HEMOLYTIC STREPTOCOCCUS LYMPHADENITIS IN GUINEA PIGS

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The observations to be reported are concerned with a naturally occurring chronic infection in guinea pigs. Studies on transmission and on the variations of the causative hemolytic streptococcus have been carried out, in addition to immunological investigations.

This disease was described by Boxmeyer (1) in 1907. It consists of chronic abscesses in the lymph nodes. The cervical nodes are most often affected although the inguinal and retroperitoneal lymphatics may occasionally be involved. The condition generally runs a chronic course; animals may exhibit very large abscesses for months with no apparent debility. Recovery may occur spontaneously following the drainage of the lymph node abscess through the skin. Occasionally death occurs due to pressure effects or to rupture of the abscess into the blood stream.

We have maintained a colony of 30 to 50 naturally infected guinea pigs in a large pen since 1931. The disease passes from infected to normal animals very readily. Fresh stock has been added from time to time in addition to the litters born in the pen. At one time a separate colony was developed in which only the young born in the group were used to keep up the population. No fresh animals from outside were introduced. This did not affect the nature or prevalence of the infection over a period of 2 years.

Transmission of the Disease

The pus from lymph node abscesses contains a hemolytic streptococcus falling into Lancefield's group C (2). Concerning the variations of this organism more will be said later. The phase which is responsible for the disease is mucoid and possesses capsules in young cultures (3–5). It will be referred to as strain 3. Young cultures ($3\frac{1}{2}$ hours old in 10 per cent horse serum, 1 per cent dextrose digest broth) injected intradermally in a dilution greater than 10^{-2} will bring about local abscess formation and enlargement of the regional lymph nodes in 4 to 8 days. The infection remains chronic, just as in the naturally acquired disease, and guinea pigs in contact with an animal thus artificially infected will contract cervical lymphadenitis.

To determine the probable method of natural transmission, 6 guinea pigs were placed in the middle of the large pen containing the infected colony, but were separated from

direct contact with diseased animals by two sheets of ¼ inch mesh wire screen spaced 1 inch apart. 6 controls were placed in the pen at the same time in direct contact with the diseased colony. The 6 screened-off pigs were completely protected from the disease although they were separated by a distance of only 1 inch. Over a period of 3 months no infections developed; all the directly exposed controls showed enlarged cervical nodes in 2 weeks to 1 month. 5 of the 6 isolated animals, subsequently turned into the pen, promptly developed the disease.

Guinea pigs were exposed for ½ hour to an atmosphere into which an undiluted culture of strain 3 had been sprayed. The container was arranged so that the animals did not touch any surface which had been exposed during the spraying. Of 6 animals, 4 died after about 1 week, with extensive pneumonia but no cervical lymph node enlargement. The remaining 2 animals survived and developed enlarged lymph nodes which were deeper in location than those involved in the natural disease. Food was withheld from 8 guinea pigs for 24 hours following which they were given oats which had been saturated with a young culture of strain 3. In 1 week all had developed enlarged cervical lymph nodes typical of the natural infection both in location and in their subsequent course.

From these results it appears that in nature the chronic cervical lymphadenitis is transmitted infrequently or not at all by droplet infection, and that the ingestion of the infective agent is the most probable means of spread.

Spontaneous Variation in the Infected Colony

Dr. Theobald Smith (6) during his study of a similar colony of diseased guinea pigs encountered a spontaneous increase in the virulence of the streptococcus. The new variant, instead of localizing in the regional lymph nodes, brought about an acutely fatal septicemia when placed on the abraded skin of guinea pigs. He found that animals having a chronic infection due to the original streptococcus were resistant to inoculation with the new virulent variant. This will be referred to as strain 1. It is more fully described below.

