STUDIES ON THE HEMOLYTIC STREPTOCOCCUS OF HUMAN ORIGIN

I. Observations on the Virulent, Attenuated, and Avirulent Variants

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(Received for publication, January 11, 1935)

It could be said with little fear of contradiction that the hemolytic streptococcus presents a more hazy picture to bacteriologists and immunologists than does an organism like the pneumococcus. Its study presents many difficulties. In the first place, many strains which are pathogenic for man may show little or no primary virulence for animals. In the second place, antisera cannot be produced readily and consistently against all pathogenic strains. In the third place, although it is certain that many different types exist, typing by agglutination is full of pitfalls, both in technique and in the interpretation of results.

It seemed to us that the foundation for any work on the hemolytic streptococcus was a careful study of the characteristics of the organism on first isolation from the body, and the ways in which it changed on mouse passage or culture passage; and the results of this part of the work are recorded in this first paper. We were aided in this investigation by the studies of several workers in this field, although it was sometimes difficult to correlate their descriptions of the variants of the hemolytic streptococcus with our own observations.

Cowan (1) was the first to correlate cultural characteristics with virulence. She described a virulent S colony and an avirulent R colony. These variants were apparently found in stock laboratory cultures.

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Todd (2), working with freshly isolated strains from cases of puerperal septicemia, noted three variants. He found that the organism on primary subculture was virulent for mice, and developed into matt surfaced colonies on chocolated human blood agar plates. After serial cultivation on blood agar, the virulence was diminished, but the colony form remained unchanged. The virulence could be restored by mouse passage or cultivation in human serum. The variant characterized by decreased virulence was designated the matt attenuated form. Complete and permanent loss of virulence resulted from prolonged subcultivation of the matt attenuated form. The colony of this avirulent variant had a glossy surface.

Loewenthal (3) isolated a strain of hemolytic streptococcus from a mouse epidemic. From the original form isolated, he derived three additional variants. The organism obtained directly from the epidemic was mouse-virulent, produced small, compact, conical colonies which grew flocculently in serum broth, and was designated the N (*Nadelkopf*) variant. The N form, on subsequent mouse passage, threw off two variants: the M (mucous) and the O (*ohne Typspecificität*). The M was mouse-virulent, had a slimy colony, and grew diffusely in serum broth. The O was mouse-virulent, and resembled the pneumococcus in its cultural and antigenic characteristics. It lacked type specificity. The R, or fourth, variant was obtained by prolonged cultivation and was distingished by its avirulence for mice and a cultural similarity to *Streptococcus viridans*.

Dawson and Olmstead (4), in studying strains of recent human origin, found three variants. The colonies of these three variants on neopeptone rabbit blood agar plates were (1) mucoid, (2) smooth convex, (3) smooth flat. They confirmed Loewenthal's observation that the mouse passage of a non-mucoid variant frequently changed it into the mucoid form. It was also noted that the mucoid form was more virulent for mice than the non-mucoid forms, and they thought that human infections from which they isolated the mucoid type were more severe than those caused by the smooth or non-mucoid variants.

In our own work on the variants of the hemolytic streptococcus, we have laid the chief emphasis on the virulent variants and the characteristics which distinguish them from the avirulent forms, in the hope that we would gain a better insight into streptococcal infection, but we have attempted to make the study as comprehensive as possible in the time available.

The details of the experimental methods and results are as follows:

Experimental Methods

(*a*) *Source of Material.*—The majority of the organisms were obtained from blood cultures and direct platings of pus from human lesions. A few strains were isolated from normal throats. The strains were preserved by picking a colony

into a meat tube, incubating for 24 hours, covering the culture with mineral oil, and storing in the refrigerator.

In every case, whether blood culture or laboratory culture, the strain was plated out and carefully studied to determine whether the culture contained one or more variants.

