THE ARONSON STREPTOCOCCUS

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The origin of this classical streptococcus, with which much experimental work has been done in Germany, is now uncertain, and, moreover, strains designated "Streptococcus Aronson" which have been obtained from different laboratories have not always been identical. My first strain was sent to me in December, 1927, by Prof. Neufeld, whose kindness I wish to acknowledge; it was in the form of a dried mouse's spleen which produced fatal septicaemia when injected into a fresh mouse. On plating the blood of the mouse on fresh horse blood agar plates I was struck by the difference in the appearances of the surface colonies from those with which I had become familiar in my study of scarlatinal and puerperal streptococci. Further observations on the virulence and on the immunological properties confirmed me in the view that the Aronson strain could be differentiated from haemolytic streptococci of the *Streptococcus pyogenes* group.

Later I acquired, through the kindness of Dr L. Colebrook, two other strains designated Streptococcus Aronson. One which came from Prof. Wamoscher's laboratory was identical in its high virulence for mice and in its serological characters with that which I had received from Prof. Neufeld. The other was a strain from Prof. Schnitzer's laboratory, and was the laboratory strain from the Serum Department, Chemische Fabrik Schering. It was also of high virulence for mice and resembled *Str. pyogenes* both in the appearance of the colonies and in the formation around them of large clear haloes on fresh horse blood agar plates, but I was unable to identify it with any of the twenty-seven serological types of streptococci described in my previous paper. In January, 1931, Prof. Schlossberger kindly sent me his strain which I found to be identical with the Neufeld and Wamoscher strains. I have also tested Dr G. H. Eagles' strain, Aronson R2, and this I found to be a *Str. pyogenes*, Type 5.

The observations which I have described in this paper relate chiefly to the Aronson type of streptococcus from Prof. Neufeld's laboratory, and in order to distinguish it from Prof. Schnitzer's strain, with which it will be frequently compared, I have used for the former the designation Aronson N and for the latter Aronson S.

Yoshioka (1923) and Killian (1924) have shown that active immunity in mice and passive immunity in rabbits can be obtained against the Neufeld strain of Streptococcus Aronson. Lancefield (1933), differentiating haemolytic streptococci from various animal sources by precipitation tests, places the Aronson Streptococcus (Wamoscher), O90 in her series, in Group B, a group comprising mainly streptococcal strains of bovine origin, and the Aronson

Streptococcus (Schnitzer), O89 in her series, in Group A, which contains strains chiefly of human origin. In a later paper, 1934, she showed that Group B haemolytic streptococci can be differentiated into specific types by the method of the precipitation reaction, and that the type-specific substances are polysaccharides. Loewenthal (1932) described four different colony forms which may be assumed by haemolytic streptococci. He observed that the Aronson strain of the "Robert Koch" Institute grew in the "O" form which produced slightly haemolytic, moist, white colonies and was highly virulent for mice by intraperitoneal inoculation; immune sera prepared with it protected mice against many lethal doses and agglutinated the "O" form with the formation of clumps characteristic of a type-specific carbohydrate reaction. He remarked that the "O" form occurred in laboratory strains which had undergone many passages through mice, though recently he had found it in freshly isolated strains.

Colonial characters

The surface colonies of Aronson N on horse blood agar plates after incubation for 24 hours resemble pneumococcus colonies. When the culture is derived from the blood of a mouse which has died of septicaemia, they appear as smooth, shiny, circular discs, which are of a soft consistency and spread like paint. There may be a slight clear halo around the colony, not more pronounced as a rule than that around a Type 1 pneumococcus colony, and around and under it is a deposit of fine greenish granules. (There was more lysis around the surface colonies of the Wamoscher strain.) The Aronson N colonies differ from those of pneumococci in being whiter and less translucent, and in the absence of autolytic change, *i.e.* there is no flattening of the colonies, though the margins of the colonies may become heaped up after longer incubation. The absence of a clear zone (*beta* haemolysis) around the surface colonies differentiates them from colonies of *Str. pyogenes*. There are morphological differences also, the colonies of the latter being generally more opaque, thicker in consistency and difficult to emulsify; sometimes, however, *Str. pyogenes* colonies which are tending to become mucinous may form translucent discs very similar to Aronson N colonies.

On mouse and rabbit blood agar plates Aronson N, when first received, produced surface colonies with large clear areas around, which were hardly distinguishable from *Str. pyogenes* colonies except for a deposit of brownish pigmented granules close to the growth; it lost this haemolytic property after subcultivation.

The colonies of Aronson N in the depth of horse blood agar developed perfectly clear haloes and were indistinguishable from similar colonies of Aronson S and other *Str. pyogenes* strains. A curious change was noted in shake cultures of Aronson N in mouse blood agar due to the diffusion of brownish pigment similar to that which forms around the surface colonies on this medium.

Aronson S invariably produced on suitably moist agar plates made with horse's blood large mucinous colonies (third variety) surrounded by pronounced clear zones.

