INTRODUCTION

Bacterial variation has, since the dawn of modern bacteriology, been a subject of more than passing interest, and within the last decade it has ventured to occupy the front stage. The recent awakening of interest was stimulated by new approaches in this field of research, and by vigorously renewed claims regarding the occurrence of so-called "life cycles" and "filtrable phases" of bacteria.

The present investigation was suggested partly by the work of Smeaton and Rettger (1924) in which a strain of B. mesentericus isolated from a case of clinical pulmonary tuberculosis was observed to undergo extreme pleomorphism and variability in staining properties, under certain conditions of cultivation and incubation. It was stimulated further by the writers' observation of marked colonial variation in a laboratory stock strain of B. mesentericus.

Very few references to papers dealing with variation of B. mesentericus and B. vulgatus are found in the literature. Two publications, one by Seligmann (1919) and the other by Kerkhoff (1927) describe mucoid forms of B. mesentericus. Gee (1927) observed smooth and rough forms of an aerobic spore-producing organism resembling B. vulgatus, and Wahlin (1930) reported a dissociation study of a vulgatus-like bacillus. A few attempts have also been made to demonstrate filtrability of members of the subtilis group. The most recent were those of Rettger and

1 This paper covers in part the work done by the senior author as partial requirement for the degree of Doctor of Philosophy in Yale University.
Gillespie (1933), who failed to demonstrate filter-passing properties. Haag (1927), claims to have observed a filtrable phase or form which was derived from a culture of *B. anthracis*.

The main interest in the present investigation lay in attempts to demonstrate filtrable phases or stages of *B. mesentericus* and *B. vulgatus*. For a more extensive review the reader is referred to the doctorate thesis of the senior author, deposited in the Yale University Library.

**SOURCES OF STRAINS USED, AND COLONIAL TYPES EXHIBITED AT THE OUTSET ON BEEF-INFUSION AGAR AFTER FOURTY-EIGHT HOURS INCUBATION AT 30°C.**

*B. mesentericus*-F. Obtained originally from Ford, and carried for several years as a laboratory stock culture. This was split into the four major variants more easily than were any of the other original strains, and the variants were more easily stabilized. Colonies of the rs colonies yielded only the rs type. The smooth intermediate was regarded as the so-called "normal" type.

*B. mesentericus*-Am. M. Obtained originally from American Type Culture Collection, and carried in stock for about ten years. It showed more variation at the beginning than any of the other strains. A great majority of the colonies were of the rs type. This type was readily preserved by direct transfer to new agar.

*B. mesentericus*-4519. From American Type Culture Collection. About 95 per cent of the agar colonies were of the rs type, at the outset.

*B. mesentericus*-M. From H. J. Conn. Obtained originally from Dr. Meyer, Marburg. It produced only the rs type of colony, but differed from the previous rs strains in that the colonies were more dry and were not so easily removed from the agar plates. Strain M was the most stable of all of the organisms studied.

*B. mesentericus*-JH4 and *B. mesentericus*-JH4A. From Ford. These proved to resemble very closely the F strain. The rs type was readily established as stable.

*B. vulgatus*-123–4529. From American Type Culture Collec-
tion. It produced two types of colonies, rS and Rs. The rS greatly predominated, and was regarded as the "normal" type for this strain. It resembled very closely the rS form of the Marburg strain (M).

* B. vulgatus-JH3 and B. vulgatus-JH3A. From Ford. The rS type of colonies of these two strains greatly predominated. They were easily established as stable or permanent.

All of the nine strains, when grown on the routine agar medium at 30°, and when stained by the Gram method, showed characteristic Gram-positive rods. Most of the cells were single; occasionally there were short chains. The cells were moderately motile. Spores were observed after twenty-four hours incubation at 30°C.; many were present at the end of forty-eight hours. The spores were oval and equatorial, and did not cause bulging of the sporangium, except rarely. Frequently, small portions of the sporangium were seen on the free spores.

MEDIA AND METHODS

The media used for most of the routine procedures were standard beef-infusion broth containing 1 per cent bacto-peptone and 0.5 per cent NaCl, and an agar medium of the same composition plus 1.8 per cent agar. The final reaction was pH 7.5. Other media employed were prepared according to standard methods.

