THE MORPHOLOGY OF BACTERIUM SHIGAE CULTIVATED ON VARIOUS MEDIA FAVORABLE TO THE DEVELOPMENT OF FILTERABILITY AND LIFE CYCLE FORMS

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Plates 6 to 9

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Practically all knowledge of bacterial morphology has been gained from fixed and stained material. The usual disadvantages of this method of study are present in an aggravated form when dealing with bacteria. Artifacts are numerous not only within the cells themselves but in the surrounding medium. Stained figures, particularly those which have been fixed by heating, may be much smaller and may have shapes very different from the living organisms. The most important limitation of this conventional procedure does not, however, lie in these disadvantages, but rather in the static character of all information that can be obtained from dead material. No better evidence of this could be found than the fact that it is still possible, 50 years after they were first seen, to debate the significance of the so-called life cycle and involution forms.

It was early shown that bacteria could be satisfactorily cultivated in microcultures on hanging blocks of agar. For many organisms such preparations will grow as well for 12 hours or longer as the corresponding Petri dish cultures. The individual bacteria in these microcultures are readily seen with oil immersion objectives and often show as much intracellular structure as can be found after staining. While it is possible to follow the growth in these preparations by direct microscopic observation, micro motion pictures provide the ideal record. It is frequently stated that such pictures can be obtained only with an equipment which is costly to buy and difficult to operate. We have constructed for less than the cost of a good microscope an
apparatus which gives satisfactory records of bacterial development and which provides finished negatives at not more than fifteen cents an hour of photography. Inasmuch as these micro pictures can be made with very little special skill and experience they could reasonably be prepared as one of the routine procedures of the average bacteriological laboratory. The following study of the morphology of the Shiga bacillus shows the character of some of the information that can be gained in this way.

The cell types appearing in our photographs have previously1 been seen in stained preparations. Their further investigation is of especial interest at the present time because of the light it can throw upon the important questions of filterability and life cycle phenomena. Experiments have been described in which B. shigae, as well as other members of the colon-typhoid group, has been filtered through Berkefeld candles after cultivation in media containing lithium chloride.2 The same kind of growth from Berkefeld filtrates3 of B. typhosus living in a purely protein medium has recently been largely discussed. It happens that the same media that render the Shiga bacillus supposedly filterable also give rise to all of the various non-rod-like forms described by those who believe in the existence of bacterial life cycles. Our motion pictures show the formation and the subsequent fate of these unusual cells.

Technique

Three strains of B. shigae were used. Two were from the Chicago American Type Culture Collection; for the third we are indebted to Dr. K. Landsteiner of The Rockefeller Institute. The three proved indistinguishable in their manner of growth upon the several media used. At numerous steps in the experiments the purity of our cultures was checked by their sugar reactions. Incubators were held at 37°C.; preparations being photographed were maintained at this temperature.

All photographs have been made of bacteria growing in microculture on solid media. According to the earlier technique of Hill4 the seeded surface of an agar

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1 See for example Gotschlich, E., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, Gustav Fischer, 3rd edition, (Kolle, W., Kraus, R., and Uhlenhuth, P.), 1929, 1, 33; and also the various papers on life cycle phenomena referred to below.
3 Kendall, A. I., Science, 1931, 74, 129; 1932, 76, 295.
block was inverted, bacteria-to-glass, upon a cover-glass before mounting on a depression slide. This gives excellent optical conditions but allows only a limited growth of aerobic organisms. Where, as in the present experiments, only moderate magnifications are needed it is better to inoculate the free surface of the medium. To make a culture of this type a block of nutrient agar has been formed on a 22 x 30 mm. cover-glass by using two pipettes, one to introduce a drop of melted medium, the other to aspirate away most of this drop before it has solidified. The resulting thin layer has a comparatively flat surface and is sufficiently homogeneous so that it will not seriously disturb the microscopic image viewed through it. It must not be too thin or it will be insufficient to yield a suitable growth; on the other hand of course it must not exceed in thickness the free working distance of the objective used. In the present experiments this distance was about 0.4 mm. Since the thin agar layers dry up very quickly they are seeded immediately using a small drop of fluid inoculum held in a platinum loop of ca. ½ mm. diameter. The cover-glass carrying this preparation is inverted over a thin depression slide and sealed in place with melted paraffin (m.p. ca. 60°C.). Necessary precautions for sterility are maintained in preparing the slides, cover-glasses, pipettes, etc. With reasonable care these microcultures will rarely be infected by foreign organisms. Among the several hundred made during the present study only one contaminant has been found.

