FATAL SEPTICEMIA IN MACACUS RHESUS CAUSED BY A STREPTOCOCCUS DECOLORIZED BY GRAM’S METHOD.¹

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The interest which is being displayed in the group of pathogenic cocci which are decolorized by Gram’s method of staining justifies the description of a streptococcus obtained from three monkeys (Macacus rhesus) which died spontaneously while in the laboratory. A further interest and importance attaches to the description of the streptococcus in view of the far greater use at the present time of monkeys in experimental laboratory work. The nature and causes of the diseases of the lower animals are only less important than of those of man, and we require particularly, in our experimental work, to understand fully the diseases of laboratory animals.

The monkeys from which the streptococcus was obtained had been previously used in an experimental study of syphilis. For several weeks before they were taken ill they were apparently in perfect health and had been returned to the house where stock animals are kept. All three died during the first few days of February, 1908—before the fifth. The first one became ill during the latter part of January and died after about ten days. The other two died much more quickly, the whole duration of illness in each being only two or three days. Of the two which died so soon after showing signs of illness one occupied the same cage with the one which was taken ill first. The third animal occupied a separate and removed cage, but it was attended by the same keeper who looked after the others.

In none of the animals was the portal of entry of the infecting streptococcus ascertained; nor were special pathological foci present in any. The visible lesions consisted of punctate hemorrhages in

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The serous membranes, congestion of the lungs, moderate enlargement of the spleen, and cloudy swelling of the viscera.

I shall describe the streptococcus fully not because its intrinsic importance may justify a detailed description of its properties but in order that future observers may be able to identify it. I have myself suffered from the very inadequate descriptions, in the literature of the subject, of what may be allied forms. It is highly improbable that the organism in question has been noted before; at least there is no description from which it may be identified. Those organisms mentioned in the literature which bear any resemblance to the streptococcus will be briefly considered after its characteristics have been set forth. Here it is sufficient to say that no previous description deals with a Gram negative streptococcus which was obtained from monkeys or which is so highly pathogenic for other animals as this one. Even when compared with Strep-tococcus pyogenes this streptococcus possesses a high degree of virulence for rabbits and white mice which it retains unimpaired after many months of cultivation upon artificial media.

DESCRIPTION OF THE MICROORGANISM.

Source.—Three cultures, designated (1), (2) and (3) and later found to be strains of the same organism, were obtained at autopsy from the heart's blood of three monkeys (Macacus rhesus) which had died under natural conditions. Except for less vigor of growth and less resistance to untoward influences on the part of (3), no material differences can be detected between the three strains. All statements in the description apply alike to each strain unless otherwise stated.

Morphology.—There is least irregularity in size and intensity of staining in preparations made from cultures upon glucose-sheep-serum agar kept for eighteen hours at 37°. Under such conditions the organism appears as a coccus usually arranged in pairs, sometimes in short chains of from four to eight or ten elements. Only exceptionally may a single isolated coccus be seen. In most of the short chains the arrangement in pairs is preserved, although in the long chains which are formed in liquid media (see below) it is often wanting. In stained preparations the individual elements are round,
or elliptical with the long diameter at right angles to the axis of the
chain. Very often the opposed surfaces are less convex, more
nearly flat, than the removed ones. There is little variation in size,
the smallest measuring perhaps 0.7μ, and the largest about 1.2μ,
in diameter. The average size is about 0.8μ or 0.9μ. In impres-
sion preparations made from colonies no chains are observed, but
along the thin edges of the preparation the arrangement in pairs
may be seen. In hanging-block preparations there are isolated pairs
and short chains made up of from two to four or five pairs. Here
the individual cocci are larger than in stained preparations and aver-
age about 1μ in diameter. In the hanging drop made from eighteen
hour old bouillon cultures at 37° the coccus does not appear singly
but in pairs or, most often, chains containing from ten to forty or
more elements. Here, too, the size is larger than in stained prep-
arrations. In the water of condensation of glucose-sheep-serum
agar cultures, where vigorous growth takes place, very similar ap-
pearances obtain as in bouillon except that isolated pairs are very
rare and that the chains often contain hundreds of elements. These
long chains are winding and they often attain a length greater than
the diameter of the field of a one-twelfth objective (Oc. Leitz 3).
In bouillon containing sugar there is more tendency to the formation
of long chains than in plain bouillon and growth is more rapid and
profuse.

No appearances suggestive of spore formation have been encoun-
tered.

Motility has been absent in preparations made from cultures
fourteen, eighteen, twenty-four, thirty, and thirty-six hours old at
37°, the surface growth upon glucose-sheep-serum agar, the water
of condensation, bouillon and milk being utilized for the examina-
tion. Attempts at capsule staining by the methods of Welch and of
Buerger in cover-glass preparations made from the body fluids and
tissues of animals killed by the organism, and from eighteen and
twenty-four-hour cultures upon glucose-sheep-serum agar, upon
Löffler’s medium, in milk, and in Hiss’s sugar serum waters, have
failed to reveal the presence of a capsule.