PRODUCTION OF VARIANTS

Various attempts to derive comparatively stable variants from the "normal," rS, type by continued incubation in deep infusion broth failed; nor did the addition of 1 per cent normal rabbit's serum help to bring about definite transformation of type. Some variation was observed when platings were made on nutrient agar, but the organisms tended quickly to revert to the rS type. Rapid and frequent transfer to infusion broth also yielded practically negative results.

By far the most satisfactory method of producing and stabilizing the variants was the selective isolation of colonies from agar plates. Until a desired colony type had been obtained and purified, re-isolations were made every twenty-four hours. After
a new type was once stabilized, transfers were made only every four or five days. The method was as follows: the “normal” stock (rS) strains were streaked on infusion agar plates and, after incubation for twenty-four hours at 30°C., colonies were selected which were either “smoother” or “rougher” than the “normal.” These were streaked on fresh plates. Characteristic rS colonies were carried forward at the same time. After the plates had been incubated for twenty-four hours the same procedure of selection and transfer was repeated. In this way distinct colony types were well separated from one another after many subcultures. At first these types were not thoroughly stable. When they were not transferred at the end of twenty-four hours they frequently reverted to their original form. On the other hand, after numerous selective transfers, the derived types showed marked stability.

Following this method, the four major colony types (S, rS, Rs and R) were developed without much difficulty. The Rs type was obtained most easily of all. A stabilized Rs form was produced within three or four transfers from all of the nine original strains. The extreme smooth or S type was the most difficult of all to obtain. At least ten or twelve transfers were required for the production of an even moderately stable S colony. In one culture (Marburg) a smooth type was never stabilized, although it was occasionally observed. The R colonies were very easily developed, but the most careful selection was necessary to maintain their extreme roughness throughout the series of subsequent transfers. The different variant colonies were carried through 150 culture generations.

No great effort was directed toward producing phantom or mucoid forms. Mucoid colonies were very frequently observed, however. These, except for their mucoid character, were quite similar to the smooth intermediates in appearance. Phantom types were also found quite often. They were suggestive of the types described by Soule for B. subtilis (1928), and by Nungester (1929) for B. anthracis, except that they reverted to the normal type more readily than theirs.
Description of four major colonial variants

The S colony of these species has an even compact margin, is free from surface markings, and is elevated above the surface. It has a diameter of about 2 mm., is grayish white in color and of a rather soft or butyrous consistency. With the exception of strain JH3, which was dry and smooth, the colonies are quite moist. They are opaque throughout and glisten more or less when examined under a bright light. The S type was obtained from all of the nine parent strains, and was stabilized in all except the "Marburg" B. mesentericus strain (see fig. 1).

The rS colony is quite similar to the S type in several respects. It is opaque, glistens in a strong light, is raised and is grayish-white in color. Its margin is entire, but is characterized by small indentations which give it a rather wavy appearance. The surface of the colony is marked by fine convolutions (see fig. 2).

The Rs colony is noticeably flatter than either of the two types just described; it is dull and somewhat grayer in color; it shows, on examination with a hand lens, the "ground-glass" appearance frequently described for members of the group. Its margins are extensively indented, and its surface is lined and furrowed. Occasional small groups of cells are seen along the margin of the colony, producing an irregular, ragged appearance. Colonies of this type are, as a rule, somewhat larger than the smooth or S type (see fig. 3).

The R colony is very much like the Rs, but more exaggerated in every detail; it is flatter, more spreading and duller than the Rs colony. Examination with the low power objective shows a very uneven, irregular margin which closely approximates in appearance the "Medusa-head" growth described classically for B. anthracis. The whole surface is furrowed and lined, as are those of the Rs colonies. With a hand lens the "ground-glass" character is seen to be particularly marked (see fig. 4).

Stability of variants

A brief study was made to determine the degree of stability which had been developed in the variants. The different types were inoculated into several different media, incubated, and
examined at various intervals. The media included plain nutrient broth, glucose and mannitol broth, broth containing 2 per cent normal rabbit serum, and broth tubes of different H-ion concentrations (pH 5.0, 6.0, 7.0 and 8.0 respectively).