HAEMOLYSIN

A soluble haemolysin is formed in broth cultures of Aronson N incubated for 24 hours; its activity is increased if the culture is allowed to stand at room temperature for 3 days.

VIRULENCE

Aronson N is highly virulent for mice and rabbits. In mice an intraperitoneal inoculation of 10^{-8} c.c., occasionally 10^{-9} c.c., of serum broth culture causes fatal septicaemia within 2 days. In rabbits subcutaneous doses, ranging from 0·25 c.c. to 10^{-4} c.c., set up fatal septicaemia in 2–4 days; the duration of life was longer after smaller doses, *e.g.* a dose of 10^{-8} c.c. killed in 7 days, the rabbit showing, in addition to septicaemia, whitish masses composed of cocci in the wall of the appendix. Vegetative endocarditis was produced in a rabbit which had been immunised with heat-killed homologous culture. The animal had been treated with dead culture injected intravenously for 5 months and, as the serum caused only a trace of agglutination in a dilution of 1 in 2, inoculation of living organisms was begun. After thirteen doses, ranging from 0·1 to 5 c.c. of broth culture, during a period of 7 weeks the rabbit died; Aronson N streptococci were recovered from the vegetations on the mitral valves, and from the kidney and spleen.

Capsule formation. In the animal body Aronson N develops typical capsules, which are well stained by Muir's method. On the other hand with Aronson S, although it is of equally high virulence for mice and rabbits, I have not been able to demonstrate capsule production in the animal, but when smear preparations are made from the large mucinous colonies on a blood agar plate and suitably stained, the mucinous substance around the cocci gives the appearance of ill-defined capsules; a similar appearance has been described in the case of the third colonial variety of *Str. pyogenes.*

FERMENTATION REACTIONS

Tested on sugars contained in an ascites broth medium Aronson N exhibits the same fermentative activity as the majority of *Str. pyogenes* strains, *i.e.* it produces acid with lactose and salicin but not with mannite, inulin and raffinose. Aronson S belongs to the rarer variety of *Str. pyogenes* which ferments mannite in addition to lactose and salicin.

SERUM REACTIONS

Agglutination. The rabbit responds slowly to immunisation with Aronson N, and the highest agglutinin titre reached has been 1 in 160. The strain is type-specific and gives no agglutination with heterologous sera prepared with haemolytic streptococci, streptococci of the viridans group, or pneumococci. The interaction between the virulent culture and its homologous antiserum results in the formation of firm masses which cannot be broken up, a type of reaction which is obtained with type-specific pneumococci and their antisera.

Precipitation. Aronson N forms in the peritoneal cavity of the mouse a soluble substance which precipitates with the antiserum. The peritoneal washings of a mouse which has died after an intraperitoneal injection of virulent culture is centrifuged until quite clear. On addition of 0·2 c.c. of immune serum to 1·0 c.c. of clear supernatant fluid, the latter becomes opalescent, and if the mixture is allowed to stand overnight in the refrigerator, granules form which collect into firm masses at the bottom of the tube.

Occurrence of Streptococcus Aronson N in man

Since the Streptococcus Aronson N has been under subcultivation for so many years, during which it has been subjected to animal passage in order to maintain its virulence, the question may be raised as to the possibility of its having acquired its special characters in the laboratory. It is well known that a haemolytic streptococcus may under certain cultural conditions lose its ability to form zones of haemolysis around the colonies on fresh blood agar plates. I have observed that a strain of *Str. pyogenes* Type 1 became non-haemolytic after being grown for many generations at 40° C. The surface colonies on blood agar caused green pigmentation of the medium and there was no lysis of the blood cells around the deep colonies. The strain had retained its serological characters and could still be identified as Type 1. To quote another instance of a similar change in my own experience, a dense mass of culture of Aronson S, heated to 56° C. for half an hour in a sealed tube, was found still to contain living cocci. Subculture in horse blood agar showed that the strain had lost the capacity to produce haemolysis around surface colonies, although deep colonies exhibited *beta* haemolysis.

There appears, however, to be no doubt that the Aronson N strain is a distinct species of streptococcus which is different from *Str. pyogenes* though it may occur naturally in human beings; I have obtained similar cultures from throat swabs on several occasions. The first two cultures were obtained from the throats of two healthy boys in the course of an investigation into the variation of the pharyngeal flora in school boys. The discovery was the outcome of the technique employed. In addition to direct plates from the throat swabs blood broth cultures were made and these were injected after incubation overnight into the subcutaneous tissues of mice. When death occurred it was generally due to the pneumococcus, but on one occasion smear preparations from the mouse's blood revealed round cocci instead of the usual lanceolate diplococci. The appearance of the plate colonies was a little different from that of pneumococcus colonies and reminded me of the Aronson strain, a suspicion which was confirmed by the immediate coarse flocculation obtained on emulsifying them in a dilution of Aronson N antiserum. A second swab taken a week later from the boy yielded the same organism, but re-examination after some months gave a negative result. A similar organism was obtained from the throat swab of another boy at the same school.