Motion picture records 6000 feet in length covering a total period of about 600 hours have been made of the growth of 90 of these preparations. The details of construction of the simple micro motion picture camera with which these photographs were made will be described in another place. Essential optical conditions were as follows:

- Objective: Beck 3 mm. N.A. 0.95 dark field apochromat
- Ocular: Leitz 5 × periplan
- Light source: 100 watt pointolite or 5 V concentrated filament bulb
- Filter: Dilute CuSO₄ solution
- Film: Plain positive
- Exposure: 1/2 or 1/5 second
- Magnification on film: ca. 300 ×
- Frequency of exposures: From one every 20 seconds to one every 2 minutes depending on rate of bacterial growth

All the photographs accompanying this paper are enlargements of single frames selected from these negatives. They are not all equally sharp because they have been chosen from study negatives for which adjustments for focus were only occasionally made.

**EXPERIMENTAL**

Studies have been carried out of the growth of *B. shigae* for varying lengths of time (1) in the ordinary beef infusion medium, (2) in certain other familiar laboratory media and (3) in some of the foods which
like "K" broth and LiCl infusion broth are supposed to induce filterability and the development of "life cycle" forms.

1. Growth in Ordinary Nutrient Medium.—Young dysentery bacilli are rods which average about 1 μ in diameter by 5 μ long (Figs. 1 and 2). The protoplasm in some of these cells is homogeneous; in others it is definitely granular. If the organisms are from a culture which has been subjected to several daily transfers, they will not show polar granules. If the seeded bacteria are polar, their immediate progeny may have poles. Sometimes structure can be seen in the region where a cell wall is about to form; in most cases, however, there is no such index of a coming cell division.

As individual dysentery bacilli age their poles become increasingly opaque and the intervening space clearer and clearer. Bacteria with well defined polar granules are numerous after a few hours of active growth on agar (Fig. 3). Most of the cells of a several day culture seemingly consist of nothing but granules and surrounding cell membranes. Since none of these extremely polar organisms (Figs. 4, 7, 17) has ever been seen to grow on any film, it is possible that they are no longer alive.

Our three _B. shigae_ strains produced smooth colonies. Even amongst these S organisms there was a considerable variation in what might be called the average cell size. Rapidly growing and multiplying bacilli are much larger than those which continue alive after the stage of most active proliferation is past. Cultures which during several transplants gave colonies decidedly rough in appearance have also been photographed. The R organisms show a pronounced tendency to form chains (Fig. 2) but otherwise no definite morphological distinction could be drawn between them and the truly S organisms. It would be necessary, however, to make experiments upon a long stabilized R strain before concluding that R and S Shiga bacilli are morphologically alike.

Moderately old cultures on normal agar frequently contain many viable bacteria so short that they are practically coccoid (Fig. 5). The fact that these coccus-like forms planted on fresh media grow into the usual _B. shigae_ rods (Fig. 6) indicates that they are to be considered as starvation forms rather than as some sort of cell variant.

Old growths in broth usually exhibit (1) many of the very polar and perhaps dead bacilli previously mentioned and (2) filamentous and branched forms. When inoculated to fresh agar it is these filaments which develop and ultimately produce colonies of normal bacilli. Within limits, the filament length increases with the age of the culture; in _B. shigae_ broth cultures 3–5 weeks old single organisms several tenths of a millimeter long are not rare. Such bacteria are shown in Figs. 4, 7, 8–9. In media less favorable to the growth of dysentery than plain infusion broth, branched and mycelial forms appear within 3 or 4 days of seeding. After several transplants of _B. shigae_ in gelatine or LiCl-containing broth the formation of branching cells can be directly photographed (Figs. 10–12, 13–14, 15–16). These
organisms usually disintegrate before the colonies of which they are the starting point have attained large size (Figs. 2, 17–19). It sometimes happens that when long and irregular organisms are planted on fresh medium they swell till they burst (Figs. 20–21).

2. Growth in Protein Media.—In order to see what morphological changes can be brought about by media containing little food material besides unaltered protein, \( B. shigae \) has been cultivated (1) in "K" medium made from hog intestines after the formula of Kendall\(^5\) and (2) in a medium composed of gelatine and Ringer’s solution.