It seemed from these observations that the stability of the types is a function not only of the purity of the type, but, even more, of the character of the medium upon which the organisms are growing.

While all of the derived types were quite stable, when they were once established, the two intermediate forms showed by far the greatest degree of stability. Strangely enough, however, the rough-intermediate type (Rs) was noticeably more stable than was the normal smooth-intermediate (rS), when this was produced from other definite major types. In several instances there was a repeated swinging back and forth from one type to another as incubation continued, the tendency being toward a reversion to the original type. The cultures seemed to be somewhat more stable on alkaline than on acid media.

**Physiological and biochemical properties of parent strains and variants**

A comparative study was made of all of the parent strains and the types of variants described above, with regard to their action on gelatin, litmus milk and potato, the reduction of nitrates and the production of indol and hydrogen sulfide.

While there was some difference between different original strains in the type and rapidity of gelatin liquefaction, the variants resembled the respective parent strains in their action, except that the rough and intermediate rough (Rs) variants brought about the liquefaction somewhat more slowly than did the smooth and intermediate smooth (rS) types.

There was considerable difference between the parent strains in their ability to reduce nitrate to nitrite, some being positive and others negative. Corresponding differences were observed in the variants. None of the original strains and variants produced indol, and all failed to show evidence of hydrogen sulphide production.

All of the original strains and variants slowly peptonized the
casein of litmus milk, with or without preliminary rennet coagulation. The different variants reacted essentially alike. The litmus was completely reduced in all of the cultures.

On cooked potato the smooth and rough types appeared to differ from each other somewhat during the first forty-eight hours of incubation at 30°C. However, all of the growths showed the characteristic dirty grayish-brown color and pronounced wrinkling after the fourth or fifth day. The potato became progressively more discolored as incubation continued. After ten days it was impossible to observe any difference between the different types.

_Growth in extract broth at 30°C._ In eighteen hours a faint general turbidity was evident in all of the cultures; a delicate and incomplete pellicle was ordinarily found on the cultures of the R types of all strains at the end of twenty-four hours. Occasionally there were small amounts of flocculent sediment. The S types did not show the production of a pellicle as rapidly as did the R types, but incubation for a period of six or seven days would ordinarily cause the development of a very delicate surface film on the broth. The production of the pellicle was always accompanied by gradual clearing of the broth.

_Carbohydrate fermentation._ The activity of the various strains was studied in the following carbohydrates, alcohol and glucoside: glucose, levulose, maltose, sucrose, lactose, inulin, mannitol and salicin. The reactions of the variants were practically identical with those of their parent strains. Glucose, levulose, sucrose, inulin and salicin were fermented by all, with distinct acid production. All strains and variants attacked mannitol, but with different degrees of intensity. Some of the original strains appeared to exert a very slight action on maltose, while others were entirely negative. There was little or no difference, however, between the different variants of individual strains. Lactose was not attacked by any of the organisms.

_Colony types and cell morphology_

While bacterial cell morphology and type of colony generally bear a definite relation to each other, and one would expect, for example, rough colonies to be made up largely of long chains or
filaments of the organism in question, there are many notable exceptions, as for example in the long chain-forming streptococci, and in certain single cell variants of *Bacterium pullorum*.

No striking correlation could be established between the types of colony and the length of cells and of chains of *B. mesentericus* and *B. vulgatus*. While there was a greater tendency, on the whole, for the individual bacteria to develop chains and elongated cells in the rough than in the smooth colonies, the difference was not such as to establish a full correlation, in spite of the fact that the rough colonies had the appearance under the low-power lens of containing chains lying parallel to each other, particularly in the irregular border and in runners which extended from one portion of the colony to another (see fig. 4).

**FILTRATION EXPERIMENTS**

The major interest in the present investigation lay in the possible demonstration of a filtrable stage in the life history of the organisms under study. While many papers have appeared in recent years on so-called filter-passing forms of ordinary bacteria, there seem to be no published claims of such properties for *B. mesentericus* and *B. vulgatus*. Deeply-staining granular material has been observed in the cells of these organisms by various workers. Smeaton and Rettger (1924) observed extreme cellular morphology in a mesentericus-like organism which was isolated from a case of clinical tuberculosis. Granular and bacillar forms of widely different size and shape, which at times were acid-fast, were frequently seen. According to some investigators, there is a direct relation between filtrability and the presence of granules in a culture.