The organism stains readily and well with dilute solutions of
the aniline dyes ordinarily used for staining bacteria, and more
sharply with basic than with acid ones. It is not acid fast. It is
decolorized by the method of Gram and by Nicolle's modification.
In view of the occurrence, frequent in the older literature and still
occasional in the newer, of such expressions as "stains well by
Gram's method," "stains poorly," "almost completely decolorized,"
etc.; of the contradictory statements regarding the behavior of many
organisms to Gram's method; of the confusion which has resulted
from the use of such expressions and statements; and, finally, in
view of the fact, that of the Gram negative organisms some de-
colorize more readily and more rapidly than others, it would seem
pardonable to add that the observation is made after maximum de-
colorization as apparent to the unaided eye. While the streptococ-
cus in question does not decolorize with the rapidity with which
Micrococcus intracellularis or Micrococcus catarrhalis do, still in
thin sections it is always decolorized in two or three minutes' treat-
ment with alcohol, and in thinly spread cover-slip preparations
within one and one-half minutes, usually within from forty to sixty
seconds.

Involution Forms.—In twenty-four cultures upon glucose-sheep-
serum agar at 37° there is a moderate degree of irregularity in size,
most of the elements which are not of the average normal size being
larger and only a relatively small number being smaller. "Arthro-
spore" formation is not infrequent. Sometimes a short chain is
made up wholly of large forms which may reach a diameter of 1.5μ.
Usually the larger forms stain more intensely than the normal ones,
while the smaller forms show less avidity. After forty-eight hours' 
incubation abnormal forms are more numerous than after twenty-
four hours but they are still far outnumbered by normal ones.
There may be seen a moderate number of pairs in which one element
is of normal or slightly enlarged size and well-stained, the other
much smaller and less sharply stained. Even after ten or twelve
days when well protected from drying the appearances are very
much the same as after only forty-eight hours, there having taken
place very little increase in the ratio of abnormal to normal forms.
After remaining three months in the incubator, when the medium is
quite dry and the growth dry, shrunken and glazed in appearance,
the cocci are quite sharp in outline and stain fairly well. Preserva-
ion is remarkably good after five months in the ice-chest (5°–8°). Under each of these conditions viability has been lost.

When grown upon glucose-rabbit-serum agar, plain rabbit-blood-agar, and Löfler’s medium involution occurs as above described. But in bouillon it takes place somewhat more rapidly and is manifest in a proportionately large number of individuals. Even after eighteen hours many pairs in which one element is half as large again as the other may be seen. In bouillon cultures which have been kept for a few days at 37°, there are many large round, oval and ovoid forms which may reach a size several times the average normal. Often they are situated in the middle of a chain but frequently they are seen at one or both ends, and sometimes at various points between the ends and the middle of the chain. Not infrequently a whole chain of ten or more elements is composed of cocci twice the average normal size. After eleven weeks the state of affairs is much the same.

Upon agar, plain and with glucose, involution occurs more rapidly and is more marked than in any other medium. It is extensive even after eighteen hours when the growth is still slight and has not yet reached its maximum.

In milk there is much less tendency to involution than in bouillon although in it after several weeks nearly all of the cocci are very large. Upon potato involution occurs fairly rapidly and is moderate in its extent but less marked than upon agar.

In suspensions of the growth upon glucose-sheep-serum agar in normal salt solution with toluol added the organisms are well preserved after forty-eight hours in the incubator as well as at room temperature.

*Cultural Features.*—The organism is a facultative anaerobe.

Stroke cultures upon glucose-sheep-serum agar; twenty-four hours, 37° : growth moderate in amount; thin, slightly raised, glistening, opalescent streak with edges made up of semi-confluent colonies. (When a very small amount of growth has been transferred in making the inoculation the colonies may remain discrete.) They are small, punctiform up to 0.5 mm., convex or pulvinate, smooth, glistening, opalescent. Chromogenesis is wanting and no odor is produced. To avoid needless repetition it may be said here
that this applies to cultures in all other media in which the organism has been grown. The growth may be scraped away with ease but it remains very finely granular and is never viscid or slimy. With the growth which has been scraped away a fine, smooth emulsion may be made, but unless it be judiciously and thoroughly well rubbed up in the beginning with only a trace of moisture the emulsion is prone to be uneven and to contain small clumps. In cultures forty-eight hours old of which there has been some drying this tendency to cohesion and to the formation of clumps is very difficult to overcome.

Clouding of the medium, presumably due to precipitation of the contained albumin by the acid set free in the fermentation of the glucose, is present to a slight degree. It may indeed be noticed in cultures only eighteen hours old. The cloudiness steadily increases until within the course of a few days the medium is opaque and white throughout. In the water of condensation growth is profuse. A coarsely granular grayish-white precipitate is formed. At first it is evenly distributed throughout, sometimes forming a thin pellicle, later it has fallen to the bottom leaving the fluid above clear. Upon microscopical examination a considerable proportion of this precipitate is seen to be made up of coagulated albumin. The water of condensation is coagulated in a jelly-like mass quite late, usually after some ten or fifteen days.