We are indebted to Dr. Lancefield of The Rockefeller Institute, who kindly sent the matt virulent, matt attenuated, and glossy avirulent variants of three strains with which she and Dr. Todd (5) worked some years ago. To Dr. Loewenthal we owe an N and an O variant.

(*b*) *Cultivation.*—

1. Blood Agar Plates.—These have the following composition:

Saline	100.0 cc.
Neopeptone (Difco)	2.0 gm.
NaOH N/1	0.5 cc.
Glycerine	1.0 cc.
Agar	1.5 gm.
Horse blood	15.0 cc.

The first five ingredients are sterilized at 10 pounds for 15 minutes, cooled, the horse blood added, and the plates poured. These plates are soft, but if more agar is added the colony forms of some of the variants are not characteristic. This medium dries out quickly, even in the ice box, and must be used immediately after pouring. The plates are incubated at 37°C. for 15 hours. If a longer incubation is more convenient, a 30°C. incubator should be used. The colonies are examined by reflected light under the colony microscope.

2. Serum Neopeptone Water.—The neopeptone water has the following composition:

Distilled water	100.0 cc.
Neopeptone (Difco)	2.5 gm.
Dextrose	0.05 gm.

The mixture is boiled, adjusted to pH 7.3, passed through a paper filter, tubed in appropriate amounts, and autoclaved at 10 pounds for 15 minutes. For primary cultivation from blood cultures or meat tubes, 5 per cent horse serum is added to the neopeptone water. The characteristics of the growth (diffuse or flocculent) are observed after overnight incubation at 37°C. Secondary cultures for use in phagocytosis are made by adding 1 drop from the primary culture to 4.0 cc. of a mixture of equal parts of neopeptone water and horse serum. These are grown at 37°C. until the first obvious turbidity appears. This usually occurs after about 2 to 3 hours. Such cultures may be used immediately for phagocytosis, or may be stored on ice for as long as 6 hours. It has been our practice to use only freshly autoclaved or boiled neopeptone water to eliminate any growth-lag.

(*c*) *Phagocytosis in Human Blood.*—

1. Source of Blood.—It has been shown that normal adult human blood contains specific opsonins for virulent pneumococci, but that these opsonins are absent in infant's blood (14). We have obtained similar results with virulent streptococci, so that, whenever possible, we have used defibrinated infant's blood to exclude the effect of such natural specific opsonins. Satisfactory results, however, may be obtained by using a mixture of equal parts of adult serum diluted eight times with saline, and washed human blood cells.

2. Technique.—1 drop from a capillary pipette (equivalent to about 0.03 cc.) of the secondary culture described above is added to 0.25 cc. of infant's blood in a pyrex glass tube; the tube is sealed and rotated for 30 minutes at 37°C. The tube is then flamed, broken open, and 1 drop of the contents smeared as a blood film on a glass slide. The dried smear is flooded with 2.0 cc. of Wright's stain,[1] and, after 6 minutes, 2.0 cc. of distilled water is added and left on for 4 minutes. The slide is then washed in running water and allowed to dry. Further details of the phagocytic technique are given in a paper by Ward and Enders (14). Counts are made of the number of organisms phagocyted by 25 or 50 polymorphonuclear leucocytes, and the percentage of these cells taking part in the phagocytosis is noted. The extracellular organisms are studied for the presence or absence of capsules.

(*d*) *Mouse Phagocytosis.*—Normal mice are prepared by injecting 0.5 cc. of saline into the peritoneum in order to induce a cellular exudate in advance of the injection of organisms. 4 hours later 0.25 cc. of the secondary culture of the variant to be investigated is injected into each mouse. One mouse is killed ½ hour later, and a drop of the peritoneal exudate is withdrawn with a wide bore capillary pipette, smeared on a glass slide, and stained with Wright's stain as described above. A second mouse is killed after 1 hour, a third after 2 hours, a fourth after 4 hours, and the peritoneal exudate similarly smeared and studied for phagocytosis and encapsulation of free organisms.