Two different methods of demonstrating filtrable forms were employed: the first was the one used by Hadley, Delves and Klimek (1931) for the production of "G" colonies, in which the organisms were subjected to serial passage through broth containing small amounts of lithium chloride; the second involved exposure of the organism to the action of bacteriophage.
Serial transfer in 0.5 per cent LiCl broth

In the preliminary experiment a large typical rS colony of the F strain was selected from a beef infusion agar plate, suspended in a tube of infusion broth, and the culture incubated for four hours at 30°C. Of this culture, 1 cc. was inoculated into a second tube of broth and the tube incubated for four hours. One cubic centimeter amounts of the last culture were then introduced into each of four tubes of broth, two of plain extract broth (pH 7.6), and two tubes of extract broth containing 0.5 per cent lithium chloride (pH 7.6). Two of these tubes, one of the plain and one of the LiCl broth, were incubated at 22°; the other two were held at 37°. After twenty-four hours, subcultures were made into fresh media, plain sterile broth being inoculated from the plain broth cultures, and LiCl broth from the LiCl tubes. This procedure was repeated serially at intervals of twenty-four hours through seventeen subcultures.

At the time of every culture transfer agar plates were streaked with material from each of the four twenty-four hour broth cultures. Duplicate sets of plates were made: one on plain extract agar, the other on agar to which 0.5 per cent LiCl had been added. Plates made from the 22° cultures were incubated at 22°; those from the 37° cultures, at 37°.

"G-form colonies"

The plates were examined, after twenty-four hours growth, for the presence of colonial variants which might correspond to Hadley's "G-form" colonies. After four or five serial passages, the agar plate cultures of the 22° series showed colonies which were thought to be similar to the "G-forms" described for B. dysenteriae (Hadley, Delves and Klimek, 1931). They were very small, often groups of only a few cells being observed which were either barely visible to the naked eye or, as was more often the case, visible only with the aid of a strong hand lens or the low power objective of the microscope.

Microscopic examination of these colonies showed them to be composed of a single flat layer of cells on the surface of the agar. There was not the slightest evidence of a tendency toward
heaping-up or dense massing of the cells. Some of the colonies were composed of as few as eight or ten individual cells; others, of a hundred or more. Slide mounts were made of a few of the larger colonies of this type and stained with either methylene blue or the Gram stain. The majority of the cells were fairly uniform rods which were slightly longer and thinner than the normal. Many irregular cells were observed, however. These included “spindle” and “club” forms, in particular. There were also occasional large globular forms which were from three to five times as large as the ordinary cells.

The “G-type” colonies first appeared in the LiCl agar plates which were streaked from the 22° LiCl broth cultures. They were observed on the fifth passage. On the fifth transfer plate very few of the “G” colonies were seen. On each succeeding plate of the series, however, more and more of them appeared until the tenth series, when only so-called “G-colonies” were noted. On the eleventh, twelfth and thirteenth plates of the series only this type of colony developed during twenty-four hours incubation; but from the fourteenth series on the number of such colonies steadily diminished until the nineteenth series, when only normal colonies appeared on the plates.

All of the plates which showed “G” colonies at the end of twenty-four hours were held for an additional period of forty-eight hours. In this period a marked change was observed in the character of the growth on the plate. “G” colonies were no longer present. Some of them, the positions of which had been carefully noted, were no longer visible. They seemed to have been autolyzed. Others, which had resembled typical “G” colonies by the end of twenty-four hours incubation had continued development until they were indistinguishable in appearance from the so-called “normal” colonies on the plate. A few “G” colonies were still found on occasional plates after the first forty-eight hours of incubation, but by the end of seventy-two hours all had invariably disappeared.

“G” colonies first appeared on plain agar plates made from the LiCl broth series, on the sixth serial transfer. In the plain broth series “G-forms” first appeared on the LiCl agar plates
made from the eighth subculture; on the plain agar plates made from plain broth cultures, on those made from the tenth series. While rapid serial passage in plain extract broth eventually produced "G-form" colonies, the addition of LiCl to the medium induced the development of the "G" character in a much shorter time. "G" colonies were never obtained from the 37°C series of subcultures.