Two strains were allowed to age in “K” broth and were carried through a number of daily transplants in it. Photographs were made of microcultures obtained by inoculating 24 hour, 48 hour, 3 day, 5, 7 and 9 day “K” cultures to dextrose agar. A 3 day record also was made of the development of \( B. shigae \) on solid “K” medium (“K” broth + 2 per cent agar).

Growth in “K” media is much less and the bacilli are smaller than in beef infusion broth or agar. In 48 hour or older preparations many of the living organisms are almost coccoid in appearance (Fig. 22). Transferred to a fresh medium these dwarf cells elongate into normal rods (Figs. 22–23) and multiply as normal bacilli. In common with all the other small forms observed they have longer lag periods than normal sized organisms. Inoculations from day-by-day transplants in “K” broth have been photographed. In its morphology the 12th passage is not unlike the 1st; it departs from normal only in the reduced cell size. From these observations there is reason to look upon the action of “K” media as due to their low content of nutrient material available to Shiga bacilli.

Gelatine is a far richer food. When first transferred to gelatine, \( B. shigae \) produces many short rods but after frequent transfers the cells are normal in appearance. Aging takes place more rapidly than in beef broth and results both in the long and branched forms (Figs. 8–9, 10–12) already described and in bloated cells of various shapes (Figs. 17–19). In other respects growth in gelatine does not seem to be of great morphological interest.

3. Growth in Lithium Chloride Media\(^6\)—The forms produced when small amounts of LiCl are added to ordinary media are far more varied and instructive than those obtained in other ways. Two kinds of LiCl media have been used. In one, to be called LiCl normal broth or agar, the LiCl was added to ordinary beef infusion medium; in the other the base was a 2 per cent solution of the hydrolyzed amino peptone of Fairchild made up in Ringer’s solution. Such a peptone solution without LiCl is a most favorable medium for dysentery bacteria. Bacilli growing in it are similar to those in beef infusions and the morphological changes occurring in LiCl peptone media are very like those observed in LiCl

normal media. For this reason it is unnecessary to describe separately the experiments made with these two media.

Two types of experiment were made. In one *B. shigae* cultures were carried by means of daily transfers through many passages in a broth containing a small and constant amount of LiCl. For this purpose the best salt content is 0.25 per cent LiCl although amounts as high as 0.50 per cent LiCl can be used without too great an initial inhibition in growth. In the other experiment the LiCl content of the medium was gradually increased with the number of transplants until it amounted to six or eight times the initially tolerated concentration. All steps in both of these procedures were photographed.

Usually the most interesting morphological changes in dysentery bacteria transplanted daily in LiCl broth have taken place by the 10th or 12th passages. This does not mean, however, that the organisms in succeeding cultures are necessarily normal bacilli for some of the best examples of irregular bacteria and branched forms have been observed in the 19th and 20th LiCl peptone broth passages. Our motion pictures include records of the growth of each of the first 10 LiCl passages when inoculated to normal agar and of a number of analogous growths on LiCl-containing agars. They thus provide morphological data upon the same kinds of cultures that have previously been studied for colony structure and for their “filterability” through Berkefeld filters.

Ordinary Shiga bacilli when planted upon normal agar containing 0.25 per cent LiCl immediately swell to several times their original volumes (Figs. 25-27). Many of the large coccoid and balloon forms thus produced (Figs. 13-14, 28-30) are able to divide and multiply indefinitely. It is important to notice, however, that the succeeding generations of the progeny of these cells become more and more normal in size and shape. At first the protoplasm within the bloated organisms is quite structureless and homogeneous but later many of them contain numerous absorbing or refractile bodies. These granules are apparently identical with the substances which, staining strongly with Giemsa and similar stains, have suggested that the macrococci may be conidial-like bodies playing a definite rôle in a bacterial life cycle. Photography has not added weight to such ideas. The granules are found in old cells of all shapes. Like the polar bodies of normal bacilli which they resemble both in optical characteristics and staining reactions they form gradually as a cell ages. When they are fully developed and the rest of the intracellular spaces have become clear and transparent, no growth has ever been observed. It thus seems natural and in accord with all available information to consider that in *B. shigae* they are merely coagulated bits of protoplasm without specialized organic function.

Most of the organisms found in LiCl media are not normal bacilli. Besides the macrococci already mentioned there exist both roundish and elongated cells which protrude from many points (Figs. 13-16, 28-30) and which on favorable media grow into a variety of branched forms.