The chronic infection in a group of guinea pigs which we have had under observation was started from a different source in 1931. Up to 1938 no change in the nature of the infection was seen. Animals would occasionally die, from causes mentioned before; the organism isolated from these animals invariably reproduced a chronic infection on injection into normal guinea pigs. However, in January, 1938, the mortality suddenly increased, and during the following month about half of the group of 40 succumbed. From the heart's blood of most of these a new streptococcus was obtained. It differed from the chronic strain in having very much longer chains and larger capsules. It usually occurred in the blood along with the strain 3 or chronic type; sometimes it was in pure culture. At the end-point of dilution of this strain intradermal injection killed guinea pigs in 5 to 10 days,

with the organism in the heart's blood at postmortem. It will be referred to as strain 4.

During this fatal epizootic fresh animals were added to the infected group as usual and during the next month 2 more died, with the new virulent form in the heart's blood. Thereafter the acute infection subsided leaving the usual chronic disease apparently unaltered. It has not recurred during the subsequent 18 months, nor has the virulent form been found again.

It is of interest that in two different groups of animals carrying a chronic disease a similar alteration in the nature of the causative organism should have occurred. While it is possible that the new form might have been introduced from outside, this is unlikely since the stock of guinea pigs employed to maintain the colony has never shown any streptococcus infection either during or after the occurrence of the fatal epizootic. The same animal man cared for the colony during the entire time, and there was no contact with other infected animals. Furthermore, there are certain similarities between the chronic strain and the new virulent strain which indicate that they may be closely related.

Mixed Infections with Chronic and Virulent Strains

The new virulent form was, under natural conditions, superimposed on a chronic infection, and mixed cultures of chronic and virulent strains were frequently encountered in the heart's blood. For these reasons the effect of the two strains on each other was investigated.

6 guinea pigs received 8 to 10 chains of the virulent strain 4 intradermally in the left flank. Another series of 6 received in the same way about 400,000 chains of the chronic strain 3. These two groups served as controls; the first group died in 5 to 10 days with strain 4 in the heart's blood, while the second developed chronic abscesses and survived 2 to 8 months. At the same time 6 guinea pigs were injected with a mixture of the two strains, and 6 others received the two strains on opposite flanks. The two last groups died in 5 to 10 days. Those animals that received the chronic and virulent strains on opposite flanks showed only the virulent strain in the heart's blood. Those receiving the mixture in the same inoculum showed both forms in the heart's blood.

These findings make plain that the presence of the virulent strain in a lesion will carry the chronic strain into the blood stream, although the chronic strain tends to localize when alone. This accounts for the presence of the two forms in the blood stream of naturally infected animals.

Dissociation of the Chronic and Virulent Strains

Our observations on the dissociation of these organisms are in close agreement with those of Dawson, Hobby, and Olmstead (5). The mucoid (M)

phase has a rather large, viscid colony, with capsules (3–5) in young cultures. It grows with uniform turbidity in 10 per cent horse serum, 1 per cent dextrose digest broth which has been the routine medium employed. The smooth (S) phase has a smaller, smooth, non-viscid colony and shows no capsules in young cultures. The rough (R) phase has a flat, very wrinkled colony with filamentous edges and is also non-capsulated. Only the M forms possess invasiveness. Matt forms have not been observed in this group.

The variants as well as the parent strains are described in detail in the following section, which is summarized in Table I.

TABLE I

Summary of Strains

Strain No.	Source	Colony	Virulence	Capsules in young cultures	Growth in 10 per cent horse serum, 1 per cent dextrose digest broth
1	Appeared in a chronically infected colony of guinea pigs	Stable mucoid	Very great	+++	Diffuse
2	Strain 1	Stable smooth	0	0	Granular
3	Causative agent in chronic guinea pig lymphadenitis	Unstable mucoid	Moderate	++	Diffuse
4	Appeared in a colony chronically infected with strain 3	" "	Very great	+++	Slimy rope
5	Strain 4	" "	Slight	++	Diffuse
6	Strain 4	Unstable rough	0	0	Fibrous
7	Strain 3	Unstable smooth	0	0	Granular
8	Strain 6	Stable smooth	0	0	"