For the major experiment of the series, all nine of the original strains of *B. mesentericus* and *B. vulgatus* were seeded into tubes of LiCl broth. These were incubated at 22°C and serial transfers and platings made according to the method described above. Growth was normal on all plates through the fourth series, although the colonies were rather small. On the plates of the fifth and sixth series a few scattered "G-form" colonies were observed. On the plates of the tenth and eleventh series, only "G" types were apparent at the end of twenty-four hours. Incubation of all plates beyond this period caused the disappearance of the "G" colonies, either by autolysis or transition to the "normal" type.

*Filtration experiments with "G-form" colonies*

Since Hadley reported that the "G-form" is indicative of the presence of filterable elements in a culture, his methods of demonstrating such a filterable stage were followed to a certain extent. Numerous "G" colonies were selected from plates of the series. They were removed from the plates with a flattened platinum needle, small chunks of agar on which the colonies rested being cut out, placed in tubes of sterile broth, and thoroughly macerated to free the bacterial cells. These new cultures were incubated at room temperature for varying lengths of time. At stated intervals, they were admitted to a Chamberland L5 candle. Filtrates were held at room temperature and examined at frequent intervals. Also, agar plates were streaked with small amounts of the different filtrates, and material from such plates washed forward according to the method suggested by Hauduroy (1927).

Seventy-two sets of "G" colonies were so treated. Two were
selected from each of the plates of the ninth series, three from each of the eleventh series, one from each of the thirteenth, and two from each of the fourteenth.

Of the cultures of the "G" colonies made from the plates of the ninth series, one of the two for each strain was filtered at the end of twenty-four hours incubation. The other was filtered two weeks later. Of those prepared from the eleventh series one set was filtered at the end of twenty-four hours, a second set after two weeks, and the last after two months. All of the "G" colony cultures of the thirteenth series were filtered at the end of twenty-four hours incubation. One set of those from the fourteenth series was filtered after seventy-two hours; the other after two weeks.

One tube of sterile broth was filtered with each set, serving as a control for the whole lot. This gave a total of eighty filtrates to be observed.

All of the eighty filtrates were treated according to Hauduroy's directions, being washed forward with broth from one agar slant to another. (Large agar slants prepared in tubes of one inch bore were used, instead of Petri plates.) In addition, quite frequent platings were made directly from the filtrates. On none of the slants was any growth ever detected. Extensive and irregular drying of the agar surface caused the appearance of many dull, film-like areas on the slants. These were equally frequent on the two series, the culture-filtrate and sterile-broth-filtrate series. Smears of material from these dull patches were entirely lacking in organized cellular elements. No contaminants were found on the agar slants, even when they were held for ten weeks or longer.

A duplicate set of washings was made on Petri plates with seven of the filtrates. The characteristic dullled areas were observed on all of these plates. Contaminants were found on about 3 per cent of the Petri dishes.

Since attempts to demonstrate the presence of filtrable forms by growing them on meat-extract agar had met with failure thus far, media which were believed to be somewhat more favorable for their development were used. The filtrates were streaked on infusion agar and on agar to which 1.5 per cent glucose had been
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added; they were seeded into glucose broth, into alkaline extract broth (pH 7.6), and into infusion broth. The results were again negative; no filtrable forms could be discerned at any time.

In addition to these filtrates from so-called "G" colony cultures, another set of filtrates was prepared and studied. After the serial subcultures had been made from the thirteenth LiCl broth cultures into the fourteenth set of tubes, and after the usual set of plates had been streaked with material from the cultures, all nine tubes of this thirteenth subculture and one tube of sterile broth were filtered directly. These filtrates were handled in the same manner as the others had been, and all of them proved to be consistently negative.

Incidentally, the plates which had been streaked from these cultures just before filtration showed, after twenty-four hours incubation, only the "G-type" colonies. It was evident, therefore, that at the time of filtration there had been present in the cultures many cells with the potential characteristics of the "G" forms. Since the "G" colonies are supposed, according to Hadley, to indicate the presence of filter-passing elements in a culture, and since so-called "G" forms were present at the time of filtration, it was logical to expect that filtrable forms might be found then, if ever. Many of the filtrates were held for ten months and still showed no evidence of growth.