When rabbit serum is substituted for the sheep serum growth is the same, sometimes slightly more profuse, but a very striking difference is noted in the appearance of the medium. The cloudiness above referred to does not appear until after four or five days; and then is very slight in degree, and is limited to the immediate proximity of the growth, while the sheep serum medium under the same conditions is white and opaque throughout. This is what occurs when the glucose agar before the addition of the rabbit serum is faintly alkaline to phenolphthalein. When the reaction is acid the phenomenon appears sooner and increases in intensity with the degree of acidity as well as with the age of culture; but it never approaches in intensity that which occurs in the presence of sheep serum no matter how acid in reaction the medium may be or how old the cultures.
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Upon sheep-serum agar without glucose the growth is similar to that described above but is not so profuse and is more delicate in appearance, the edges being slightly raised above the level of the middle portion of the streak.

Upon plain agar with rabbit’s blood growth is like that upon glucose-sheep-serum agar except that it is more nearly transparent and that there is less precipitate in the water of condensation. "Hemolysis" does not occur.

With Löffler’s medium and with solidified egg to which veal broth has been added growth occurs as upon glucose-sheep-serum agar. It is more difficult to remove.

Upon plain agar and upon glucose agar growth is moderate in amount but decidedly less than upon any of the media considered above. The streak is narrow and flat, the edges being slightly higher than the interior, and made up of punctiform, glistening, translucent, semi-confluent colonies. The medium remains unchanged. There is a small quantity of a finely granular white precipitate at the bottom of the water of condensation. Upon shaking it diffuses evenly. The maximum amount of growth is reached before thirty-six hours; indeed there is very little increase after twenty-four hours.

Upon potato growth is moderate in amount. It is invisible to the unaided eye. When the surface is scraped the growth comes away readily in small, white, rather dry, finely granular masses. The medium is unchanged.

In agar stab cultures growth is slight and most marked along the track of the wire where it appears as a fine, grayish-white, filiform streak, sometimes beaded at the bottom. Surface growth is very scant and is confined to the immediate edges of the incision.

Gelatin stab cultures 20°-22°: Growth is slight and slow, appearing after from thirty-six to forty-eight hours as a fine grayish white, filiform track, beaded for the lower two-thirds of its extent. Surface growth as in agar stab cultures. The amount of growth very slowly increases for five or six days until it may be said to be moderate. Liquefaction does not occur. Also gelatin cultures kept continuously at 37° for six weeks suffer no impairment of their power to solidify when placed in the ice-chest.
Bouillon: twenty-four hours at 37°: surface free; slight uniform cloudiness. On shaking a small amount of finely granular whitish sediment arises and diffuses evenly. The reaction has become faintly acid. Acidity increases slightly as growth advances. The amount of sediment slowly increases for three or four days, after which it remains stationary. During this time the liquid has gradually become clear, all growth having settled to the bottom. In bouillon containing one per cent. of glucose, maltose, or lactose, much more growth occurs than in plain bouillon. This is especially true of glucose broth in which there is profuse growth. The precipitate is coarser than in plain bouillon and acid production is very much more rapid and pronounced. The addition of sheep serum to bouillon favors growth. Glycerin bouillon proved the poorest of the media employed for the organism. In litmus milk, which is a favorable medium, slight acid reaction may often be detected within eighteen hours. This increases until the maximum intensity of pink color is reached in from five to seven days. Beginning coagulation is indicated at the end of two weeks or during the third week. It progresses very slowly requiring several days for its completion. The coagulum is pink and made up of large coarse flakes. The whey is clear and colorless. The reaction remains acid. Peptonization does not occur.

Gelatin plate cultures 20°-22°: colonies are just visible to the naked eye upon the third day. Under the low power they appear round or oval, grayish translucent, and much lighter and somewhat more coarsely granular than upon glucose-sheep-serum agar. The edges are fairly sharp but irregular in outline. Subsequently the colonies increase slightly in size but they never exceed a diameter of 0.5 mm.

Glucose-sheep-serum agar plate cultures at 37°: colonies may be seen at the end of twelve hours. They increase rapidly in size up to twenty-four hours; slightly from twenty-four to thirty-six; none after thirty-six. At twenty-four hours: superficial colonies are round, varying in diameter from 0.3 to 0.8 mm., convex or pulvinate, smooth, glistening, nacreous. Under the low power they are brown and finely granular without a nucleus but with a narrow circumferential zone which is of a lighter brown color than
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The interior. (Occasionally after forty-eight hours a colony may show a nucleus.) The edge is almost entire and very thin. There is slight whitening of the medium in the immediate vicinity. It is seldom that a colony attains a diameter of 1 mm., although upon very moist plates containing only three or four colonies a diameter of 1.5 mm. may sometimes be attained.

The deep colonies appear in two forms according as they are situated within the substance of the medium or upon its lower surface against the glass. Those in contact with the glass are very similar to the superficial ones except that they are flat, of a lighter grayish color, and more nearly translucent. The others are smaller than the superficial ones, either round or elliptical, whiter by reflected light, more coarsely granular and of a darker brown color by transmitted light. The elliptical ones are usually sharply defined at the poles but poorly so along the sides. None show the narrow light, circumferential zone of the superficial colonies.