(*e*) *Mouse Virulence.*—A 24 hour 5 per cent horse serum neopeptone water culture is serially diluted 10^{-1} to 10^{-6} in neopeptone water, and 0.5 cc. of each of these dilutions injected intraperitoneally into normal mice. 0.1 cc. of the 10^{-6} dilution is plated out on blood agar, and the number of colonies counted after incubation. The mice are observed for 4 days, and the cause of death verified by heart blood culture.

(*f*) *Spontaneous Agglutination.*—A culture in 5 per cent horse serum neopeptone water or in infusion broth without serum is incubated for 18 hours, well shaken up, and 0.5 cc. of this emulsion is mixed with an equal quantity of saline and placed in a water bath at 55°C. After 3 hours, the tubes are read as for agglutination.

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[1] 0.3 gm. of powder, obtained from the Coleman and Bell Co., Norwood, Ohio, is added to 100 cc. of absolute methanol, and allowed to stand for 1 week, being shaken at intervals.

RESULTS

A description of the variants necessarily entails the use of a nomenclature. In so far as possible, we have tried to retain the terms of other writers, introducing new terms only when it seemed necessary. To avoid confusion, we have attempted to correlate our own with previous classifications.

In all, we have studied 75 strains, and have encountered four main variants. Two variants are virulent for man—the F and M variants. A third is an attenuated form of the M variant. The fourth, or C variant, is avirulent. The characteristics of each of these variants are given below. These have been summarized in Table I to facilitate comparison, and photographs (Figs. 1, 2, and 3) of the different colonies are included.

F Variant

We have isolated this variant from blood cultures in septicemias, from the throat in scarlet fever, from acute sore throats, from localized infections, and from normal throats. This variant is culturally stable, although occasionally the M variant develops spontaneously in meat tubes.

The F variant is not itself mouse-virulent, but if a large amount (1.0 cc.) of a 5 per cent horse serum neopeptone water culture is injected into the peritoneum of a normal mouse, the mouse may die. If death occurs within 2 days, the M or mouse-virulent variant is frequently recovered in pure culture from the heart's blood. With certain strains, however, the mouse may survive indefinitely, or die eventually with the F variant in the heart's blood.

This variant is resistant to phagocytosis in infant's blood, and small or fragmentary capsules are usually demonstrable in the blood films. If the organisms, in the phase of growth in which they resist phagocytosis in infant's blood, are introduced into the peritoneum of a prepared mouse, they are fairly resistant to phagocytosis during the first ½ hour. At the end of an hour, however, practically all the cocci have been phagocyted. The few cocci that remain free show no capsular structure.

The colony on the special blood agar plates has an irregular contour, with a marked crater-like central depression (see Fig. 1). It grows

flocculently in 5 per cent horse serum neopeptone water. It agglutinates spontaneously in infusion broth without serum, but remains in even suspension in 5 per cent horse serum neopeptone water.

We have experienced difficulty in correlating the F with any previously described variant. It conforms most closely with the published description of Loewenthal's N variant (3), except that the N was found to be highly virulent for mice. Unfortunately, the single N culture which we were able to obtain from Germany did not conform to Loewenthal's description, and was apparently an M variant. It is likely that the F corresponds to Dawson and Olmstead's (4) smooth convex variant. Todd's classification does not include this form.

Atypical F Variant

This variant has been isolated from the blood stream of two cases of mastoiditis. It differs from the typical F only in having a conical colony without central depression, and forming heavier capsules. It may possibly be a transition form between the F and the M, since it has been isolated only in combination with the M.

M Variant

The sources of isolation include blood cultures from cases of septicemia, acute sore throats, localized infections, and normal throats. This variant is apt to change into the attenuated M variant when kept under laboratory conditions.