As the following comparison of their cultural and serological characters will

show, these two new strains are not absolutely identical with Aronson N or with each other. The strain isolated on two occasions from the first boy, St. 33, most nearly resembled it, while the second strain, St. 13, differed in several respects.

Cultural characters. St. 33 produced shiny opalescent dome-shaped colonies with definite green pigmentation and no lysis of horse blood cells around the colonies; around the deep colonies, both in horse and rabbit blood agar, were well marked clear zones; there was only a trace of soluble haemolysin in broth cultures.

St. 13 produced on horse blood agar whitish shiny discs, slightly raised in the centre, some of which were more translucent than others; there were partially cleared areas around the colonies and no green pigmentation; there were clear zones around the deep colonies, both in horse blood and in rabbit blood agar, but not all the blood cells were lysed; there was only a trace of soluble haemolysin in broth cultures.

Virulence. Aronson N has a high virulence for mice, causing fatal septicaemia in doses of 10^{-8} c.c. of broth culture. St. 33 was of medium virulence, M.L.D. $= 10^{-6}$ c.c.; St. 13 was of low virulence, M.L.D. $= 10^{-3}$ c.c. In smear preparations (stained by Muir's method) of the blood of the mice all three strains were shown to develop small well-defined capsules. St. 13 was inoculated intravenously into a rabbit which was killed when very ill 21 days later; the blood of the rabbit yielded a few colonies agglutinating with Aronson N serum, and a profuse culture was obtained from a wrist joint.

SEROLOGICAL REACTIONS

Agglutination titre v. *Aronson N serum*

	Slide method			
Strain	1 : 26	1 : 52	1 : 100	1 : 200
Aronson N	+	+	+	–
St. 33	+	+	Trace	–
St. 13	+	–	–	–
	Water-bath at 50° C.			
Strain	1 : 26	1 : 52	1 : 100	1 : 200
Aronson N	+	+	±	–
St. 33	+	+	±	Trace
St. 13	+	±	Trace	–

AGGLUTININ ABSORPTION OF ARONSON N SERUM

Method. 0·1 c.c. of Aronson N serum was added to the deposit of 100 c.c. of broth culture contained in 1·25 c.c. of broth. Test of agglutination was made by mixing equal loops of absorbed serum and homologous suspension on a slide. After the first treatment with St. 13 culture the absorbed serum was poured on to the deposit of a fresh 100 c.c. of culture; this was repeated a third time.

Test of absorbed serum diluted 1 *in* 27 v. *homologous suspension*

Absorbing strain	First treatment	Second treatment	Third treatment
Aronson N	–	.	.
St. 33	–	.	.
St. 13	Trace	Trace	Trace
Nil (control)	+	.	.

Result. St. 33 removed the type-specific agglutinin of Aronson N serum as efficiently as the homologous strain; St. 13 failed to remove the whole of the agglutinin even after three treatments, though the clumps formed were much reduced in size.

Precipitation. Clear fluid was obtained by centrifuging the peritoneal washings of mice which had been inoculated intraperitoneally with each of the three strains, and 0·2 c.c. of Aronson N serum was added to 1·0 c.c. of each. After the mixtures had stood in the refrigerator overnight, the fluid from the homologous strain had formed large firm masses, that from St. 33 smaller firm clumps, and that from St. 13 still smaller firm clumps. It will be observed that the amount of the precipitum, which is probably related to the amount of soluble substance produced, varied with the degree of virulence of the strains for the mouse (see above).

The third strain was obtained in June, 1931, from the throat of a boy at a different school from the above. A mouse injected with a mixed culture from a swab in blood broth died within 24 hours. The blood of the mouse yielded a pure culture of pneumococcus-like colonies with greenish granules in the medium on the surface of horse blood agar and *beta* haemolytic colonies in the depth. The culture grew uniformly in broth and agglutinated with Aronson N serum diluted up to 1 in 160. A mouse inoculated intraperitoneally died in 1 day and showed abundant capsulated diplococci in the blood; the turbid peritoneal washing agglutinated up to 1 in 80 with Aronson N serum.

The fourth strain was from the throat of a nurse who had been a carrier of *Str. pyogenes*. She was swabbed in July, 1932, and the swab plated directly on blood agar yielded a nearly pure culture of shiny, greyish-white discs; the medium was slightly green under the colonies and there was no lysis of the blood cells except where the growth covered a pit in the medium. A mouse inoculated intraperitoneally with the culture died within 24 hours and showed numerous chains and diplococci in the blood. The peritoneal washing was centrifuged and the turbid supernatant fluid formed firm masses when mixed with Aronson N serum diluted up to 1 in 160. The culture was virulent for mice in a dose of 10^{-3} c.c. inoculated intraperitoneally. A second swab was taken 5 days later from the nurse's throat and the same organism was obtained by mouse inoculation.