Upon plain and glucose agar plates the colonies are as above described, only smaller with edges sharper in outline and very finely serrated.

The most favorable solid medium for the streptococcus is glucose-sheep-serum agar. One-half per cent. glucose favors growth more than one per cent., 1.5 per cent., or two per cent. Of the liquid media milk and one per cent. glucose, plus 0.5 bouillon (before sterilization) are alike the best suited for its growth.

Physical and Biochemical Features.—In fermentation tubes containing bouillon (originally sugar free) plus one per cent. of glucose, lactose, maltose, saccharose, glycerin and mannite gas formation does not occur. Acid is produced, however, in those tubes containing glucose, saccharose, lactose and maltose, but not in those containing glycerin and mannite. The acidity is greatest in the presence of dextrose, yet marked in broths containing the disaccharids. Growth occurs in both arms of the tubes.

By the use of Hiss's sugar serum waters the fermentative abilities of the organism are found to be slight in degree and range. With dextrose and levulose acid production is noticed in twenty-four hours; but coagulation does not occur until between the sixth and tenth days. The attack upon the disaccharids is very weak.
Lactose water acquires a sharp color only after several days and solidification does not occur until after three weeks. Sometimes maltose water is coagulated after two or three weeks; sometimes not at all. But it is always slowly reddened. The same is true with saccharose. Of the polysaccharids, starch and dextrin waters are turned pink very slowly and never coagulated; while inulin is not attacked at all. Nor are glycerin or mannite split.

Indol production occurs but very slowly and the reaction obtained is slight.

Influence of Reaction upon Growth.—Growth occurs in media having before sterilization a reaction slightly alkaline to phenolphthalein; also at a reaction of $+1.5$; and at intermediate points. The optimum reaction is $+0.5$.

Vitality.—The organism preserves its vitality for a long time upon artificial culture media. Longevity is greatest in glucose-sheep-serum agar cultures kept in the ice-chest at a temperature of from $5^\circ$ to $8^\circ$. After three months under such conditions vigorous growth occurs in the transplantations. At room temperature, $22^\circ$ to $27^\circ$, viability does not persist for so long a time. When carefully protected from drying cultures upon glucose-sheep-serum agar, plain agar, and in bouillon often remain alive, for from six to eight weeks; almost always for four weeks. In the incubator vitality seems to be preserved longest in milk, most cultures giving growth after four weeks although sometimes growth has ceased to occur in the transplantations during the third week. In bouillon the average length of time during which vitality is preserved is about three weeks. Upon glucose-sheep-serum agar it is still less—about two weeks, often not more than ten or twelve days.

The relation which the reaction of the medium bears to the life of the organism is well shown in a number of vitality experiments. These were made in a parallel manner with specimens of bouillon slightly alkaline to phenolphthalein and having a reaction of $+0.5$. One may be quoted.

April 7, 1908. Transplantations upon glucose-sheep-serum agar were made from one culture of each strain in plain slightly alkaline bouillon and in plain $+0.5$ bouillon. All cultures were eleven days old at $37^\circ$. In each instance one maximum loop full of the bouillon
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cultures was transferred. After twenty-four hours incubation at 37°:

<table>
<thead>
<tr>
<th>Transplantations from Slightly Alkaline Bouillon.</th>
<th>Transplantations from 0.5 Bouillon.</th>
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<tbody>
<tr>
<td>Strain (1) shows one colony.</td>
<td>(1) Considerable growth of confluent colonies.</td>
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<tr>
<td>Strain (2) shows two colonies.</td>
<td>(2) Slightly less growth.</td>
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<tr>
<td>Strain (3) shows none.</td>
<td>(3) Slight growth, many colonies.</td>
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**Temperature Relations:** Growth does not take place at temperatures below 17°, or at or above 42°. The optimum temperature is 37°. Upon glucose-sheep-serum agar (the most favorable solid medium):

- At 17.5°, growth is extremely slight and is visible only after four days.
- A 20°-21°, it is scant; visible after 48 hours.
- At 25°-27°, it is moderate; barely visible after 18 hours.
- At temperatures between 30° and 40°, growth is more marked and may be seen after twelve hours.

The organism does not survive an exposure to a temperature of 80° prolonged for fifteen minutes. There was often a considerable difference in the resistance to heat offered by various bouillon cultures of the same age; and cultures of strain (3) were invariably killed by less heat than those of (1) and (2). All, however, resisted a temperature of 65° for fifteen minutes. The relation to very low temperature has been referred to.

Cultures upon solid media in capped tubes remain viable about twice as long as those in uncapped ones.

No proteolytic action is exerted and lipase is not produced.

**Toxin Production.**—Several flasks of bouillon kept continuously in the incubator were inoculated with strains (1) and (2). They were re-inoculated every three or four days for two or three weeks after which length of time the contents were filtered. The filtrates in amounts of from two to eight cubic centimeters produced no visible effects when injected intravenously into rabbits which are extremely susceptible to infection with the living organism.