The M is more or less mouse-virulent, and the virulence, if not maximal, can be increased by mouse passage. This form is resistant to phagocytosis in infant's blood and in the mouse peritoneum. In each case, the cocci are surrounded by a well marked capsular structure. These capsules persist in the mouse, and the organisms increase rapidly in number as time goes on.

The appearance of the colony depends to some extent on the length of incubation time. The younger colonies—6 to 8 hours—are of the mucoid type, being smooth, watery, and of regular contour. The older colonies have a matt or wrinkled surface, but still retain the regular contour (see Fig. 2). The colonies are flatter and larger than those of the F or C variants. Growth in 5 per cent horse serum neo-

peptone water is diffuse, and there is no spontaneous agglutination in either plain broth or in the 5 per cent horse serum neopeptone water. This variant appears to be identical with the matt virulent variant of Todd (2), the M variant of Loewenthal (3), and the mucoid variant of Dawson and Olmstead (4).

Attenuated M Variant

This has been met with only in cultures which have been kept in the laboratory for some time. Some of the strains which are used for toxin production are of this type. Similarly, an old culture of *Streptococcus epidemicus* was an attenuated M. Unfortunately, we had no recent isolation from an outbreak of milk sore throat available for study, but the descriptions in the literature would lead us to believe that the *Streptococcus epidemicus*, when newly isolated, is probably an M variant.

It is not mouse-virulent, but according to Todd (2) the virulence can be restored by mouse passage. On the other hand, he states that, if cultivated for long periods on blood agar, it may give off a permanently avirulent variant. We believe that the latter is the C variant, described below.

The attenuated M does not resist phagocytosis in infant's blood or in the mouse peritoneum, although in both cases the capsular structure is morphologically indistinguishable from that of the M variant. One can often find capsulated forms within the cell.

The colony appearance is the same as that of the M. Its growth in 5 per cent horse serum neopeptone water may be either diffuse or flocculent. If grown in this medium it agglutinates spontaneously, but when grown in infusion broth without serum it remains in even suspension. It corresponds to the matt attenuated variant of Todd (2).

C Variant

We look upon this as the avirulent variant, although it was on one occasion isolated from a case of mastoiditis with sinus thrombosis, which ended in recovery. There were several negative blood cultures in this case, but one blood culture was positive, and from it the C variant was isolated in pure culture. The C was also found in normal

throats and in the glossy, avirulent cultures sent to us by Dr. Lancefield. It is not mouse-virulent, and according to Todd (2), this variant does not become virulent on mouse passage. It does not resist phagocytosis either in the infant's blood or in the mouse peritoneum, and has no capsular structure.

The colony that we have most frequently seen is conical in shape, without central depression (see Fig. 3); but occasionally we have noted a flat, rough colony form. It grows flocculently in 5 per cent horse serum neopeptone water, and agglutinates spontaneously in both infusion broth without serum and 5 per cent horse serum neopeptone water. It is not unlikely that it is this variant which causes so much trouble in agglutination tests.

The C appears to correspond to Todd's glossy avirulent variant (2), and perhaps, on the basis of avirulence, to Dawson and Olmstead's smooth flat (4).

Unclassified Variant

We have been unable to classify two strains which behaved in a similar manner. One of these was isolated from a normal throat, and the other from the blood stream in a fatal septicemia. There were two colony forms, one conical and the other mucoid. Single colony picks of either form constantly reproduced both types of colony. Growth in 5 per cent horse serum neopeptone water was flocculent.

There was abundant phagocytosis in both infant's blood and in the mouse peritoneum. In each case, some of the free cocci had no capsule, while others were heavily capsulated. The culture from the heart's blood, after mouse passage, again yielded the two colony types. This variant is apparently a very unstable M, but we prefer to leave it unclassified.

COMMENT

Although, as we have stated, our chief interest in this investigation centered around the question of virulence, it was first necessary to devise methods, which we have described, by which we could identify the different variants.