Strain 1.—Dr. Theobald Smith (6) during his study of a group of guinea pigs carrying cervical lymphadenitis obtained a streptococcus having an invasiveness much greater than that of the usual chronic disease strain. The new form killed normal guinea pigs in 5 to 10 days after being spread on the shaven skin of the belly. This strain has been maintained in the laboratory since then with occasional passage through guinea pigs. It has shown no decrease in virulence. At the end-point of dilution, mice are killed 72 hours after intraperitoneal injection, and guinea pigs 5 to 10 days after intradermal injection. The growth is mucoid; in the absence of serum it is very scanty. After serial transfer on such serum-free medium, restoration of the culture to serum or blood agar again gives large typical mucoid colonies. For this reason strain 1 is referred to as a stable mucoid type. It, as well as its variant strain 2, has a slower growth rate than is ordinarily found in this group. Young cultures examined with Wright's stain show enormous capsules (3) which persist longer than the average mucoid streptococcus capsule. It is unique in having a clump-like arrangement rather than the typical chain morphology. Unfortunately, the chronic disease strain, which presumably gave rise to the invasive one, is no longer extant.

Strain 2.—During the routine plating of strain 1, a variant form was observed having a smaller, non-mucoid colony with a wider zone of hemolysis. It showed no capsule at any age, but preserved the staphyloid morphology of the parent strain. Injection of large numbers into mice and guinea pigs was without pathogenic effect. From a great many platings of strain 1, this non-mucoid smooth form was never observed again. Over a period of 3 years strain 2 has shown no tendency to revert to the parent form.

Strain 3.—From infected guinea pigs brought in by a local breeder for diagnosis, a new colony was begun in 1931, which is still carrying the disease. The streptococcus responsible for this is similar to an unclassified group obtained from man by Ward and Lyons (4). If the pus from one of the abscesses is plated out, two colony forms are invariably present. One is large and mucoid; the other is small, smooth, and more hemolytic. In an effort to obtain pure lines of these two forms, the following facts have been derived. If mucoid colonies are transferred serially on blood plates, one very soon establishes an apparently pure strain of mucoid organisms. Young broth, especially serum broth, cultures show capsules. This, as well as all the succeeding strains, has typical chain-like arrangement of the individual cocci. During 98 serial blood plate transfers of single mucoid colonies, none of the smaller, smooth forms have been seen. However, if the mucoid form, even after 98 transfers is passed serially in broth and each broth culture plated out, the non-mucoid smooth form promptly appears. If 10 per cent horse serum broth is used, the appearance may be delayed to the third or fourth broth passage. In serum-free broth, one usually finds the smooth form in preponderance after the first passage.

However, if the smooth form is transferred serially on blood plates, a few typical mucoid colonies are invariably present, although the smooth forms are usually very much in the majority. It has not been possible to secure pure M and S lines from this strain, but for practical purposes the transfer of several M colonies from blood agar to 10 per cent serum broth gives a culture which is almost entirely M. A second transfer from this to fresh serum broth results in the development of usually less than 1 per cent of S forms. Beyond this one may expect large numbers of S forms. Similarly, it is possible to fish several S colonies from a blood plate to serum broth, and until the second or third broth transfer the culture is essentially S. Such an S culture gives a granular growth in broth and is not capsulated at any age, in distinction to the diffusely growing, capsulated M phase.

Strain 3 will refer to the mucoid phase of this strain. A 2 hour culture diluted 1:100 injected intradermally in guinea pigs gave rise to the chronic abscesses described above. The administration of larger numbers was likely to result in death within 2 or 3 weeks. Animals dying from infection with this strain showed only M forms in their heart's blood, although, as we have said, the local lesions exhibited both M and S forms. Less than 10 chains of strain 3 occasionally killed mice when introduced intraperitoneally, but to secure regular deaths between 1000 and 10,000 chains were necessary. If one was fortunate enough to obtain a culture which was entirely S, it was without effect when injected into mice or guinea pigs. In Table I the S phase of this strain is listed as strain 7.

Strain 4.—This refers to the strain which appeared in association with the spontaneous fatal epizootic in 1938 which has already been mentioned. In serum broth it grew in a twisted mat which could be spun into a rope by swirling the tube. Once shaken up it remained suspended. Young cultures diluted to the end-point and injected intra-

dermally into guinea pigs invaded the blood stream and brought about death within 5 to 10 days. One or two chains were fatal to mice 72 hours after intraperitoneal injection.