Hemolysis has not been observed in vivo. Of many bouillon cultures which were tested in vitro for hemolysin only a few produced hemolysis and then only when large amounts were used. The corpuscles of the rabbit, guinea-pig and hen were employed.
Pathogenicity.—Belgian hares and white rabbits are alike very susceptible to artificial infection with the streptococcus and, as a rule, die quickly as a result of it. In white mice also the organism produces septicemia and death, but proportionately larger doses are required. White and hybrid rats, especially old ones, are very refractory but in them death sometimes follows intra-abdominal and intracardiac injections of large amounts of cultures. Subcutaneous injections of twenty times an amount fatal for a rabbit weighing 1800 gr. do not produce death, but are followed by some loss in weight, and sometimes by a slight indurated swelling at the site of injection. The swelling slowly subsides and health is regained. Subcutaneous, intra-abdominal and intra-cardiac injections of cultures into guinea-pigs are followed inconstantly by only a slight loss of weight which is quickly regained; except when excessively large amounts are introduced directly into the circulation when the loss of weight is more pronounced and persists longer, and when there are some evidences in the behavior of the animals of illness. But death does not follow even intracardiac injections of these large amounts. In the pigeon injections of large amounts of cultures into the pectoral muscles produce a more or less pronounced local reaction—redness, swelling, induration—which persists for several days and only slowly subsides. Abscess formation has not been observed. Appearing a few hours after the injection there is a slight general disturbance manifested by reluctance to move, apparent inability to maintain a firm hold of perch and consequently falling off when placed upon it, refusal of food, and finally, by a slight loss of weight. These manifestations disappear quickly and health is completely regained. The hen responds in a similar manner.

Mice.—Subcutaneous injections in white mice are followed by death within from forty-eight to seventy-two hours, usually after about sixty hours, except when a minimal fatal amount (about 0.1 or 0.2 c.c. of a twenty-four hour bouillon culture) is injected. Then death is sometimes delayed until four or five days after the injection. At the site of injection is a rather dry, plastic, fibrinous exudate moderate in amount, and a necrosis of the surrounding subcutaneous tissue often extending into and involving the con-
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tiguous muscles. Abscess formation has never been observed. Usually the spleen is slightly enlarged, soft and moist. In one mouse hemorrhagic infarction occurred in the spleen. The lungs are always of an intensely bright scarlet color as soon as exposed; they crepitate throughout. Microscopically the congestion is seen to be marked and many hemorrhages into the alveoli to have occurred. In the dead mice the streptococcus is always readily found pure in smears and by culture from the local exudate, the heart’s blood, lungs and spleen. In the exudate the cocci are very numerous and appear mainly in isolated pairs, although occasionally a very short chain of three or four pairs or a small clump is observed. There are few polymorphonuclear leucocytes in the exudate, and only exceptionally is phagocytosis seen. In the heart’s blood the organisms are fairly numerous, and usually grouped in short chains containing ten or fifteen elements although a few isolated pairs may be seen. In the lungs they are present in large numbers within the blood vessels and mixed with the blood which has escaped into the alveoli. Likewise large numbers are found in the spleen. Cultivation from each of these sources succeeds easily, the resulting colonies tending to remain discrete.

Intra-abdominal injections produce a general peritonitis with a small amount of serofibrinous exudate. Septicemia occurs and death follows in two or three days.

Rabbits.—Belgian hares and white rabbits are both very susceptible to infection with the streptococcus. For them the organism possesses a high degree of pathogenicity—higher than for any other animal upon which it has been tested. After subcutaneous injection septicemia develops and death occurs in about three days. The local reaction is like that which occurs in mice. Intra-abdominal and intravenous injections alike produce death with great rapidity when the amount of culture injected is moderate or small. The minimal fatal dose for rabbits weighing from 1,700 grams to 2,000 grams has been approximately about 0.2 c.c. or 0.3 c.c. of a twenty-four hour bouillon culture; or less than one fiftieth of a very small loop (made of No. 24 platinum wire and having an inner diameter of 2.5 mm.) of a twenty-four hour growth upon glucose-sheep-serum agar. When such small amounts have been injected intra-
abdominally in some rabbits and intravenously in others, death has often followed more quickly after the intra-abdominal injection and sometimes it has seemed as though a smaller amount was fatal. Death is usually delayed after a very small dose for five or six days. With small doses of 0.4 c.c. or 0.5 c.c. of a twenty-four hour bouillon culture, or a fortieth to a thirtieth of such a loop as mentioned above of a twenty-four hour growth upon glucose-sheep-serum agar, death usually occurs within from forty-eight to seventy-two hours, while when larger amounts are injected death occurs before the lapse of twenty-four hours, often before eighteen.

Following an intra-abdominal injection there is a general peritonitis with a hemorrhagic sero-fibrinous exudate. As a whole the peritoneal surfaces are very red, moist, dull and lusterless, with here and there fibrinous deposits. Frequently the fibrin has been greatest in amount about the spleen. There is a small amount—5 to 10 c.c.—of rather thick, turbid, yellowish-red fluid containing smaller and larger flocculi of fibrin. Microscopically the fluid exudate contains only a moderate number of pus cells and a great many cocci. The common arrangement is in isolated pairs and small groups of pairs although chains of four or five elements may be seen. The position is almost always intra-cellular, phagocytosis being very rare and when present slight in degree. The changes in the viscera are like those to be described as following intravenous injection except that the spleen is larger, softer and more moist.