BACTERIOLYSIS AND FILTRABILITY EXPERIMENTS

Isolation of bacteriophage

Considerable difficulty was experienced in isolating a bacteriophage active against B. mesentericus and B. vulgatus. Seven different attempts to procure a phage from sewage gave negative results. Water taken from a stagnant pool in which mesentericus-like organisms were abundant apparently did not contain a lytic agent active against any of the nine stock strains used in this investigation.

A B. anthracis phage was obtained from a co-worker in the laboratory. He reported this as being active against one of his stock cultures of B. mesentericus, but no activity against any of
the strains used in this investigation was observed for it. An unsuccessful attempt was made to "adapt" it to the test strains.

By mere chance, all of the negative filtrates, including that of \textit{B. anthracis}, had been held. Some of them had been stored between two and three months; the most recently prepared were three days old. They were all pooled and set up with an equal amount of sterile alkaline extract broth, seeded with a mixture of the nine bacterial strains, and the tubes incubated at 30° for twenty-four hours. A filtrate of this mixture was found to lyse nearly all of the mesentericus and vulgatus strains actively. Lysis was demonstrated in both the filtrate-culture tubes and on agar plates. Numerous plaques were produced in the plates of each of the sixteen cultures tested by this method. The exact source of the phage remains a question. It may have been present in any one or more of the filtrates of the pool sample; it may have been an instance of very slow adaptation of the anthrax phage, or it may be that some factor which previously inhibited the activity of the phages was destroyed during the period of storage. In any event, active lysis of the strains was procured.

\textit{Filtration of phage-lysed cultures}

The various cultures were subjected to the action of this lytic agent for eighteen to twenty-four hours at 30° and then filtered. The filtrates were held at room temperature for varying lengths of time. They were examined daily; platings were made from them at different intervals. In a series of two hundred such filtrates no growth was observed except in two of the filtrates, which were grossly contaminated.

A fine white precipitate was occasionally found in filtrates which had been held for ten days or longer. This was apparently not composed of living organisms. It produced no growth when inoculated into a series of different media, and in stained preparations it failed to show any organized cellular structure.

Some of the filtrates were carried serially on agar slants, according to Hauduroy's method. Results were consistently negative.
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Filtration of cultures exposed to phage action for varying lengths of time

Two sets of cultures were studied in this experiment. In the first, ten separate tubes of alkaline extract broth, each containing 8 cc. of medium, were inoculated with equal amounts of a heavy suspension of the original Am M strain. The amount of inoculum used was just sufficient to yield a faint turbidity in the broth. To nine of the tubes 1 cc. amounts of active bacteriophage were added. The tenth tube was left as a control. The cultures were incubated at 30°. One bacteriophage-containing culture was filtered at the end of every hour during the first eight hours of incubation. The ninth culture and the control were filtered at the end of twenty-four hours. The filtrates were all held at room temperature.

Twenty-four hours after filtration small amounts of the different filtrates were seeded into tubes of meat-extract broth which had been adjusted to pH 5.0, 6.0, 7.0 and 8.0, respectively, into broth containing 1.5 per cent glucose, and onto nutrient agar plates. This set of sixty cultures was held under observation for ten days. All of the broth tubes failed to produce any visible growth.

The American Type Culture Collection strain of B. vulgatus was used in the second set of cultures, which was prepared and handled in exactly the same manner as the first had been. The results of these filtrations were also negative, no filter-passing forms of the organism being detected. Many of the filtrates were held for nearly a year and still showed no evidence of growth, when plated out on routine media.

DISCUSSION

In view of the similarity observed in this investigation between B. mesentericus and B. vulgatus, and of the newer conceptions of bacterial variation, the question may well be raised as to whether these two organisms should not be regarded as one species.

