INTRODUCTION.

The microorganism, the culture and significant qualities of which it is the purpose of this paper to describe briefly, has the interest of a type to those concerned with attempts to cultivate the filterable viruses. From an immediately practical standpoint it may well be regarded as a saprophyte and an accidental contaminant arising in a series of cultures primarily directed toward the study of the filterable virus of hog cholera.

From the beginning almost of our knowledge of filterable viruses as the cause of disease in man and animals it has been a debatable question as to how far filterability is to be regarded as signifying essential invisibility. It has come to be generally recognized that particles in order to pass the coarser earthen filters ordinarily effective for the removal of bacteria must have a smaller diameter of not to exceed one- or possibly two-tenths of a micron. Length, if combined with flexibility, need not be a limiting factor to the same degree. It would doubtless be granted that extensibility within reasonable limits must also be an important factor and that this might be influential in either permitting the passage of somewhat larger particles whose extensibility was great or preventing the passage of very rigid smaller ones. Naturally the state of agglomeration and the condition of the surface, whether adhesive or not, would be additional factors which might determine the actual ability of living particles of smaller dimensions than this to pass a filter. Optically, it is known that particles of about 1/10 micron or less in their longest diameter must appear...
FILTERABLE YEAST-LIKE MICROORGANISM

round under even the highest powers of the theoretically perfect microscope. Such particles unless characterized by some peculiar state of agglomeration or by some microchemical reaction must remain indistinguishable from inert particles of the same general size. Unrecognizable they would be essentially invisible.

Within these limits various well known examples may be mentioned. The filterable virus of pleuropneumonia of cattle, long cultivated, is visible without doubt, but admittedly pleomorphic, and this to a degree which combined with the factor of difficult visibility leaves a considerable latitude of uncertainty in the descriptions which have been given of it (1, 2). The virus of "Hühnerpest" propagated by Marchoux (3) and by Landsteiner and Berliner (4) was not seen by these authors. More recently described there are the visible and filterable B. pneumosintes of Olitsky and Gates (5), the essentially invisible virus of mosaic disease of Olitsky (6), and the likewise optically unrecognizable virus cultivated by Noguchi (7) from the tick Dermacentor andersoni. Considering the nature of their growth in culture these microorganisms may probably be minute bacteria. It may well be recalled here that Borrel (8) recognized that certain filterable saprophytic bacteria could be cultivated from water in his locality.

Borrel (9) also gave a brief account of a filterable microorganism from water which he regarded as a protozoan. Schaudinn (10) recognized that certain protozoa might have spirochete-like forms as a stage in a complex life cycle, and, as a suggested application of this postulate, indeed, predicted that yellow fever, of the virus of which it was at that time known that it was transmitted by a mosquito and that it was filterable, would be found to be such a spirochete. The actuality is that the Leptospira icteroides of Noguchi (11) is filterable by reason of its inherent properties but there is no evidence of its being a stage in the life cycle of any more complex parasite. These observations of Borrel and Schaudinn served to introduce into the literature of the subject the postulate that some of the filterable viruses might be very small stages in the life cycle of complex parasites, other stages of which might be clearly visible. The thought recurs in the reviews of the subject but concrete examples are so far lacking. Nearest to fulfilling the conditions comes the case of the trypanosomes. Novy (12) described cultures of these protozoa as becoming very small under cer-
tain conditions so that they could pass filters which had been ground thin, and more recently Reich (13) has found that the liver and spleen of animals infected with Trypanosoma brucei may frequently yield infectious filtrates. While these observations do relate clearly to protozoan parasites, yet they scarcely apply to the question in hand since there is little or nothing to suggest that the trypanosomes as a group are cyclic in constitution. The culture now in hand, which is in all probability to be classified as a yeast or yeast-like fungus, seems to fulfill quite perfectly the requirements of this very general postulate.

OBSERVATIONAL.

In connection with the study of hog cholera virus it was thought to apply to its cultivation a method developed by Gates (14) during the study of Bacterium pneumosintes. Collodion sacs were sealed to a glass tube 1 cm. in diameter and 14 cm. in length (ordinary bacteriological test-tubes with the bottom cut off). These were fitted into larger test-tubes in such a way as to project through and be supported by a tight cotton plug of the latter. The tube with sac attached was also plugged with cotton. Water was put in the inner tube to the height of 10 cm. or more and in the outer tube to cover the collodion, and the whole sterilized in the autoclave. The appliance is then, two tubes, an inner and outer, the fluid in which is separated by a collodion sac 1 cm. in diameter and about 2.5 cm. in length. Either may be separately treated as a bacteriological culture tube.