When death has resulted from an intravenous injection the most striking changes are afforded by the lungs and kidneys. The lungs are intensely congested and their appearance at once suggests minute hemorrhages. Sometimes there is marked edema and the frothy fluid is blood stained. The spleen and liver are engorged. The kidneys are enlarged, dark in color, and show throughout numerous fine hemorrhages about 1 mm. in size. The cortex is increased in thickness and the pyramids are livid. There is cloudy swelling of the epithelial cells, not only of the convoluted but often also of the collecting tubules, and sometimes coagulative necrosis is observed. The urine contains (and this holds true after intra-abdominal injections also) a moderate amount of albumin, usually a moderate number, sometimes many, casts, a few blood and epi-
thelial cells and many organisms which upon cultivation are found to be the coccus unassociated with other bacteria. Most of the casts are coarsely granular, a few hyaline and a few epithelial. Often after an intravenous injection hemorrhagic transudations have occurred into the serous cavities and the streptococcus may be cultivated from the fluid. Attempts at cultivation from the spleen, liver, kidneys, lungs, heart's blood and urine succeed easily. Likewise the coccus is found in smears from these sources. Attempts to recover the organism from the bile have been uniformly unsuccessful.

In microscopical sections of the organs the cocci are readily seen. They decolorize by the Gram-Weigert method. Pairs and short chains occur. For the most they are situated within the blood vessels although in the lungs many may be seen with the extravasated blood in the alveoli. Also they may be found within the parenchyma of the kidney and often free in the lumina of the tubules.

Scratches were made upon the ear in two rabbits and a small amount of culture rubbed in. An erysipelatous condition confined to the ear occurred in both. In one animal it was very severe. The reaction subsided in ten or twelve days and was followed by desquamation of the skin and the loss of considerable hair. When the inflammation was at its height the animals appeared to be ill.

Immunity and Serum Reaction.—Rabbits were immunized by intravenous injections of pure cultures. For the first injection bouillon cultures were killed by being exposed to a temperature of 80° for fifteen minutes. Then live organisms were employed, at first heated to 60° for fifteen minutes and in very small quantities, later unheated and in larger amounts until in one instance at least fifty times an otherwise certainly fatal amount was injected. This plan was adopted because it overcame the very high degree of virulence of the organism for the rabbit, and because it at the same time afforded a serum of a fairly high agglutination titer.

By means of agglutination tests with the serum of such an immune rabbit the streptococcus was easily differentiated from the streptococcus of "lumps" in guinea-pigs, five strains of *Streptococcus pyogenes*, two of *Micrococcus intracellularis*, and from three
unclassified Gram negative cocci obtained at autopsies upon horses suspected of having meningitis. The serum of one animal (Rabbit No. 23) immunized with strain (1) agglutinates that strain, as well as strains (2) and (3) at a dilution of 1 to 250. The same serum in a dilution of 1 to 20 does not agglutinate any other of the organisms named above except *Streptococcus pyogenes* which is slightly agglutinated at this strength. At a dilution of 1 to 40, however, there is no trace of agglutination of *Streptococcus pyogenes*.

No bactericidal effect of the immune serum was detected.

Until the present time (October 20), after having been maintained upon artificial culture media for eight months, none of the three strains of the streptococcus has suffered any appreciable loss of virulence for white mice or rabbits. This holds true for cultures kept most of the time at room temperature as well as for those preserved in the ice-chest.

*Habitat.*—Concerning the habitat of the streptococcus nothing has been found out. Several attempts to find it in the mouths and throats, axillae and groins, and about the anus, of normal monkeys have failed. In autopsies upon four monkeys which died while the study was in progress it was not encountered.

In reviewing those organisms, mentioned in the literature, which might be considered as more or less similar to the streptococcus above described one is confronted by meager notes, often of a single observation only, and frequently by contradictory statements concerning important characteristics, so that in many instances it is impossible to gather even a fair idea as to the nature of the organism mentioned. Such observations are almost useless for comparative study and will be only cited and not dwelt upon. But there are a few organisms which have been studied more closely and of which there exist better descriptions, although concerning certain of these contradictory statements are made. The latter group will be considered first.

In the present state of our knowledge the organism most like the streptococcus just described is the streptococcus of "lumps," a chronic infectious lymphadenitis of the guinea-pig. It was described by Boxmeyer (1) in 1907 and said to stain by Gram's
method. Before the date of Boxmeyer’s paper Dr. Flexner (personal communication) had observed a coccus associated with a disease in guinea-pigs apparently identical with “lumps.” He noted, however, that the coccus decolorized by Gram’s method. I have made observations upon seventeen strains of this organism and have found it to be quite similar to the one described by Boxmeyer, except mainly in regard to its behavior to Gram’s method and in regard to the nature of the growth upon solid media. Stroke cultures upon solid media almost always appear as a raised, glistening, vitreous slimy and viscid streak which after twenty-four to forty-eight hours has dried into a thin, flat, grayish-white film. In addition to well-defined cultural differences this streptococcus may be differentiated from the one obtained from the monkeys by agglutination tests (see above).