Recognition of Variants.—Our experience in studying numerous strains and their variants has taught us that no single method is abso-

lutely reliable. Dawson and Olmstead (4) have pointed out the value of using neopeptone in differential media, and we have used this as the basis of all our media. The colony form on neopeptone blood agar perhaps gives the most information of any one test, and with a little experience, the appearance of the colony is a fairly accurate guide in the majority of cases. The type of growth in serum neopeptone water is a useful confirmatory test, and in case of doubt we have found the phagocytic test to be reliable (with the possible exception of one strain) in distinguishing the virulent from the avirulent variants. The phagocytic test is particularly useful in distinguishing the M variant from the attenuated M variant.

Virulence.—Since the discovery that the hemolytic streptococcus formed an exotoxin, there has been a distinct tendency by certain writers to correlate virulence with the ability to secrete toxin (6). We do not agree with this view, but believe that virulence and toxigenicity are independent attributes of the hemolytic streptococcus. Eagles (7) has shown experimentally that animal virulence has no relation to toxin production, and our own observations bear this out, strains which are being used for toxin production having none of the criteria of virulence.

Menkin (8) has observed that there is delayed fixation of streptococci in the zone of inflammation in contrast to the prompt fixation of staphylococci. These experiments have been confirmed by Dennis and Berberian (9). This phenomenon is no doubt an important factor in the dissemination of this organism, but Menkin does not believe that it determines the virulence of the organism.

Tillett and Garner (10) have recently shown that hemolytic streptococci of human origin produce a soluble substance which lyses human clot, and this again may be a factor in virulence. We, however, have found that avirulent variants also produce this lytic principle, and as Tillett and Garner have pointed out, the filtrate of a mouse-virulent culture does not lyse mouse clot.

Although the foregoing properties of the hemolytic streptococcus are no doubt important supplementary factors, we do not regard any of them as the fundamental factor which determines the virulence of the organism. To us, this appears to depend upon the capacity of the organism to resist phagocytosis and consequent destruction by the

TABLE I

Variant	F	M	Attenuated M	C
Source	Blood stream, local lesions, inflamed and normal throats	Blood stream, local lesions, inflamed and normal throats	Laboratory cultures	Normal throats ? Blood stream (one case)
Virulence	Virulent for man, non-virulent for mice	Virulent for man, virulent for mice	Non-virulent for mice	Non-virulent for man, non-virulent for mice
Resistance to phagocytosis of young culture in infant's blood	Resistant	Resistant	Non-resistant	Non-resistant
Resistance to phagocytosis of young culture in mouse peritoneum	Somewhat resistant at first. Later, non-resistant	Resistant	Non-resistant	Non-resistant
Capsule formation in young culture	Small capsule	Large capsule	Large capsule	No capsule
Colony formation on special medium	Irregular, with central crater-like depression	Regular, flat, watery surface in young colony	Regular, flat, watery surface in young colony	1. Conical ?2. Rough, flat
Growth in 5% horse serum neopeptone water	Flocculent	Diffuse	Diffuse or flocculent	Flocculent

Behavior of cultures after shaking up and incubating for 3 hrs. at 55°C.				
(*a*) Culture in 5% horse serum neopeptone water	(*a*) Remains in suspension	(*a*) Remains in suspension	(*a*) Agglutinates spontaneously	(*a*) Agglutinates spontaneously
(*b*) Culture in broth	(*b*) Agglutinates spontaneously	(*b*) Remains in suspension	(*b*) Remains in suspension	(*b*) Agglutinates spontaneously
Variant produced by mouse passage	M variant or no change	M variant	M variant	No change
Correlation with variants described in literature	? Smooth convex of Dawson and Olmstead	M of Loewenthal; matt virulent of Todd	Matt attenuated of Todd	Glossy of Todd; ? smooth flat of Dawson and Olmstead