The experiments in question consisted in replacing the water in the inner tube with mixtures of nutrient bouillon, blood serum of either rabbits or swine, and adding pieces of fresh kidney of either rabbit or swine. A commercial hog cholera virus (unfiltered) was diluted 1/100 with sterile water and the outer tubes of a number of these preparations were inoculated with 1/10 or 2/10 cc. of this. The tubes were then incubated in various ways, aerobically and anaerobically, at room temperature and at 37.5°C., in all possible combinations. A number of these tubes developed growth of ordinary bacteria within a few days and were discarded. Others remained perfectly clear and limpid for weeks and were likewise eventually discarded. A few of the tubes after a week’s incubation aerobically at room temperature or 37.5°C. showed a very slight but definite turbidity which remained, but did not increase to the point of sedimentation. Transfers to other similar tubes did not develop. All of these tubes sooner or later developed growth of easily visible microorganisms and were discarded, it being considered that the repeated opening of the rather cumbersome tubes rendered eventual contamination inevitable.

As transfers were made, samples for microscopic examination were reserved in small test-tubes (agglutination tubes) and these were kept on the desk. They were repeatedly examined both in hanging drop and by the application of various
staining methods. There was from the first a reasonable doubt as to whether the particles responsible for the turbidity, which could be clearly seen in hanging drop, might not be living. The reexamination remained a matter of interest for weeks and in the meantime the fluid gradually evaporated. It was several times restored to approximately the original volume with sterile distilled water. Some few days after such an addition of water three tubes in quick succession developed a much greater turbidity with some tendency to the formation of a flocculent precipitate easily redistributed by shaking. This was about 10 weeks after the segregation of the samples and approximately 3 months from the planting of the original cultures. The tubes were now found to contain undoubted microorganisms. So much detail has been given in order to make plain the uncertainties which surround the actual source of the culture.

The slightly turbid samples as segregated from the original tubes and in the succeeding weeks showed moderate numbers of particles, ill defined in optical character. They ranged from those barely visible, through a considerable number just clearly visible, to a small number which may have been 0.5μ in their larger diameters. The smaller particles appeared to be perfectly round; those somewhat larger also were round, and while usually single were sometimes in pairs. The largest of the particles were sometimes round but were often definitely angular suggesting the longitudinal section of an irregular but generally ovoid body. All the particles had active Brownian motion and the larger ones presented a definite halo.

Stained with methylene blue, even with heat or for a long time, and after various methods of fixation, nothing could be seen of these particles. When preparations were fixed in methyl alcohol and stained with full strength carbolfuchsin for from 3 to 5 minutes, or when Giemsa stain was applied, stained particles showing a general correspondence to those seen in the fresh were easily made out, but they presented no qualities sufficiently definite to distinguish them with certainty from precipitated stain or stained detritus. If, as is possible, there was any gradual development of more definite forms such as later appeared suddenly in abundance, it passed unnoticed in spite of repeated examinations.

Examination of hanging drops immediately the sudden increase in turbidity was noticed revealed very considerable numbers of a yeast-like microorganism. This was small as compared to the usual yeast, presenting as characteristic forms more or less round bodies about
1.5μ in diameter associated with longer or shorter structures of the type of abortive mycelia. The number of these was not sufficient to account for the turbidity and they were associated with many much smaller forms. Some of the constituents of the apparent mixture were very actively motile. It seemed impossible at first sight that there could be other than a gross contamination of the fluid but it remained of apparent interest to see whether the smaller forms presented might represent a more active multiplication of the forms previously seen. Suitable preparations were stained and it appeared that there was very little in the material stainable with methylene blue, while carbol-fuchsin and Giemsa stains quite adequately represented the picture presented by fresh preparations. Cultures made on the usual media emphasized the apparent peculiarities in that there was no immediate growth. The material was therefore subjected to a more systematic study with the following final results.