Strain 4, like its parent strain 3, shows marked instability under certain cultural conditions. If it is kept on 10 per cent horse blood agar it may be transferred serially without any change in morphology or virulence. To date it has gone through 147 such transfers. However, if it is brought over to 10 per cent horse serum broth it begins to develop rough forms within the third or fourth passage. Decreasing the amount of serum causes this dissociation to take place much more rapidly. When no serum is present, either in broth or agar, it is not possible to find any M forms remaining after two generations. Heating serum to 56°C. for 15 minutes does not affect its ability to maintain the M phase. However, heating to temperatures above 65°C. destroys this property. 10 per cent raw egg white, or 0.5 M phosphate buffer at pH 7.6 added to agar or broth will not prevent this rapid dissociation. These findings are similar to those of Oppenheim (7).

Strain 5.—This strain was obtained from the highly virulent mucoid strain 4. Although the usual dissociation of strain 4 results in a rough form, referred to as strain 6 and described below, a small smooth colony with more extensive hemolysis has been obtained from broth cultures on two occasions. This smooth form is unstable. On 10 per cent horse blood agar, over a period of 99 serial transfers of single smooth colonies, a few M forms have invariably appeared on each plate. After the 46th transfer of S colonies, one of these M forms was fished and transferred serially on 10 per cent horse blood agar. Only the M form has been seen in the series during 59 transfers. Reintroduction of this "pure" M line into serum-free broth immediately gives rise to many smooth forms which on passage in broth usually dominate the culture.

The striking resemblance to strain 3 is obvious. The M form of this strain will be designated strain 5. It is considerably less invasive than strain 3. Undiluted young cultures only occasionally give rise to chronic abscess formation in guinea pigs. In mice the intraperitoneal injection of young cultures is fatal in 72 hours at a dilution of 1:10 (about 800,000 chains). Higher dilutions result in only occasional deaths, and beyond 1:1000 all the mice survive. In the mucoid phase, this strain shows rather small capsules in young cultures.

Strain 6.—This is the rough strain regularly obtainable from strain 4 by withholding serum from the culture medium. It shows a fibrous tangled growth which is very difficult to break up and which settles rapidly. It is non-capsulated and entirely avirulent in mice and guinea pigs. Since the length of the chain is as great as that of the parent strain 4, one cannot correlate this factor either with mucoidness or virulence. There has been no change in the strain during 147 serial transfers on 10 per cent horse blood agar. If the local lesions due to strain 4 are cultured, one finds many such R forms although the heart's blood contains only M forms. Thus the M → R dissociation occurs *in vivo* in the lesion although the R forms are probably destroyed in the blood.

Strain 7.—Unstable smooth. See strain 3.

Strain 8.—When strain 6 is grown in broth, especially in the absence of serum, one commonly finds smooth forms on plating. Occasionally the entire culture appears to have lost its rough character. Aside from the small smooth colonies and somewhat shorter chains, this strain resembles strain 7 in its lack of both capsule and invasiveness. It is extremely stable under any cultural conditions, showing no tendency to change during 76 serial passages on 10 per cent horse blood agar, or 15 serial transfers in broth.

The marked differences in the stability of these strains is apparent. The importance of serum in maintaining the M phase is also made clear by these observations. The fact that the mucoid strains 3 and 4 as they develop in a local lesion undergo dissociation to avirulent non-capsulated forms is of some interest, and the failure to isolate these avirulent forms from the circulating blood indicates that they are probably phagocytized and destroyed as rapidly as they enter the blood stream.

Immunity to Virulent Strains

It may be stated at the outset that immunity to infection with the virulent streptococci under consideration is provided only by an already existing infection with a chronic strain. All attempts to immunize mice or guinea pigs with killed virulent strains, living non-pathogenic strains, or "aggressins" have met with failure. Occasionally immunized animals will show a slightly longer survival time, but usually death occurs at about the same time as in the controls, or even more rapidly. Passive protection of guinea pigs and mice with precipitating sera from rabbits or with serum from chronically infected guinea pigs is unsuccessful. Most of these studies have been concerned with the acutely lethal strain 1; less extensive experiments with the similar strain 4 have given the same results. The following data are representative.