Fifth strain. A culture designated Phillips was sent to me in October, 1931, by Dr L. Colebrook, who had obtained it from a uterine swab from a case of puerperal sepsis. A mouse inoculated intraperitoneally with 0·01 c.c. of a broth culture died within 24 hours and the blood produced on the surface of a horse

blood agar plate an abundant pure culture of dome-shaped, greyish-white colonies with green pigment and no lytic areas in the medium around them. The peritoneal washing from the mouse was slightly cleared by centrifuging and was mixed with Aronson N serum; agglutination occurred promptly with the formation of firm masses. The culture from the mouse grew uniformly in broth and agglutinated with Aronson N serum in dilutions up to 1 in 160.

Sixth strain. A culture, No. 78, was sent to me by Mr R. Lovell, London School of Hygiene and Tropical Medicine, in July, 1931; it had been obtained from a normal throat swab. The colonies on the surface of horse blood agar were conical in shape and of a thick porridgy consistency, and there were incompletely cleared though fair-sized haloes around them; in the depth of the medium the colonies were of the *beta* haemolytic type. The culture was of moderate virulence for mice, killing in a dose of 0·01 c.c. intraperitoneally in 2 days. A mouse inoculated with ascites broth culture died after 20 days and yielded a culture from the blood which agglutinated with Aronson N serum. The peritoneal washing from an inoculated mouse, centrifuged until almost clear, gave very fine clumps when mixed with Aronson N serum in dilutions up to 1 in 80.

VARIANTS OF ARONSON N

A virulent culture of Aronson N produces on blood agar plates colonies which resemble pneumococcus colonies. Under certain influences Aronson N, like the pneumococcus, undergoes biological changes which result in the formation of matt, coherent or R colonies in place of the typical S colonies of the virulent culture. In contrast with the R variant of the pneumococcus the peculiarity of the Aronson R colonies is that virulence is rarely completely lost, and reversion to the S form occurs readily both in culture and in the mouse.

A completely attenuated strain was obtained from Aronson N in the following circumstances. A virulent culture from the mouse was sown in pure homologous immune serum, incubated overnight and plated. The first generation in serum produced smooth colonies which occasionally developed marginal rough patches. The colonies of the second generation were apparently all smooth. In the third there were a few colonies which had a porridgy instead of the paint-like consistency of the S colonies. The fourth generation showed a moderate number of the coherent or R colonies. The rough patch from the first generation colony was touched and plated; there were produced from it two varieties of colonies, (1) smooth discs, (2) irregular colonies with a matt surface: neither was examined further. One of the R colonies from the fourth generation was subcultured and tested on mice; there appeared to have been no attenuation of virulence associated with the change to the R type of colony, since all the mice died and cultures from them grew in the S form. It was found also that reversion readily took place in culture; when R colonies were sown in blood broth and this was plated after incubation overnight, the plates showed a mixture of R and S colonies.

From one of the R strains, after repeated subculture in broth and incubation

at 40° C., a colony was obtained which appeared permanently attenuated. This colony, R7, had a matt surface, irregular margin and raised centre, resembling somewhat a limpet shell, and there was a slightly cleared area in the medium around it. On further subcultivation the colonies in general became less irregular, although there were invariably on the plate a few of the original limpet shape. R7 colony culture, injected into mice in large amounts, either alone or together with killed cultures of virulent strains, invariably failed to kill mice; reversion to the cultural characters or virulence of the parent culture never occurred. Agglutination tests with the R strain were difficult, since the cultures grew in a granular fashion. A usable suspension was obtained from glucose agar, the growth being washed off in distilled water and added to an equal quantity of broth. With this suspension the antiserum from the S strain gave dispersible clumps, but the R antiserum failed to agglutinate the type-specific S strain. In antigenic structure the R and S strains were dissimilar; each failed to remove the homologous agglutinin from the serum prepared with the other. In the absence of reversion one cannot assert with confidence that the R strain was actually derived from the Aronson culture with which the passage experiment was begun and was not of extraneous origin. But there were two facts which supported this supposition: (1) the same sugars, lactose and salicin, were fermented, (2) the R strain was agglutinated by the type-specific Aronson serum, while twenty-two rabbit sera, prepared against viridans strains, had no effect.

A second trial was made of the influence of homologous immune serum on the growth of Aronson N. A virulent culture was grown overnight in pure immune serum, and from it a fresh blood agar plate was sown. The plate was incubated for 4 days and on examination it was found that most of the colonies had developed daughter colonies and one showed a rough fan-shaped outgrowth. This outgrowth was touched, and a plate was made directly from it; the result was a mixture of (1) greyish-white discs, and (2) grey rough irregular colonies. The R and S colonies were not only totally dissimilar in appearance but also in smear preparations; (1) showed short chains, (2) showed long chains of much larger cocci. Six of the R colonies were subcultivated and given three successive platings, during which the colonies changed a little in appearance, becoming less irregular in shape. After the third plating a colony from each of the six R strains was grown in serum broth which was tested on a mouse in a dose of 5 c.c. subcutaneously. All the strains proved to be virulent, killing the mice in 2–4 days.

