even though at this time the protective action is entirely lost. No relation between the agglutination titre for avirulent R pneumococci and the antipneumococcus protective power of fresh serum was evident. Specific absorption of the protective principle with virulent S pneumococci produced no significant change in the capacity of the serum to agglutinate avirulent R pneumococci. The serum of certain other normal animals was also found to agglutinate avirulent R pneumococci, but never in as high dilutions as did swine serum.

The principles in swine serum responsible for the agglutination of both R and S pneumococci, are also contained in the globulin fraction of the serum (Table VI).

Other Immunological Properties of Swine Serum

Swine serum seems to render virulent pneumococci susceptible to phagocytosis. Stained films of the peritoneal exudate of infected mice which have received serum show active phagocytosis by the polymorphonuclear leucocytes. Cultures of the exudate made at intervals show a gradually decreasing number of pneumococci, and after 4 to 6 hours the cultures are sterile.

Virulent pneumococci previously treated with normal swine serum stimulate little or no specific antibody response when injected intravenously into normal rabbits. On the other hand, animals given pneumococci treated with heated swine serum show the same degree of specific antibody response as do animals given an equal number of untreated pneumococci.

Neither virulent S nor avirulent R pneumococci showed any change in their morphology or in the character of their colonies after repeated transfers in media containing normal swine serum in various dilutions.

No precipitation occurs in mixtures of pneumococcus polysaccharides and swine serum, at least not with the amounts of the two

substances employed in our tests. Serum, in the amounts it was possible to use in complement fixation studies, when mixed with the polysaccharide of Type II did not fix complement. However, the presence of swine serum does not interfere with the precipitating action of immune horse or rabbit serum on the homologous polysaccharide, nor does it modify the fixation of complement in mixtures of immune serum and the homologous polysaccharide.

When normal swine serum is mixed with the nucleoprotein derived from avirulent R pneumococci, precipitation occurs, and this takes place when the nucleoprotein is in a dilution as high as 1:50,000. On the other hand, fixation of complement did not occur in these mixtures. The presence of swine serum, however, did not interfere with the fixation of complement in a mixture of nucleoprotein and the serum of a rabbit artificially immunized to this substance.

SUMMARY

Certain interesting facts have been obtained regarding the action of normal swine serum, but in the light of present knowledge it is not easy to interpret them. It has been found that the normal serum possesses the property of protecting mice from infection with virulent pneumococci, and of agglutinating virulent S pneumococci and also avirulent R pneumococci.

The protective action of the serum is specific, that is, absorption of the serum with pneumococci of one type removes or destroys only the property of the serum which is responsible for its protective action against pneumococci of that particular type, leaving the serum still active against pneumococci of other types. In this particular, therefore, the swine serum resembles a polyvalent antipneumococcus serum produced by artificial immunization.

In other particulars, however, the swine serum differs from that produced by artificial immunization. In the first place, the protective action exhibited by normal swine serum against type-specific pneumococci is very slight compared with that of animals which have been artificially immunized, even though the latter have received only a very few injections of the specific antigen. Moreover, the protective properties of the swine serum are destroyed by heating to 65°C., and they disappear after a few weeks, even if the serum is kept in the cold. Also

the protective action of the serum is diminished after the serum has been mixed with non-type-specific avirulent R pneumococci and the latter have been removed by centrifugalization. A further difference between the action of swine serum and that of the antipneumococcus serum produced by artificial immunization is that although the swine serum is type-specific in its action in protecting mice, nevertheless when this serum is mixed with a purified type-specific polysaccharide, neither precipitation nor fixation of complement results; and, finally, the action of the serum in protecting mice against pneumococci of a particular type is not inhibited by the addition of the homologous polysaccharide to the serum. These latter facts relating to the specific polysaccharide, and especially the fact that the specific protective action disappears after applying the so called absorption technique with non-type-specific organisms, are most difficult to harmonize with the present conceptions of the nature of pneumococcus type specificity. It is not proposed at the present time to offer any theoretical explanation of these phenomena.

The studies have also confirmed the observations of Robertson and Sia, that, if a proper technique be employed, normal swine serum exhibits the property of agglutinating type-specific pneumococci, and that the agglutinins for pneumococci of one type may be specifically absorbed, leaving those for pneumococci of other types unchanged.

Finally, it has been shown that swine serum causes the agglutination of avirulent R pneumococci. This property of the serum is not destroyed by heating at 65°C., and therefore probably depends on factors other than those responsible for the agglutination of S pneumococci, and for the protective action of the serum in mice.

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