After several passages through LiCl broth the number of unswollen rod-like
bacteria gradually increases. Especially at first these rods are short and smaller than actively growing dysentery bacilli. By the 5th or 6th transplant, when B. shigae cultures begin to be "filterable," such small forms, which resemble in appearance the "starvation" cells found in poor media, are already numerous.

 Cultures of B. shigae apparently normal in the presence of relatively high concentrations of LiCl have been developed by gradually increasing the LiCl content of the medium after every few passages. This production of an LiCl-resistant strain has been hastened by making every 2nd or 3rd transplant a dilution plating on LiCl agar, choosing for reinoculation the largest B. shigae colony occurring on the plate. Organisms thus obtained grew rapidly and as normal bacilli in 2 per cent LiCl media whereas the living cells in an untreated B. shigae culture show important morphological changes when introduced into 0.25 per cent LiCl broth. There is no reason to believe that dysentery could not be caused to adapt itself to still higher concentrations of LiCl. It is important that not only do these resistant bacilli on LiCl media grow like ordinary B. shigae on normal media but they show no profound morphological changes when returned to normal LiCl-free infusion broth or agar. The photographs make it clear that these normal appearing rods are not obtained merely by the selection and multiplication of organisms originally resistant to LiCl. Instead there occurs a real adaptation which proceeds through many generations and ultimately gives a strain seemingly unaffected by the unfavorable environment in which it has been placed.

A very striking feature of the growth of B. shigae on LiCl substrates, especially during the period of supposed ready filterability, is the production of many micrococci. Some of them are shown by the motion pictures to be bits of protoplasm extruded presumably through minute holes in cell walls but others arise from large bacilli by a process which seems identical with ordinary fission. Three cocci are shown in Fig. 31; the way they result from certain "budding" bacilli is illustrated by Figs. 32-33 and 34-36. Their function and fate are not yet well known. The smallest cocci have never been seen to grow though the development of somewhat larger coccoid forms has been photographed several times. This inertness of the micrococci does not necessarily mean that they are dead, especially when it is borne in mind that with B. shigae the small organisms customarily have a longer lag period than the larger ones. Individual cocci have, however, been photographed and observed in micrcultures for several days without any apparent changes occurring and it seems legitimate to conclude that the small ones if they germinate at all do so infrequently or only after a long time.

**DISCUSSION**

The foregoing observations and especially the films upon which they are based have important bearings on many long discussed problems in bacteriology. They could be used as a basis for quantitative studies of rates of bacterial multiplication in young and older cultures, of
measurements of initial lag periods, of the change in cell size with age and of other aspects of growth and cell destruction. They could throw light on the effect of surface tension and osmotic pressure upon cell size and shape, on the connection between these and other physical influences and what are known as the plasmotyphsis and plasmolysis of bacteria, and they provide an interesting starting point for hypotheses concerning the more or less specific action of lithium ions upon bacilli of the colon-typhoid group. But at the present time their greatest interest lies in the information they furnish concerning the possible existence of bacterial life cycles and the supposed filterability of bacterial forms. Either of these phenomena, if real, would revolutionize large sections of both the thought and the practice of bacteriology. The fact that after years of debate and experiment this reality is still vigorously discussed indicates that conventional methods are not well adapted to answering these questions. The detailed and permanent records of all morphological changes taking place in cells as furnished by micro motion picture photography provide new and unequivocal data.

**Life Cycle Phenomena.**—The idea that bacteria exhibit various types of life cycles which ally them with one another and with higher living forms is as old as bacteriology itself. Throughout his life Naegeli advocated such an extreme pleomorphism and each succeeding generation has contained workers who believed that an organism can appear and grow as a bacillus, as a coccus or as a spirillum and that it will multiply not only by transverse fission but also by the production of mold-like conidia and by various sexual and pseudosexual processes. The repeated exposition of these theories and the description of experiments which have been thought to prove them have built up a voluminous literature. Löhnis has reviewed the papers written to 1918; many more recent articles are cited by Hadley and by Kuhn. By some, bacterial life has been pictured as existing in an amorphous state as well as in the familiar formed stages. The idea that viruses constitute another and submicroscopic manifestation of bacterial

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7 Fischer, A., Z. Hyg. u. Infektionskrankh., 1900, 36, 1.
pleomorphism has been repeatedly urged. Its connection with the related question of bacterial filterability is obvious. Amongst the published theories of life cycles there are all sorts of gradations from a simple pleomorphism which sees bacterial changes occurring only under the influence of adverse environmental conditions to beliefs in fixed and immutable cycles through which normal bacteria must pass if they are to multiply indefinitely.