The effect on Aronson N of frequent subcultivation on different media and incubation both at 37 and 40° C. was tested. In all cases R colonies appeared, *i.e.* matt coherent discs; they were most readily differentiated from the S colonies on chocolate-blood agar. The virulence of twenty-three colonies was tested by the subcutaneous inoculation of 5 c.c. of serum broth culture, and all but two caused fatal septicaemia. The mice inoculated with the two exceptional strains, R12 and R23, survived.

R12, which was obtained from the fourth serum broth culture incubated at 40° C., was inoculated a second time subcutaneously into a mouse in a dose of 6 c.c. without causing ill effects. Nine days later a fresh serum broth culture was tested on mice in subcutaneous doses of 0·5, 6·0 and 100 c.c. and fatal septicaemia was produced in each case. The cultures had increased in virulence, although the colonies used for inoculation were still R in appearance. Each R colony culture was plated three times, the succession being continued with an R colony. Finally serum broth cultures from the colonies on the third plate were tested on six mice in subcutaneous doses of 0·25 c.c. Two of the mice survived and four died in from 6 to 26 days. The mouse dead 26 days after inoculation yielded a plate culture from the blood, which consisted of a mixture of matt and typical smooth colonies. R12 was apparently a partially attenuated strain with the capacity to revert to the original S form.

R23 was obtained from a culture which had been grown at 37° C. for twenty-four generations on plain nutrient agar slants. It formed small coherent colonies when plated from serum broth, in which it grew in the form of a deposit, the supernatant broth being quite clear; on standing, the deposit assumed an orange tint. Tested with Aronson N serum R23 formed small dispersible clumps, very different in appearance from the firm masses characteristic of agglutination with the virulent culture. Mice inoculated with doses of 0·5, 6·0 and 100 c.c. survived. It appeared that a really attenuated strain had been obtained. To eliminate any possibility of an S organism remaining in the culture it was plated three times, the succession being continued each time with an R colony. From the third plate a colony was sown into serum broth which was tested on mice. Eight mice received 50 c.c. subcutaneously, and all died in 2–6 days with profuse septicaemia; two mice received 0·5 c.c. and two 1·0 c.c. intraperitoneally, and of these one died and one survived under each dose; six mice received 0·5 c.c. subcutaneously and all survived. But when 0·25 c.c. of R23 broth culture was inoculated subcutaneously together with the deposit (killed by heating to 60° C.) of 50 c.c. of virulent Aronson N culture the mouse died in 5 days and showed capsulated diplococci in the blood. Also the same dose of R23 (0·25 c.c. subcutaneously) was inoculated together with the deposit of 50 c.c. of Type 1 *Str. pyogenes* culture killed at 60° C.; the mouse died in 8 days and showed capsulated diplococci in the blood.

IMMUNISATION WITH ARONSON N AND WITH *STR. PYOGENES*

Active immunity against the Aronson N strain is readily obtained by intraperitoneal injection of heat-killed suspensions of homologous culture (Table I), whereas subcutaneous injections are wholly ineffective (Table III), even when large doses are used. Grinding of the cocci in an agate-ball mill to facilitate absorption gave no better results by the latter method of inoculation (Exp. 5).

The Aronson N vaccine, given intraperitoneally in small and large doses, induced no immunity against the heterologous Aronson S, although a slight

resistance, viz. against one and ten fatal doses, after subcutaneous vaccination with large amounts of culture was recorded.

Active immunity against Aronson S with homologous vaccine was not readily achieved (Tables II, IV and Exp. 6), and not the least resistance was induced by the methods and doses which were successful in the case of Aronson N. With larger doses of vaccine, employed subcutaneously as well as intraperitoneally, resistance was obtained, though not consistently, against one, ten and even one hundred fatal doses of the homologous culture.

Aronson S vaccine, in large intraperitoneal doses, produced some heterologous immunity against the Aronson N strain, *e.g.* mice receiving 10^{-7} c.c. (one fatal dose) and 10^{-6} c.c. (ten fatal doses) survived.

The resistance exhibited against even the smallest doses has some significance, since there were no survivals in the various experiments among the sixteen untreated control mice receiving a dose of 10^{-8} c.c. of Aronson N, or among the fourteen receiving 10^{-8} c.c. of Aronson S; one only of thirteen control mice inoculated with 10^{-9} c.c. of Aronson S survived. One may conclude that natural resistance in mice to fully virulent cultures of the above two strains is rare.