Perhaps as next in importance from the standpoint of similarity should be considered the “Drusestreptococcus” or *Streptococcus equi*, which is the cause of “strangles,” “Druse,” or “gourme” in horses. The disease is characterized by a chronic inflammation of the mucous membrane of the nose and throat usually complicated by abscess formation in the anatomically related lymph nodes. Occasionally pyemia occurs with multiple abscesses in the liver, spleen and lungs. The streptococcus was described almost at the same time but independently by Schütz (2), Sand and Jensen (3) and Poels (4). Only Sand and Jensen made mention of the behavior to Gram’s method and they stated that the organism retains the stain. Of later investigators all except Rabe (according to von Lingelsheim) seem to agree that it stains by Gram’s method, although Bongert (5) writes:—“The decolorization in alcohol must not extend too long as otherwise the Drusestreptococcus gives up the stain.” He has seen complete decolorization after forty-five seconds’ treatment with alcohol. According to him growth occurs in milk without causing a change of reaction or coagulation, while Nocard and Leclainche (6) state that milk is coagulated in from twenty-four to forty-eight hours. Except for this difference there is a close agreement as to the characteristics of the organism. In liquid media it forms very long chains. Perhaps its most striking single culture feature is the appearance of stroke
cultures upon agar and solidified blood serum. The streak is at first raised, glistening, vitreous, mucoid. After three or four days it has become a thin, dry, shrunken, iridescent deposit. Sometimes superficial, well isolated colonies upon agar attain a diameter of three or four millimeters. White mice die within three or four days after subcutaneous inoculations of cultures. Guinea-pigs and rabbits are refractory, subcutaneous injections having no effect, or, at the most, producing only a slight local reaction in the rabbit. Intravenous and intra-abdominal injections of very large amounts, however, produce death in both. As to just what relationship may exist between the Drusestreptococcus, the streptococcus of "lumps," and the streptococcus described above further work is necessary for a determination.  

Closely related to, and perhaps identical with, Streptococcus equi is the organism which Schütz (7) found associated with pneumonia in the horse, and which has been called "Brustseuchecoccus." Apparently it has not been as much studied as Streptococcus equi; and concerning many of its characteristics there are wide diversities of opinion. In his original communication Schütz states that the organism is a "small, capsulated, oval bacterium." It decolorized by Gram's method, and to this fact he attached diagnostic significance. Rust (8) confirmed the statement of Schütz as regards morphology and staining reactions. Later writers refer to the organism as a coccus and do not consider it capsulated. Lignières (9) holds that it is a streptococcus, that it stains by Gram's method (as Schütz is said to have later admitted) and that it is identical with Streptococcus equi. Bongert (10), on the other hand, does not agree with Lignières concerning the identity of the two organisms. Until more work has been done it can not be said just what the "Brustseuchecoccus" is.  

Since the paper was written I have had an opportunity of obtaining six strains of Streptococcus equi from horses having "strangles." All decolorize by Gram's method. Without discussing at length the characteristics of the three Gram negative streptococci, streptococcus obtained from monkeys, the streptococcus of "lumps," and Streptococcus equi, it may be stated that a comparative study reveals close similarity between the streptococcus of "lumps" and Streptococcus equi and well-marked differences between these and the streptococcus obtained from monkeys which are sufficient to establish the streptococcus obtained from monkeys as a distinct organism.
Ostertag (11) has described as the cause of the infectious aborting of mares a streptococcus which decolorizes by Gram's method. It occurs in short chains. Cultivation upon artificial media is very difficult and growth is less active with each succeeding transplantation. Upon serum agar a very delicate, scarcely visible streak is formed. Growth does not occur in gelatine or milk. For mice, guinea-pigs and rabbits the organism is not pathogenic. These facts serve to differentiate it from the streptococcus obtained from the monkeys.

The same is true of the streptococcus which Hecker and Ostertag (2) have described as the cause of a specific vaginitis in cows. It, too, decolorizes by Gram's method but is pathogenic for cows alone.

In 1884 Nocard and Mollereau (13) observed in the milk of cows affected with a chronic contagious mammitis a streptococcus which they believe to be the cause of the disease. Hess and Borgeaux (14) in 1888 described a streptococcus which they considered the cause of the same disease (mammitre streptococcique de la vache; gelber Galt). Later the two organisms have been shown to be identical. In regard to the behavior of the streptococcus to Gram's method Nocard and Mollereau wrote originally that if the action of the alcohol be prolonged it decolorizes. Later Nocard and Leclainche (15) stated that it "stains poorly by Gram's method." Recently Steiger (16) in a study of a series of cases of chronic mammitis in the cow, apparently the same disease, gives a description of a streptococcus which tallies with that of other observers except for the statement that it stains by Gram's method. The organism described by Nocard and Mollereau coagulates milk solidly within from twenty-four to forty-eight hours. Introduced directly into the udder of a cow or goat a culture produces a mammitis indistinguishable from that arising naturally. For other animals, no matter how introduced, it is not pathogenic. Klein's (17) "Streptococcus radiatus" found in a serofibrinous exudate from the udder of a cow stained by Gram's method. He considers it a different organism from that of Nocard and Mollereau.