The morphological evidence for all these hypotheses is based on the examination of stained preparations. In order, therefore, to relate the motion pictures to earlier observations, several hundred stained slides have been made of dysentery bacteria. In this way it has been established that all the forms usually taken as illustrations of life cycle stages have been seen both in smears and in living preparations.

For convenience in discussion it is desirable to consider the predictions of a typical cycle. That of Löhni, based on his work with the azotobacterium, is especially complete. Text-fig. 1 shows it simplified. All stages have not been found with B. shigae but those designated as A, B, C, D, E, F, H, K and L are frequently seen and are pictured in the figures. There is no evidence in the motion pictures that they transform from one to another after the scheme of Text-fig. 1, or in any similar way. On the other hand the large cocci of A and irregular protoplasmic masses indistinguishable from D are obviously extruded from decomposing organisms. Sometimes they stain fairly uniformly, sometimes they are granular. Macrococci, like B, which are frequent in cultures freshly treated with LiCl, are mere bloated cells. If they can multiply, their progeny become more and more like normal rods. If conditions do not favor their division they may grow larger and as they age their protoplasm will segregate into granules which are colored with Giemsa and similar stains. These granules (C) do not seem to differ from those (H) which are to be found in aged cells of other shapes or from the deeply staining polar bodies of the short bacilli that are numerous in old cultures. They are to be seen in many of the fields photographed both within intact organisms and more or less free. In no instance has one of them shown any evidence of being alive and capable of growth. There is thus from the motion picture records no reason whatsoever for considering that the macrococi that can be produced in B. shigae are comparable in func-
tion with the conidia of molds. There is equally no indication that the protoplasmic masses A and B are anything but the débris of dead cells. Occasional macrococi have been found to protrude at several points of their surface and have the appearance of K (budding conidia). Those which have been followed disintegrate explosively. The long branching organisms that are abundant in all old cultures arise through the outgrowth from several points of cells which sometimes are roughly coccoid. On media which sustain the growth of such cells, the "bud-

![Text-Fig. 1. A typical bacterial life cycle (after Löhni,--- simplified).](image)

nings" elongate into rods that eventually split from the parent cell by ordinary fission. It is hard to see in this a special reproductive process.

It must be concluded that our motion pictures have presented no evidence for the existence of life cycles in the Shiga bacillus. Grown in a novel or somewhat deleterious medium it exhibits the shapes described as life cycle forms but the organisms manifesting these abnormalities, if alive and in a favorable environment, will invariably multiply in such a way that their progeny tend to become normal bacilli again.
The fate of the micrococci that are split by fission from certain bacteria is the only incompletely answered problem in the morphology of dysentery bacilli grown under the conditions of these experiments. Though frequently photographed these cocci have never been seen to develop bacilli. Efforts to give them better conditions for growth by separating them from their rapidly multiplying neighbors either by filtration and ultralfiltration or by fractional centrifuging were not successful. If the observation that members of the colon-typhoid group have a permanently coccoid modification should be correct, it is conceivable that it is formed in this manner. Slightly larger roundish rods have been photographed as they separate from large cells and have been seen to grow later into normal bacilli but in some respects they differ from the micrococci and it may not be legitimate to expect similar behaviors from the two forms.

Filterable Bacterial Forms.—Much work has been done to show that there are modifications of the Shiga bacillus capable of passing through the finer grades of Berkefeld filters. The present experiments, especially those involving the use of “K” and of lithium chloride media, have a direct bearing upon this question. To supplement the photographic records many tests have been carried out to ascertain the sterility of treated \textit{B. shigae} cultures drawn through V and W Berkefeld candles. In discussing the bearing of these results upon the question of filterability it is convenient to distinguish between filtrates which show many bacilli after not more than 48 or 72 hours incubation and those which contain numerous organisms only after 2 or 3 weeks growth. In the following discussion these reversions will be designated as “quick” and “slow,” respectively.

All filters were frequently tested by submerging them in water and applying compressed air. Those which did not show air leaks under 300 mm. Hg pressure and which gave sterile filtrates with young cultures of normal \textit{B. shigae} were chosen for further use. At first the method of getting reversions recommended by Hauduroy was followed. It consists in planting the filtrate upon nutrient agar.