Immunity in the Presence of Chronic Infection.—A chronic streptococcus infection was set up in 12 guinea pigs by the intradermal injection in the flank of 0.1 cc. of an 18 hour culture of strain 3 diluted 1:100. After 1 week, 3 of these were reinoculated on the opposite flank with the virulent strain 1, 0.1 cc. of a 6 hour culture diluted 1:100,000 representing 25 to 50 clumps. 4 were similarly reinoculated 2 weeks after establishing the chronic infection, and 5 after 3 weeks. 9 controls receiving only strain 1 died within 12 days. Of the 3 animals tested 1 week after their first injection, 1 died 6 months later, and 2 were sacrificed at the same time. Of the 4 tested after 2 weeks, 1 died after 1 month, 1 after 6 months, and the remaining 2 were sacrificed after 6 months. Of the 5 tested after 3 weeks, 2 died 1 month later; 1, 3 months later; and 1, 4 months later. The remaining animal was sacrificed at 4 months. The heart's blood of those which succumbed yielded strain 3, rather than the virulent strain 1, with the exception of 2 guinea pigs which survived for only 1 month after their test injection. The same resistance to superinfection was found in guinea pigs carrying naturally acquired abscesses.

Very rarely one encounters a guinea pig which appears to be relatively resistant to infection with the virulent strain 1. Such animals develop chronic abscesses and may live for months. Pus from these abscesses contains fully virulent streptococci. 4 normal guinea pigs were placed in a cage with such a guinea pig having large cervical and inguinal abscesses due to strain 1. In 2 weeks one of the normal animals died, another in 6 weeks. Both of these resembled acute infections at postmortem with little or no evidence of local lymphatic involvement. The heart's blood of both contained strain 1

which proved fully virulent on passage. This experiment was carried out 4 months after the carrier infection had been established. Even after 6 months, the abscess pus showed virulent organisms in large numbers.

Attempted Active Immunization of Guinea Pigs.—Heated (56°C. ½ hour) and formalinized (0.1 per cent) vaccines were prepared with strain 1, using both 6 and 24 hour serum broth cultures. Turbidity was adjusted to 1 on the Gates scale (8). Aggressins were prepared by injecting guinea pigs intraperitoneally with 0.5 cc. of an undiluted 24 hour culture of strain 1. After 2 days the moribund animals were killed and the peritoneal cavity washed out with salt solution. The very viscid turbid washings were adjusted to a turbidity of 1 and heated to 60°C. for ½ hour. For each of these preparations 5 guinea pigs weighing 300 gm. each were used. 3 cc. were injected intraperitoneally. After 2 weeks they were tested for immunity by an intradermal injection of 0.1 cc. of a 4 hour culture of strain 1 diluted 1:100,000, containing about 40 colonies by plate count. Except for the fact that the aggressin-vaccinated animals survived 1 to 2 days longer than the controls, there was no evidence of protection. This experiment was repeated using 3 weekly injections of 2 cc. each, testing 16 days after the last dose. The result was the same; in this experiment the aggressin failed to prolong the survival period. Heavy suspensions of heat-killed 4 hour organisms of strain 1 were mixed with freshly prepared alumina (9) and injected intramuscularly. Although this produced a vigorous inflammatory response, the animals succumbed as rapidly as the controls when tested 2 weeks later.

Using the chronic strain 3, aggressins and vaccines were made as described above for strain 1 and similarly administered. They failed to prevent the development of regional lymph node abscesses in guinea pigs subsequently tested with young cultures of strain 3 diluted 1:100 and injected intradermally. A heavy suspension composed of a mixture of heat-killed human tubercle bacilli and heat-killed young organisms from strain 3 was injected intraperitoneally into guinea pigs (3 doses of 0.5 cc. at 4 day intervals). This was an attempt to simulate the prolonged inflammatory response of the chronic disease. No protection against strain 1 was demonstrable 2 weeks after the final injection.