Passing from this group of streptococci which are fairly well characterized we may turn our attention to the notes concerning

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other Gram negative organisms about which less is known. Even in the imperfect reports concerning these enough is stated to make it seem highly improbable that any one of them is closely related to the streptococcus obtained from the monkeys. Only those features in which they differ widely from the organism under consideration need be mentioned.

Galtier (8) in 1891 described as the cause of "courage"— a wide-spread epidemic infection of newly-born calves, lambs, kids and pigs—a motile, Gram negative coccus. It grew upon artificial media at from 12° to 25°. Gelatin was liquefied by it.

In preparations made from the throat of a patient having pseudomembranous angina, Étienne (19) found, and grew in pure cultures, a streptococcus which decolorized with great rapidity by Gram's method. Ziemke (20) in a footnote to an abstract of Étienne's communication mentions a streptococcus which he obtained from the blood of a hog which had died of septicemia. It was very similar to the one described by Étienne, constantly decolorizing by Gram's method. Inasmuch as the notes are scanty and the observations have not been confirmed it is useless to speculate as to the real nature of the organisms.

Cottet and Tissier (21) in 1900 described a streptococcus of which the principal characteristic was that it decolorized by Gram's method. It was found in the urine of a patient having a purulent cystitis and in the faeces of a nursing infant which had diarrhoea. In diameter it was scarcely more than 0.5μ. Colonies upon agar were fine and almost transparent; after twenty-four hours, they did not exceed 0.4 to 0.5 mm. in diameter. The organism was killed by an exposure to a temperature of 60° for fifteen minutes.

Baruchello (22), 1905, found in the faeces of almost all normal horses and ass a Gram negative saprophytic streptococcus. He considered it different from Streptococcus equi, Streptococcus pyogenes and Schütz's Brustseuchecoccus.

In a study of mumps, Laveran and Catrin (23), 1893, found a diplococcus which they said decolorized by Gram's method. Later Mecray and Walsh (24) found an organism which they regarded as identical with the one observed by Laveran and Catrin, and
which they believed was only “Staphylococcus epidermidis albus.”

Crajkowski (25) in 1895 reported the finding of a Gram negative coccus in the blood (obtained from the ear) of patients having scarlatina. Large quantities of cultures injected intravenously into rabbits produced no effect.

In the descriptions of the following streptococci there is nothing to warrant their being considered as other than Streptococcus pyogenes except for the statements that the first one decolorized by Gram’s method and that the second one “almost decolorized:” Doleris and Bourges (26), pus of a pelvic abscess; Barbier (27), membraneous angina; Lucet’s (28) Streptococcus pyogenes bovis; Moore (29), suppurative cellulitis in the cow; Kutschera (30), multiple abscesses in the white mouse.

It is impossible to classify the Gram negative coccus found by Lesage (31) in the nasal and laryngeal secretions of patients having measles. There is no mention of its cultural characteristics except that it formed zoogloeae.

The diplococcus found by van Harrevelt (32) in the flesh of a horse which had been shot on account of an enteritis decolorized by Gram's method. It did not form chains. Gelatine was liquefied by it.

Babes’s statement (33), 1889, concerning a capsulated, Gram negative diplococcus found in the blood of cows suffering with an infectious hemoglobinuria is so obscure that it may not be known with what organism he was dealing.

In the communication of Eberth (34) (mycosis in the guinea-pig, 1885), and of Penberthy (35) (valvular vegetation in a horse, 1893) no cultural features are stated. Apparently only microscopical examinations were made.

Finally, many other organisms have been found associated with various infections occurring in laboratory animals. Such are those described by Smith (36), Binaghi (37), Catterina (38), Klotz (39), and others. But it is unnecessary to discuss these here inasmuch as each one possessed some distinct feature or features, such as capsule, flagella, etc., which serve to differentiate them at once from the streptococcus obtained from the monkeys.
R. V. Lamar.

SUMMARY AND CONCLUSION.

A Gram-negative streptococcus is associated with one form of fatal septicemia arising naturally in *Macacus rhesus* confined in laboratories.

This streptococcus differs from *Streptococcus pyogenes* in its behavior to Gram's method of staining; in its slight fermentative powers; in the persistence of vitality for a very long time in its cultures; in its high degree of pathogenicity for certain lower animals; and in the preservation of its virulence undiminished for many months when cultivated upon artificial media.

In the present state of our knowledge this streptococcus must be considered as not identical with any streptococcus heretofore described.

I desire to express my thanks to Dr. Flexner for the opportunity of making the study and for supervision of it, and to Drs. Jobling and Noguchi for many suggestions.

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