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which after incubation was washed over with broth. This broth after incubation was put on agar and the broth-agar transplants continued as long as desired. Such a procedure courts contamination. It has been found that some of the plate infections which can be picked up in the routine of carrying out these manipulations are morphologically not unlike B. shigae though of course they differ from it in other ways. In later experiments we have used as culture flasks 2 ounce flat-sided perfume bottles partly filled with nutrient agar. After seeding this agar with one or more drops of a filtrate the bottle was closed with a sterile rubber stopper and sealed with a cellophane cap. Since the bottle can, when necessary, be sterilized after being filled with medium the chance of contamination is greatly reduced.

A certain number of bottles seeded with V filtrates of "K" medium cultures and LiCl cultures (ca. 5th to ca. 15th passages) will show growth after 24–72 hours. Judged by their morphology and sugar reactions these organisms are ordinary B. shigae. A number of filtrates were planted in microculture and whenever quick reversions were seen in bottles it was possible after sufficient search to find organisms in the filtrate within an hour of the time of filtration. These bacteria were the short rods commonly observed in "K" and in lithium chloride cultures; planted on nutrient agar they eventually grew into normal Shiga bacilli and produced normal Shiga bacillus colonies. This fact taken in conjunction with the recent observation that "K" medium facilitates the passage of bacteria through a stone filter removes any mystery that there may have been concerning the mechanism of the quick reversions from filtrates of Shiga and Shiga-like bacilli.

The present experimental results do not warrant equally definite statements concerning the supposed "slow reversions." Bottles seeded with V and even with W filtrates have often developed roughenings of the surface agar which did not show recognizable organisms either in stained smears or when planted on agar blocks. These roughenings, which seem to correspond to the preliminary stages of reversion described by others, increase with time and can, sometimes at least, be transferred from bottle to bottle. Our experiments, however, do not prove that they consist of living matter. In a number of bottles which were never unsealed real bacterial growth has appeared after a lapse of 10 days or longer. The organisms in some morphologically re-

sembled *B. shigae* but their sugar reactions were not constant nor were they identical with those of the unfiltered dysentery bacilli. A number of attempts were made to obtain these "reversions" in microculture. Nothing was seen in any one of them, even after weeks of incubation. In the absence of further knowledge it is at least as reasonable to consider these bacteria some hardy and slowly growing contaminant introduced before sealing as to look upon them as arising from a minute filter-passing stage of the Shiga bacillus.

The writer is indebted to James R. Lucas for help in carrying out many of these experiments.

**SUMMARY**

A simple micro motion picture apparatus has been developed which is so inexpensive to construct and to operate that it can be used regularly for bacteriological research. With this equipment about 6000 feet of film representing 600 hours of photography have been made of *B. shigae* growing upon various solid media. These pictures illustrate the principal phenomena accompanying the development of this organism on ordinary nutrient media, on media consisting exclusively of either peptones or proteins, and on media containing small amounts of LiCl.

Information has thus been gained concerning the existence of a life cycle in the Shiga bacillus and concerning its filterability through Berkefeld filters. The formation and history of the various "life cycle forms" are recorded but the evidence does not point to them as phases of actual cycles. In "filterable" *B. shigae* cultures—such as those grown in the so-called "K" broth or in lithium chloride-containing media—many small and short rods are present. It has been found that these dwarfed organisms pass through filters impervious to the cells of rapidly growing normal cultures. This offers a simple explanation of "quick reversions." The present experiments do not provide conclusive information concerning the slower reversions which are supposed to occur only after many days of treatment and incubation.

\[13\] See also Zinsser, H., *Science*, 1932, 75, 256.
PLATE 6

Magnification, 760 ×.

Fig. 1. Normal Shiga bacilli 2 hours after being plated on normal agar.

Fig. 2. A similar growth of R bacilli on normal agar. The disintegrated parent organism can be seen in the middle of the picture.

Fig. 3. The slide of Fig. 1 after 3 hours additional incubation.

Fig. 4. A field from a 2 weeks old culture of B. shigae in normal broth planted on dextrose agar. The growth is from the long thick cells. Several old bipolar bacilli are to be seen within and around the microcolony.

Fig. 5. Organisms obtained from a very spreading S type colony growth on normal agar. Photographed immediately after seeding.

Fig. 6. The same field after about 1½ hours.

Fig. 7. A field from a 3 weeks culture of B. shigae in normal broth planted on normal agar. The large cell which was about ½ mm. long grew and divided at both its ends. Several strongly polar old bacilli are in the field.