The difficulty, shown in the case of Aronson S, of obtaining a high degree of active immunity is not characteristic of all strains in the *Str. pyogenes* group. An intraperitoneal vaccination with Schnitzer 87 (a culture obtained from pus of a mastoiditis case) was as successful in inducing resistance against test doses of the homologous culture as a similar experiment with the Aronson N strain (Exp. 7). For example, mice were protected against doses ranging up to 10^{-4} c.c., while the minimal fatal dose of the test culture for untreated mice was 10^{-8} c.c. Schnitzer 87 exhibited consistently a high degree of virulence, and of the nine control mice inoculated in various experiments with a dose of 10^{-7} c.c., only one survived. The immunising capacity of another strain of the *Str. pyogenes* group, C203 (Exp. 8*a*) was tested, and immunity was produced against the homologous test culture by the intraperitoneal injection of a heat-killed vaccine. The results were, however, very irregular, since one vaccinated mouse succumbed to the smallest dose, 10^{-9} c.c., whilst another resisted as large a dose as 10^{-3} c.c. Subcutaneous vaccination with C203, in which a large dose was followed 5 weeks later by a smaller dose, was wholly ineffective (Exp. 8*b*).

In general, heterologous immunity produced by strains of haemolytic streptococci was slight, the degree of resistance induced rarely sufficing to protect against more than one fatal test dose (Table V); it was also less persistent than homologous immunity (Exp. 4*a*).

The results of my vaccination experiments agree very closely with those recorded by Yoshioka (1923), in particular those with the Aronson N strain of which he also made use.

Passive immunity. Aronson N produced in a rabbit an efficient protective serum. An intraperitoneal dose of 0·14 c.c. protected mice against doses of the

homologous culture ranging up to 0·05 c.c. (Table VI). In this test the Aronson N culture was of the highest virulence, the smallest fatal intraperitoneal dose being 10^{-9} c.c. The power of producing a passively immune serum is another point of resemblance between pneumococci and Aronson N. On the other hand, the same methods of immunisation with five strains of *Str. pyogenes*, including Aronson S and Schnitzer 87, failed to stimulate the production of protective antibodies in rabbits. It must be noted, however, that the test doses of culture were inoculated soon after the serum, and this may have been a cause of failure, since, according to Lancefield (1934), it is necessary to inject the antiserum at least 8 hours before the infecting organism in the case of organisms of Group A into which *Str. pyogenes* falls.

DETAILS AND TABLES OF IMMUNITY EXPERIMENTS

Exp. 1. *Vaccination experiment with Aronson N.* Mice were injected intraperitoneally with a 6-hour old glucose broth culture of Aronson N which had been killed by heating to 60° C. for 1 hour. The culture was centrifuged and suspended in salt solution, each dose being contained in 0·5 c.c. Three doses, equivalent to 0·01, 0·1 and 1·0 c.c. were administered, 7 or 8 days intervening between injections. The resistance tests (Table I) were made on the twelfth day after the last dose of vaccine with 24-hour-old cultures in rabbit blood broth, injected intraperitoneally. The control mice injected intraperitoneally with the two smallest doses all died, except one mouse which received 10^{-7} c.c. of E 14. The cause of death was verified by smear preparations, and when necessary by culture. The mice were kept under observation for some weeks after the test doses. For the resistance tests in addition to the Aronson N and S cultures certain virulent strains of the *Str. pyogenes* group were employed, viz. Schnitzer 87, C 203 (Type 1) and E 14 (Type 10). The results show that vaccination intraperitoneally with the killed culture of Aronson N induced a high immunity against the homologous strain and no immunity against the four heterologous strains.

Table I. *Intraperitoneal vaccination with Aronson N*

	Resistance test		
No. of mice	Test culture	Doses in c.c.	Results
4	Aronson N	10^{-5} to 10^{-8}	All lived
4	,, S	,,	All died
4	Schnitzer 87	,,	,,
4	E 14	10^{-4} to 10^{-7}	,,
4	C 203	10^{-5} to 10^{-8}	,,

Exp. 2. *Vaccination experiment with Aronson S.* The procedure in respect of vaccination and test of resistance (Table II) was identical with that in Exp. 1 with Aronson N vaccine. All the control mice inoculated with the two smallest doses of each test culture died. The results show that vaccination intraperitoneally with heated culture of Aronson S produced no immunity against either the homologous or the heterologous strains.

Table II. *Intraperitoneal vaccination with Aronson S*

	Resistance test		
No. of mice	Test culture	Doses in c.c.	Results
4	Aronson N	10^{-5} to 10^{-8}	All died
4	,, S	,,	,,
4	Schnitzer 87	,,	,,
4	E 14	10^{-4} to 10^{-7}	3 died, 1 lived
4	C 203	10^{-5} to 10^{-8}	All died

Exp. 3. *Vaccination experiment with Aronson N.* The mice were injected subcuta with culture of Aronson N killed by heating to 60° C. for 1 hour, and prepared a preceding experiments. Three series of vaccinating doses were administered, the sec third series being respectively 10 and 100 times higher than the first. The result of th ance test is given in Table III.