They have essentially the same morphological characteristics, and the cultural reactions observed in the course of this investigation have been quite similar.
The following brief description characterizes both organisms to a very large extent: so-called "normal" cultures of both are made up of rods which are usually 0.5 by 2.0 to 2.7 \( \mu \) in size. The presence of long, stout cells and occasional chain and filamentous forms may be noted, particularly in cultures containing rough variants. Moderately active motility is common to young cultures of all of the strains. Gelatine is liquefied and milk is peptonized by all. Both \textit{B. mesentericus} and \textit{B. vulgatus} ferment glucose, sucrose, mannitol, inulin, salicin, and levulose, and fail to ferment lactose. Some strains do not ferment maltose, while others do to a slight degree. Nearly all of the so-called "mesentericus" strains failed to reduce nitrates, whereas the "vulgatus" strains exerted a definite reducing action.

The use of the term "mutation" seems scarcely justified, as applied to these organisms. There was a constant tendency for them to vary, but the changes were readily reversible and never strictly "permanent," under the usual cultural conditions.

One of the most interesting points observed in the course of the investigation was the surprising degree of constancy of physiological properties exhibited by all strains, both parent and daughter, in spite of the marked variations in their colony forms. These observations are essentially in accord with those of others who have studied different members of the group (Soule for \textit{B. subtilis}, Nungester for \textit{B. anthracis}, and Gee for his vulgatus-like organism).

From a series of observations made in an attempt to determine the character of the so-called "G" colonies, it seems highly probable that these forms are derived from bacterial cells which are relatively weak and inactive. The organisms are so sluggish that they do not multiply rapidly enough to produce normal, visible colonies within twenty-four hours. The very small colonies which do develop in this period are composed of a few small, almost transparent cells. On prolonged incubation of the "G" colonies either one of two things happens: the small colonies disappear entirely, or they develop into small, but otherwise "normal," colonies. In the first instance, the cells are apparently so weak that they are unable to resist the attack of enzymatic
substances present in the culture and are rapidly lysed. In the second, the cells exhibit a somewhat higher degree of vitality and are able to produce "normal" colonies if incubation is continued long enough.

In many of the investigations in which positive filtration results have been reported quite coarse filter-candles were used. It seems probable, also, that in many instances the pores of the filters became coated with materials which tended to neutralize the electric charge on the candle and yet did not clog the pores. A few organisms, especially if they were very small, may have passed through such filters, yielding so-called "filtrable forms." Finally, one cannot refrain from the conclusion that many of the so-called "filtrable forms" were probably contaminants. Refinement of technic should eliminate such in nearly every case.

SUMMARY

Four major types of variants have been described for B. mesentericus and B. vulgatus. These have been purified and stabilized by serial selection from agar plate colonies through approximately 150 culture generations.

The normal colony type has been designated as a smooth-intermediate or rS type. The extremely smooth variant has been called the smooth or S type. The other two stabilized variants have been called the rough and rough-intermediate, or R and Rs types, respectively.

While considerable variation in colony form has been observed, the organisms have shown a remarkable constancy in their physiological properties.

The stability of the different colony types was studied. The extreme rough and smooth types showed the greatest tendency to be unstable, the R type to an even greater extent than the S. The Rs type, when fully established, seemed in most instances to be somewhat more stable than the rS type. The stability of all types appeared to be markedly influenced by the medium upon which they were growing.

The development of so-called "G" colonies in lithium chloride-containing broth is reported. Attempts to demonstrate that
these "G-type" cultures possessed filtrable elements yielded negative results.

The isolation of a bacteriophage which is active against the different strains of both B. mesentericus and B. vulgatus is discussed. Attempts to produce filter-passing forms of these organisms by exposing them to the action of the bacteriophage proved fruitless.

A true filtrable form was not observed for any of the nine mesentericus-vulgatus strains studied here.

REFERENCES


PLATE 1

Fig. 1. B. mesentericus, S colony on beef infusion agar after twenty-four hours incubation at 37°. × 125.
Fig. 2. B. mesentericus, rS colony on beef infusion agar after twenty-four hours incubation at 37°. × 125.
Fig. 3. B. mesentericus, Rs colony margins on beef infusion agar after twenty-four hours incubation at 37°. × 125.
Fig. 4. B. mesentericus, R colony margins on beef infusion agar after twenty-four hours incubation at 37°. × 125.
(Catherine S. Flynn and Leo F. Rettger: Studies on B. mesentericus and B. vulgatus)