cells of the host, as was demonstrated by Bordet (11) nearly 40 years ago. This author, in the year 1897, injected guinea pigs with virulent streptococci, and noted that the majority of the organisms were phagocyted at once, but the few that remained free developed capsules, resisted phagocytosis, and increased in number until death of the animal ensued. This clue to the virulence mechanism of at least some strains of streptococci was largely forgotten until Hare (12) in 1929 showed that mouse-virulent streptococci in young culture resisted phagocytosis by human blood, whereas old cultures were readily taken up by the phagocytes. Seastone (13) has recently demonstrated that hemolytic streptococci which grow diffusely in serum broth resist phagocytosis in young culture, and he correlated this resistance with the development of a capsule. We have found that the M variant freshly isolated from human infections grows diffusely in serum broth, develops capsules in young cultures, and that these organisms resist phagocytosis. It is clear that both Hare and Seastone were working with the M variant.

On the other hand, the F variant, frequently isolated from human infections (some of them fatal septicemias), grows flocculently in serum broth, and when grown in young culture in the usual laboratory media fails to develop capsules and is readily phagocyted. Both M and F variants, however, develop capsules and resist phagocytosis if they are grown for a short time in undiluted human serum. The same results may be obtained by substituting equal parts of horse serum and neopeptone water. This is an obvious convenience. The significance of the capsule in relation to virulence will be discussed later.

Although both the F and the M variants resist phagocytosis under these conditions, only the M variant has any virulence for mice. After much study, we are unable to explain the lack of virulence for mice in the case of the F variant. Its human virulence can hardly be questioned, since it has been repeatedly isolated from the blood stream in fatal septicemias.

On the basis of these facts, we have abandoned the mouse test in favor of resistance to phagocytosis as a criterion of human virulence. It might be argued that any streptococcus, virulent or avirulent, would resist phagocytosis under these experimental conditions, but the validity of the test is substantiated by: (1) the phagocytosis of the

attenuated M and of the C variants under the same conditions; and (2) the phagocytosis of the M and F variants in the presence of specific opsonin.

Dawson and Olmstead (4) have suggested that the mucoid (M) variant is responsible for the more fulminating streptococcal infections. Although we have isolated this variant from two such cases, an examination of all the cases we have studied leaves us in doubt as to whether the M variant is really more virulent than the F variant. For example, in one outbreak of puerperal sepsis, we isolated the F variant from the vagina and blood stream of one fatal case, the M variant from the vagina and blood stream of another fatal case, and also from the vaginae of two other cases which recovered without blood stream invasion.

We have made one observation which may possibly have some bearing upon the manner in which both the F and M variants maintain themselves in the tissues of the body. The organisms will grow out in undiluted human serum when the inoculum is taken from an ordinary serum broth culture, and as has been stated, the young culture in undiluted serum is resistant to phagocytosis. Subsequent cultivation, however, from serum to serum, is dependent upon the addition of cysteine to the serum. This suggests that a lowered oxygen tension in the tissues may be an mportant factor in the rate of streptococcal multiplication in the body.

Capsules.—The resistance to phagocytosis of the virulent variants appears to be associated with the presence of capsules on the organisms, better marked in the case of the M variant. No capsule can be demonstrated on the avirulent variant. However, this structural difference between the virulent and avirulent variants cannot be the sole factor in determining resistance to phagocytosis, since the attenuated M variant, which is readily phagocyted, has a capsule which is indistinguishable from that of the M variant.

Origin of the Different Variants.—At the present time, we are inclined to regard the F variant as the parent form of the *Streptococcus hemolyticus* of human origin, since we have only encountered it in primary isolation. On the other hand, all the other variants may be derived from the F. It may be of some significance that, while the F variant was isolated from the blood stream the whole year round, the

M variant was only isolated in blood cultures during the winter months—the so called streptococcus season. Since it is known that the M variant may be derived from the F by mouse passage, it is conceivable that the winter prevalence of the M variant may be due to the high incidence of upper respiratory infections and consequent frequent passage of the hemolytic streptococcus from one case to another.