In addition, it has been noted (10) that repeated infections with strain 1, followed each time by curative treatment with sulfanilamide, failed to induce immunity in guinea pigs.

Attempted Active Immunization of Mice.—In determining the effectiveness of each of the following immunization procedures, 12 mice of about 15 gm. were used. Each experiment included 12 controls. Young cultures were diluted serially in broth and 0.2 cc. of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were injected intraperitoneally, 4 mice being used for each dilution. 0.2 cc. of the 10^{-6} dilution generally contained less than 25 colonies by plate count. Strain 1 was grown for 5 to 6 hours, the remaining strains for 3½ hours before injection.

With one exception, none of the measures listed in Table II conferred any immunity. The mice vaccinated with strain 4 and tested with the same strain lived about 24 hours longer than the control mice or those vaccinated with strains 6 and 8.

Attempted Passive Immunization of Guinea Pigs.—8 normal guinea pigs were injected intraperitoneally with pooled whole citrated blood collected from immune (chronic infection) guinea pigs. 3 of the normal animals received 3 to 5 cc. of blood from an infection of 13 days standing. 5 of the normals received blood from guinea pigs which had been carrying their chronic infection for several months. Of these, 3 received 3 cc.,

and 2 received 7 cc. They were tested 48 hours afterward with strain 1, 0.1 cc. of a 5 hour culture at 10^{-5} injected intradermally, 50 colonies by plate count. All of these treated animals and 4 controls died in less than 12 days, with strain 1 in the heart's blood at postmortem.

4 rabbit sera were prepared by the method recommended by Lancefield (11), using 4 hour cultures; 2 of the rabbits received heated, and 2 formalinized (0.1 per cent) vaccines. After the second series of injections, 1 of the formalin-vaccine and 1 of the heated vaccine animals had developed precipitins for Lancefield extracts (11) of strain 1.

TABLE II

Procedures Failing to Protect Mice Actively

Strain used to prepare vaccine	Method of preparation	Method of vaccine injection	Time elapsing between last dose of vaccine and immunity test	Strain used for immunity test
			days	
1	4 hr. culture in 0.1 per cent formalin; Gates turbidity 1	3 injections at 3 day intervals, 0.1 cc. each, i.p.*	10	1
1	As above, using 24 hr. culture	" "	10	1
1	Heated 56°C. for ½ hr. No formalin. 4 hr. culture	" "	10	1
1	" "	3 injections at weekly intervals, 0.2 cc. each, i.p.	10	1
1	As above, using 24 hr. culture	" "	10	1
1	Aggressin. Gates turbidity 1	" "	10	1
1	Heated 4 hr. organisms in alumina	Single injection 0.2 cc. s.c.* in flanks. Sterile abscess formed	14	1
2	Living 24 hr. culture undiluted	Single injection 0.5 cc. i.p.	10	1
2	6 hr. culture resuspended in 0.1 per cent formalin	3 injections at 4 day intervals, 0.5 cc. each, i.p.	7	1
3	As above, using 3½ hr. culture	" "	7	3
4	" " " " " "	" "	7	4
6	" " " " " "	" "	7	4
8	" " " " " "	" "	7	4

* i.p. = intraperitoneally. s.c. = subcutaneously.

Guinea pigs injected intraperitoneally with 4 cc. of each of the 4 rabbit sera and tested 24 hours later with strain 1 failed to show any resistance to the infection.

Attempted Passive Immunization of Mice.—12 mice were used in testing each serum, with 12 controls included in each experiment. Dilution of the test cultures was the same as in the active immunity experiments. Some of the sera had strong precipitins for Lancefield extracts of the homologous strains; those which did not were tested regardless. The data covering these attempts are summarized in Table III. No protection could be obtained with any of the sera.