Fig. 8. Part of an organism from a several day old culture of B. shigae in 5 per cent gelatine broth about 1 hour after being planted on normal agar. A bacillus of normal size and shape has already split from the lower arm.

Fig. 9. The field of Fig. 8 after another hour. Several bacilli have now been produced by the original organism.

PLATE 7

Magnification, 760 ×.

Fig. 10. These organisms growing on normal agar are from a B. shigae culture in 10 per cent gelatine broth.

Fig. 11. The field of Fig. 10 after about 1 hour. Most of the newly formed cells are not of normal shape. Some, such as the “boxing glove” in the lower left corner, are commencing to branch.

Fig. 12. A still later picture of the microcolony of the last two figures. The further development of the irregular and branching forms is apparent.

Fig. 13. Some organisms from the 4th passage of B. shigae through 0.50 per cent LiCl Fairchild’s broth when planted on dextrose agar. Branched and irregular forms, and macrococci, are present.

Fig. 14. A later picture of the preceding field showing the growth and multiplication of several of the bizarre cells.

Fig. 15. A field from a B. shigae culture on normal agar after 19 passages through 0.37 per cent LiCl broth.

Fig. 16. The same field showing how several of the branched and irregular forms develop.

Fig. 17. An organism planted on normal agar after several passages through a 5 per cent gelatine broth. The group includes an old bipolar cell. The growth
and subsequent disintegration of the other cells is shown in the two following figures. Note the vacuole in the large cell.

**Fig. 18.** A later picture of the same field. The large vacuolated cell has disintegrated but other abnormal organisms have formed.

**Plate 8**

Magnification, 760 ×.

**Fig. 19.** A still later picture of the same field. Only normal cells are left intact. Note that the bipolar rod shows no growth.

**Fig. 20.** Abnormal organisms from the 9th passage through 0.25 per cent LiCl broth 1 hour after seeding to normal agar. They are already greatly swollen.

**Fig. 21.** The field of the previous picture about 2 hours later. The protoplasm surrounding the upper ruptured cell is clearly to be seen. Organisms in the lower group are already fairly normal.

**Fig. 22.** A microculture of *B. shigae* cultivated for about 48 hours on the surface of "K" medium agar.

**Fig. 23.** A field from the 3rd passage of *B. shigae* through "K" medium broth. A coccoidal "starved" form occurs in the center of the picture.

**Fig. 24.** The coccus of the previous photograph has grown into a normal bacillus and has then undergone fission.

**Fig. 25.** Bacilli from a broth culture of normal *B. shigae* about ½ hour after planting on 0.25 per cent LiCl agar. Swelling has just commenced.

**Fig. 26.** The field of the preceding figure after about 1 hour. A few of the swollen organisms have already divided.

**Fig. 27.** The same field after further growth.

**Plate 9**

Magnification, 760 ×.

**Fig. 28.** Organisms from a 7th passage through 0.25 per cent LiCl broth planted on dextrose agar. The two following pictures show growth from the irregular cells in this field.

**Fig. 29.** A later picture of the field of Fig. 28. Several micrococci are to be seen within the colony. The large round cell at the bottom has swollen considerably and has begun to vacuolate.

**Fig. 30.** The same field after further growth. The center of the colony contains many macro- and some micrococci, the later surrounding organisms are fairly normal bacilli.

**Fig. 31.** Shiga bacteria from a 5th passage through 0.25 per cent LiCl Fairchild broth seeded to dextrose agar. The cocci which were budded from the middle Y-shaped cell did not develop during 12 hours. At the end of that time they were lost in the large colony resulting from the multiplication of the other organisms.

**Fig. 32.** Growth on dextrose agar of cells from an 8th passage through 0.25 per cent LiCl broth.
Fig. 33. A later picture of the field of Fig. 32. Comparison with the previous picture shows that the large Y cell in the center of the colony has divided to yield a large coccoid organism.

Fig. 34. As the two succeeding pictures prove, the long bacillus in the central part of this frame produces numerous micrococci by fission. The bacteria in this field, growing on dextrose agar, were obtained from a 9th passage LiCl broth small (G-) colony on dextrose agar planted in dextrose broth.

Fig. 35. The field shown in the preceding picture photographed about 1/2 hour later.

Fig. 36. The same field after another hour. The budding cell produced a total of eight cocci.
(Wyckoff: The morphology of B. shigae)