Table III. *Subcutaneous vaccination with Aronson N*

(*a*) *Doses of vaccine in c.c.*

1st series	0·04	0·08	0·18
2nd series	0·4	0·8	1·8
3rd series	4·0	8·0	18·0

(*b*) *Resistance test*

	No. of mice	Test culture	Doses in c.c.	Results
1st series	7	Aronson N	10^{-3} to 10^{-8}	All died
	4	,, S	10^{-7} to 10^{-9}	3 died, 1 lived
2nd series	7	,, N	10^{-4} to 10^{-8}	All died
	4	,, S	10^{-7} to 10^{-9}	,,
3rd series	8	,, N	10^{-3} to 10^{-8}	,,
	4	,, S	10^{-7} to 10^{-9}	1 died, 3 lived

Exp. 4. *Vaccination experiment with Aronson S.* The mice were injected subcutane with Aronson S killed by heating to 60° C. for 1 hour. The details were the same as in E to which this is a companion experiment, three series of vaccinating doses being administ the result of the resistance test is shown in Table IV. The control mice, five for Arons and six for Aronson S, inoculated with the three smallest doses all died in 1 or 2 days

Table IV. *Subcutaneous vaccination with Aronson S*

Resistance test

	No. of mice	Test culture	Doses in c.c.	Results
1st series	6	Aronson S	10^{-7} to 10^{-9}	4 died, 2 lived
	4	,, N	10^{-7} to 10^{-8}	All died
2nd series	8	,, S	10^{-7} to 10^{-9}	6 died, 2 lived
	4	,, N	10^{-7} to 10^{-8}	All died
3rd series	6	,, S	10^{-7} to 10^{-9}	3 died, 3 lived
	4	,, N	10^{-7} to 10^{-8}	All died

Exp. 4a. The mice surviving from Exps. 3 and 4 were used for a test of the duration immunity. Four mice which had been vaccinated with Aronson N, and had resisted a t dose of Aronson S, were retested 15 days later with the same doses of Aronson S; all died 1 day. Thirteen mice which had been vaccinated with Aronson N, and had resisted a dose Aronson N, were retested 15 days later with the same doses of Aronson N; ten survived a three died. Twelve mice which had been vaccinated with Aronson S, and had resisted a te dose of Aronson S, were retested 15 days later with the same doses of Aronson S; eight su vived and four died. Four mice which had been vaccinated with Aronson S, and had resiste a test dose of Aronson N, were retested 15 days later with the same doses of Aronson N all four died.

Exp. 5. *Vaccination experiment with Aronson N.* Mice were injected subcutaneously wit killed culture of Aronson N which had been grown in serum broth for 24 hours, centrifuged washed once with salt solution and dried *in vacuo* over phosphoric pentoxide. The drie culture was ground in an agate ball mill for 8 hours, resuspended in salt solution and heate for $\frac{1}{2}$ hour at 60° C.; a smear showed that there were still some Gram-fast cocci. Three dose of vaccine, equivalent to 1, 4 and 14 c.c. of serum broth culture were given.

Resistance test of vaccinated mice. Seven mice, inoculated intraperitoneally with doses o Aronson N, ranging from 10^{-3} to 10^{-8} c.c., all died in 2 days. Six mice, tested with Aronson S in doses ranging from 10^{-6} to 10^{-9} c.c., all died, except one which received 10^{-9} c.c.

Exp. 6. *Vaccination experiment with Aronson S.* Mice were injected subcutaneously with killed and ground culture; the details of the preparation of culture, etc., were identical with those in Exp. 5.

Resistance test of vaccinated mice. Seven mice, inoculated intraperitoneally with doses of Aronson S ranging from 10^{-4} to 10^{-9} c.c., all died except one which received a dose of 10^{-9} c.c. Five mice, tested with Aronson N in doses ranging from 10^{-4} to 10^{-8} c.c., all died in 1–2 days. The control mice, inoculated with the three smallest doses of both test cultures, all died in 1–2 days.

Exp. 7. *Vaccination experiment with Schnitzer* 87. A culture from mastoiditis, apparently of the *Str. pyogenes* group but the serological type was not identified. Mice were injected intraperitoneally with glucose broth culture killed by heating to 60° C. for 1 hour. Three doses of vaccine, equivalent to 1, 4 and 14 c.c. respectively, were given.

Resistance test of vaccinated mice. Seven mice were tested with the Schnitzer 87 culture in doses ranging from 10^{-5} to 10^{-8} c.c.; all survived except two, which however showed no streptococci in cultures from the organs. Four mice tested with Aronson N in doses ranging from 10^{-6} to 10^{-8} c.c. all died in 2 days. Four mice were tested with Aronson S in doses ranging from 10^{-7} to 10^{-9} c.c.; two which received the smallest dose survived and two died. Control mice inoculated with the smallest doses of each of the three test cultures all died in 1–2 days, except one mouse inoculated with 10^{-9} c.c. of Aronson S which died in 5 days.

Exp. 8. *Vaccination experiment with C*203, Str. pyogenes, *Type* 1.

(*a*) Mice were injected intraperitoneally with a young glucose broth culture killed by heating to 60° C. for 1 hour. Three doses of vaccine, 1, 4 and 14 c.c. respectively, were given.