An attempt was made to increase the effectiveness of serum by injecting 1 cc. of sterile broth intraperitoneally 72 hours before the test injection of organisms. This

preparation of the peritoneal cavity was ineffective. Although the serum from the guinea pig carrying a chronic strain 1 infection was not protective, the injection of rabbits with living organisms was attempted. Several rabbits which had been previously injected with heat-killed strain 1 were given small amounts of a young living culture intradermally. One animal survived, developing a series of small abscesses at the inoculation site. Its serum failed to protect mice when tested 1, 2, and 6 weeks after the injection of living organisms.

TABLE III

Procedures Failing to Protect Mice Passively

Strain	Source and method of preparation of serum	Precipitins for homologous Lancefield extracts	Amount used	Time elapsing between serum injection and test	Strain used for immunity test
			cc.	*hrs.*	
1	Guinea pig carrying chronic strain 1 infection	0 to ±	0.2	24	1
1	Rabbit. Heat-killed vaccine, 3 series* of injections	++	0.2	24	1
1	Rabbit. Heat-killed vaccine, 8 series of injections	++	0.1	24	1
			0.2	24	
			0.5	24	
			1.0	24	
1	Rabbit. Heavy suspension in alumina injected intramuscularly. Serum drawn after 1 mo.	+	0.2	48	1 and 3
1	" "	0	0.2	48	1 and 3
3	Rabbit. Heat-killed vaccine, 4 series of injections	+++	0.1	24	1
			0.2	24	1
			0.1	0	1
1	Chicken. Daily injections for 1 wk. of living 24 hr. culture (non-pathogenic). Serum drawn 10 days later	0	0.2	0	1
3	" "	0	0.2	0	1
1	4 rabbits immunized by the method of Loewenthal (17) using young cultures heated 12 min. at 55°C.		0.5	24	1
4	" "		0.5	24	4

* Each series of injections consisted of 6 daily intravenous doses of 1 cc. of vaccine followed by a rest period of 8 days. Bleedings were done 10 days after the last injection. Unless otherwise indicated, the vaccines were heated for ½ hour at 56°C.

Relation of Precipitin Reactions to Virulence.—Rabbits were injected with strains 1 to 7. For the most part, Lancefield's technique (11) was followed, using either heat-killed or formalinized cultures. Strains 6 and 7 failed to produce precipitins for Lancefield's extracts of homologous or heterologous strains; while strains 1, 2, 3, 4, and 5 produced precipitins for such extracts. Strain 3 was the most regular strong precipitin former, and strains 1 and 4 the most irregular. No evidence of type specificity was observed. Furthermore, all of the precipitating sera were equally active against acid extracts made from any of the group C strains under consideration, regardless of virulence. The probability that these precipitins are not correlated with the invasive-

ness of the strains used for their production was further confirmed by absorption experiments. Lancefield's method of absorption (11) was followed. The precipitin made with a virulent organism may be absorbed completely by means of totally avirulent strains, as well as by the virulent homologous strain.

Opsonins and Whole Blood Bactericidal Tests.—Guinea pig blood used in the following phagocytosis experiments was freshly drawn and citrated, since the leucocytes are badly damaged by defibrination. Human blood was defibrinated. 0.5 cc. was distributed into small test tubes and 1 to 2 standard drops of the organism suspensions washed once in salt solution were added. The organism suspensions were adjusted so that the virulent

TABLE IV

Phagocytosis in Normal Guinea Pig Blood

Tested with strain No.	Age	Phagocytosis
	hrs.	
1 (virulent)	6	0*
	18	+
2 (avirulent)	6	++++
	18	++++
3 (virulent)	3½	0
	18	0
4 (virulent)	3½	0
	18	0
7 (avirulent)	3½	++
	18	++

*0 = no organisms found in 50 leucocytes.
\+ = an occasional clump taken up.
++ = 2 to 4 chains in each leucocyte.
++++ = very extensive phagocytosis, each cell stuffed with organisms.

were slightly more turbid than the avirulent. The tubes were sealed with paraffined corks and rotated slowly end-over-end at 37°C. for ½ hour. They were then spread as ordinary blood films, stained with Wright's stain, and 50 leucocytes examined. Table IV shows the extent to which various strains, young and old, were phagocytized in normal guinea pig blood. This same amount of phagocytosis took place not only in normal guinea pig blood (3 specimens), but also in the following: normal human blood; guinea pig and human blood plus 1 drop of undiluted precipitating serum made with strain 3; 4 specimens of blood from guinea pigs infected with strain 3 and therefore immune to strains 1 and 4. No opsonizing antibody for the young virulent cultures could be detected.