Other Variants.—In his account of the variants of the hemolytic streptococcus, Loewenthal (3) mentions two other variants, the O and the R. We have not encountered either of these two variants in our own work.

CONCLUSIONS

1. Four common variants of the hemolytic streptococcus of human origin have been described. These have been designated the F, M, attenuated M, and C variants.

2. The F and M variants only have been isolated from the blood stream in streptococcal infections. Only the M, however, has any primary virulence for the mouse.

3. Both these variants resist phagocytosis in human blood under suitable conditions, and this appears to be a reliable test for human virulence.

4. The attenuated M variant, found only in laboratory cultures, has a capsule as well developed as that of the virulent variants, and yet does not resist phagocytosis.

5. The C variant has no capsule and is readily phagocyted. It appears to correspond to the avirulent variant in other species.

6. An attempt has been made to correlate these four variants with those already described in the literature.

7. The application of these findings to the problem of virulence has been discussed.

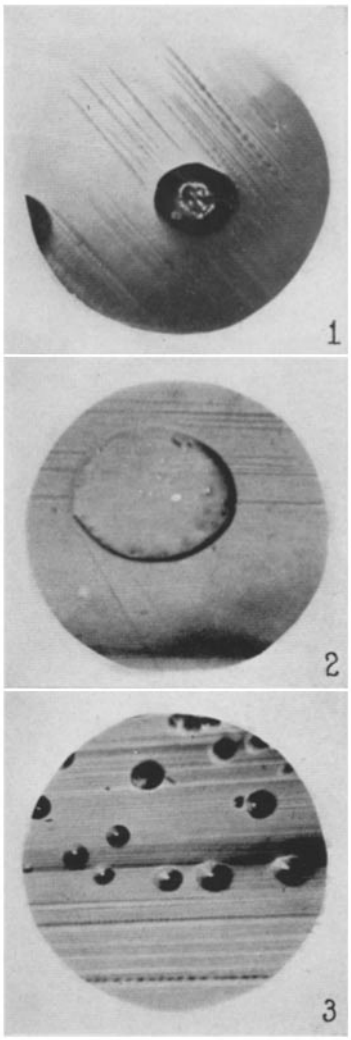
BIBLIOGRAPHY

1. Cowan, M. L., *Brit. J. Exp. Path.*, 1922, **3**, 187; 1923, **4**, 241; 1924, **5**, 226.
2. Todd, E. W., *Brit. J. Exp. Path.*, 1927, **8**, 289; 1928, **9**, 1.
3. Loewenthal, H., *Z. Hyg. u. Infektionskrankh.*, 1932, **114**, 379.
4. Dawson, M. H., and Olmstead, M., *Science*, 1934, **80**, 296.
5. Lancefield, R. C., and Todd, E. W., *J. Exp. Med.*, 1928, **48**, 769.

6. McLeod, J. W., A system of bacteriology in relation to medicine, Great Britain Medical Research Council, London, His Majesty's Stationery Office, 1929, **2,** 57.
7. Eagles, G. H., *Brit. J. Exp. Path.*, 1928, **9,** 330.
8. Menkin, V., *J. Exp. Med.*, 1933, **57,** 977.
9. Dennis, E. W., and Berberian, D., *J. Exp. Med.*, 1934, **60,** 581.
10. Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58,** 485.
11. Bordet, J., *Ann. Inst. Pasteur*, 1897, **11,** 177.
12. Hare, R., *Brit. J. Exp. Path.*, 1931, **12,** 261.
13. Seastone, V., *J. Bact.*, 1934, **28,** 481.
14. Ward, H. K., and Enders, J. F., *J. Exp. Med.*, 1933, **57,** 527.

EXPLANATION OF PLATE 23

FIG. 1. Colony of F variant.
FIG. 2. Colony of M variant.
FIG. 3. Colonies of C variant.



(Ward and Lyons: Hemolytic streptococcus of human origin. I)