Resistance test of the vaccinated mice. Eight mice were tested with the culture of C203; four which received doses of 10^{-4}, 10^{-6}, 10^{-8} and 10^{-9} c.c. survived; four which received doses of 10^{-5}, 10^{-7}, 10^{-9} and 10^{-10} died in 1–3 days. Six mice tested with Aronson N in doses ranging from 10^{-6} to 10^{-9} c.c. all died in 1–3 days. Five mice were tested with Aronson S; three died and two survived. All the control mice inoculated with the smallest test doses of Aronson N and S died; of the three control mice inoculated with C203 two died (10^{-8} and 10^{-10} c.c.) and one (10^{-9} c.c.) survived.

(*b*) Mice were vaccinated subcutaneously with C203 culture killed at 60° C. A large dose, the deposit of 40 c.c. of broth culture, was given, followed 5 weeks later by the deposit of 10 c.c.

Resistance test of vaccinated mice. Seven mice were tested with C203 culture in doses ranging from 10^{-6} to 10^{-9} c.c.; all died in 1–2 days. Three control mice inoculated with doses ranging from 10^{-7} to 10^{-9} c.c. died in 1 day.

Exp. 9. *Vaccination with* Str. pyogenes, *Types* 1, 2, 3 *and* 4. Mice were injected intraperitoneally with heated (60° C.) broth cultures of each of the above four streptococcal types on four occasions at intervals of 6, 7 and 8 days. Tests of immunity (Table V) were made, 8 days after the last dose of vaccine, against the heterologous strains, Aronson S and Schnitzer 87. Control mice inoculated with the two smallest test doses died in 1 day.

Table V. *Intraperitoneal vaccination with* Str. pyogenes *types*

(*a*) *Doses of vaccine in c.c.*

1st	2nd	3rd	4th
0·01	0·1	1·0	1·0

(*b*) *Resistance test*

Vaccine	No. of mice	Test culture	Doses in c.c.	Results
Type 1	4	Aronson S	10^{-6} to 10^{-8}	2 died, 2 lived
2	4	,,	,,	3 died, 1 lived
3	4	,,	,,	All died
4	4	,,	,,	2 died, 2 lived
Type 1	4	Schnitzer 87	,,	All died
2	4	,,	,,	2 died, 2 lived
3	4	,,	,,	2 died, 2 lived
4	4	,,	,,	3 died, 1 lived

Exp. 10. *Passive immunity against Aronson N.* An antiserum was obtained from a rabbit after eight series of injections over a period of 2 months with virulent Aronson N culture which had been killed by heating to 60° C.; its agglutination titre was 1 in 160. The protective properties of the serum were tested on mice (Table VI); the antiserum and test culture were injected intraperitoneally, the serum first and the test culture a few minutes later. The control mice inoculated with Aronson N culture in doses ranging from 10^{-7} to 10^{-9} c.c. all died in 2 days. The mice surviving the test of resistance were killed in good condition after 5 days, and plate cultures from the spleens in all cases were negative.

Table VI. *Protection test with Aronson N serum*

	Test culture	
No. of mouse	Dose in c.c.	Result
590	Aronson N, 0·2	Died, 1 day
591	,, 0·1	Died, 2 days
592	,, 0·05	Lived
593	,, 10^{-3}	,,
594	,, 10^{-4}	,,
595	,, 10^{-5}	,,
596	,, 10^{-6}	,,
597	,, 10^{-7}	,,
598	,, 10^{-8}	,,

Summary

The characters of the Aronson Streptococcus from Prof. Neufeld's laboratory at the "Robert Koch" Institute in Berlin have been described. This coccus resembles the pneumococcus in many respects, viz. the appearance of the colonies on the surface of horse blood agar, its virulence and capsule production in mice and rabbits, the production of a specific precipitable substance in the peritoneal washings of infected mice, the formation of firm clumps and masses when mixed with homologous antiserum, the ease of production of active and passive immunity in mice and rabbits by intraperitoneal and intravenous inoculation, the alteration in the morphology of colonies, *i.e.* the appearance of R forms, associated with attenuation of virulence. It differs from the pneumococcus in the following features: the round shape of the cocci, bile-insolubility and the absence of autolysis in surface colonies, the *beta* haemolysis of deep colonies in horse blood agar, the production of a soluble haemolysin in broth cultures, the difficulty of producing active immunity in mice by the subcutaneous injection of heat-killed vaccines.

I have obtained the Aronson Streptococcus (Neufeld type), which Lancefield places in a group containing chiefly streptococci of bovine origin, from human throats, but there was no evidence in any instance that it was producing disease, and it seems probable that it is not pathogenic for man.

The results of my investigation of this strain are in agreement with those of Yoshioka (1923), Killian (1924) and Lancefield (1933, 1934).

There are in existence other laboratory strains designated Aronson Streptococcus. These have been found to exhibit specific characters identifying them with the *Str. pyogenes*. It is proposed that the name Streptococcus Aronson should be confined to strains possessing the characters of Aronson N above described.

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