The killing power of whole citrated blood from chronically infected guinea pigs was examined by the technique of Todd (12), using serial dilutions of the virulent strain 1. In addition, blood from a guinea pig carrying a chronic strain 1 infection was similarly

tested. No bactericidal effect was demonstrated, although such guinea pigs are immune to strain 1.

Allergy and Immunity.—The delayed tuberculin type of skin reaction described by Moen (13) in guinea pigs having chronic streptococcus lymphadenitis was studied in its possible relation to immunity. It was found that the Lancefield acid extracts were almost as reactive as the alkaline extract employed by Moen, some of which he kindly furnished. The skin reactivity of the Lancefield extracts was much reduced or entirely destroyed by the action of purified pepsin.

It was possible to produce these skin reactions in guinea pigs following injection of heat-killed vaccines, but the reactions were less intense than in animals having actual infections. Heated aggressins were somewhat more effective, but still the reactions were less vigorous than in infected animals. As has already been shown, however, guinea pigs vaccinated with these materials are not immune to infection.

In order to test the protective effect of more vigorous allergic reactions, guinea pigs were sensitized with a pneumococcus autolysate following the observations of Zinsser and Grinnell (14). Three daily intraperitoneal injections of 0.1 cc. of a heavy suspension of pneumococcus Type II, which had autolyzed, were administered to 4 animals. 2 weeks following the last dose, they received intradermally 0.1 cc. of a 4 hour culture of strain 1 diluted 10^{-5} (about 70 colonies). Before injection the organisms were suspended in the same pneumococcus autolysate used for the sensitizations. All of the guinea pigs showed definite delayed skin reactions, two of which developed necrotic centers after 48 hours. However, they all died 2 to 3 days before the controls; the ones with the strongest allergic reactions died first. It will be noted that similar results were obtained by Rich (15).

DISCUSSION

The spontaneous appearance and subsequent rapid disappearance of the highly virulent form in spite of the presence of susceptible animals is of some interest. It is apparent that those guinea pigs which succumbed to the lethal variant must have been free of infection at the outset, since chronically infected animals are resistant. This would seem to be an example of a parasite which by virtue of its virulence is not adapted for long continued survival in its host, in marked contrast to the parent streptococcus which has moved uninterruptedly from animal to animal over a period of years. That such acutely fatal forms seldom remain long in a population has been pointed out by Theobald Smith (16).

The solid immunity exhibited by chronically infected guinea pigs to infection with the lethal variant remains unexplained. This resistance could not be produced with killed organisms though a variety of methods was used. In agreement with the data obtained by Rich (15), its presence could not be ascribed to concomitant allergic skin reactions. In view of the complete absence of any transferable protective mechanism, chronic infection with this particular streptococcus probably produces an uncompli-

cated form of so called tissue immunity and should furnish a favorable approach to that important problem.

SUMMARY

A group of guinea pigs carrying a chronic streptococcus cervical lymphadenitis has been studied. The chronic disease may be transmitted with pure cultures of streptococci isolated from the naturally occurring abscesses. Its probable mode of transmission under natural conditions was shown to be the ingestion of the infective agent.

The spontaneous appearance of an acutely fatal variant was observed. Infection with the chronic strains protected animals against the highly virulent strain. Such immunity could not be passively transferred to either mice or guinea pigs, nor could any opsonizing, precipitating, or bactericidal antibody be associated with it. The presence of allergy could not be correlated with this immunity.

The dissociation of the chronic and acute strains was investigated and non-invasive phases isolated. No precipitin reaction attributable to an antigenic virulence factor could be demonstrated. No protection was obtained with vaccines or aggressins.

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