All of those agglutination tubes which showed the sudden increase in turbidity referred to yielded the same culture. The culture grows most consistently on horse blood agar at room temperature. Growth occurs at 37.5°C. but is slower and much less voluminous under these conditions. The addition of sugar to the blood agar neither favors nor interferes with the growth. On this medium even a rather heavy planting shows no change for the first 48 hours. On the 3rd day there may appear an alteration of the surface of the slant and examination with the glass reveals an abundance of very minute, discrete, rather moist colonies. Succeeding days render these visible to the naked eye and at the end of 3 weeks, if the planting has been thin, single, brownish white, mucoid colonies may reach a diameter of 2 to 3 mm. In case the planting has been heavy the covered surface presents a thick creamy mass of the same color. Touched with the wire the colonies are soft, easily spread, but never stringy. They are never soft enough to run down the surface. These appearances are dependent on the medium being freshly prepared, moist, and on the retention of moisture by at least partially closing the tubes. In the case of old medium, or that which has partly dried before the culture is luxuriant, the growth remains flat and dry, the colonies being small and discrete. Intermediate between these extreme alternatives there develop a few mucoid colonies on a surface generally covered with flat, dry,
discrete ones. Such cultures suggest a mixture, but transfer from any part of them to favorable medium restores the described luxuriant growth.

The blood in the agar shows no characteristic changes as growth develops. The condensation water of these blood agar tubes remains clear but gradually develops a heavy sediment. Scraped into distilled water, normal saline solution, or nutrient bouillon, the growth from the blood agar slant forms a homogeneous suspension with the greatest ease. This partly sediments in 2 or 3 days but is resuspended by slight shaking. The appearance (except color) and consistency of the fully developed culture on blood agar resembles that of a luxuriant culture of ordinary yeast on the same medium.

The culture may be said to grow well on all of the usual culture media but the volume of growth attained on the other solid preparations used does not equal that on blood agar. Growth on media other than blood agar is also capricious in marked degree; that is, unless the culture from which the transfer is made is at the height of its vigor, the transfer is apt to fail or the resultant growth be very uneven. In bouillon with or without added sugar growth results in a flocculent or granular sediment tending to cling to the sides of the tube until dislodged by light shaking. There is sometimes a light ring at the top suggesting that growth takes place here and that the sediment results from the fall of this imperfect scum. When serum is added to the bouillon growth starts in the same way but the resulting sediment is much larger in amount and less granular. After several weeks there gradually develops a diffuse, heavy clouding throughout the fluid above the sediment reaching to the top and this clears but slowly over several weeks more.

Planted in a semisolid medium containing reduced amounts of bouillon constituents and with blood serum added (Noguchi’s leptospira medium), heavy growth develops on the surface and in the upper few millimeters but does not extend below this.

Neither acid nor gas is developed in fermented bouillon to which Andrade’s indicator and various sugars are added. Dextrose, lactose, saccharose, maltose, and mannitol have been tested. In the fermentation tube growth is confined to the open arm.

Growth on plain agar has always been scanty; on glycerol agar it
has sometimes been scanty and sometimes quite heavy, particularly in the lower part of the tube. On glycerol agar the growth has sometimes acquired a pale orange-yellow color with age.

Gelatin is not liquefied.

Litmus milk is not changed.

The microorganism has now been under observation for over a year. The growth characteristics as described have remained constant. The culture has been repeatedly plated and has been and is still apparently pure. Microscopically the culture has changed in that certain forms which were prominent in the early cultures are now more rarely seen. There was in the beginning a greater tendency than now for changes in the medium employed to result in a temporary predominance of particular morphological forms. The following description of the morphology of the culture is a composite based on the total experience with it.

Transfer of culture material which has aged to any medium or of a culture at the height of activity to an unfavorable medium results in a culture which for the first few days consists chiefly of what are at first sight simple micrococci of about 1.5 μ diameter. These may be single and perfectly round; more frequently they are clumped in dense masses in which the individual cocci are only apparent at the edges; sometimes they are in pairs definitely lanceolated (Figs. 5 and 6). Cultures in plain bouillon, on plain agar or on blood agar somewhat dry when the plant is made, may develop no other forms than these. The micrococci are mostly stained rather faintly with methylene blue but some individuals are intensely stained. With carbol fuchsin or with Giemsa’s stain they stain intensely. Neither in the fresh nor in the stained condition do the cocci exhibit any evidence of structure. As the cultures age the masses of cocci examined in the fresh state assume a definitely granular appearance. They now stain even less well with methylene blue. With Giemsa or fuchsin the granular condition is very evident, the particles being irregularly massed, interspersed with much poorly staining material, and varying in size from that of the original coccus to about 0.1 μ. Every effort has been made to secure preparations which might exhibit some regularity of transformation of the cocci into the smaller granules, but without success. This might be but the rather usual granular transformation in old bacterial cultures but for the following facts.
If such a granular culture, or one consisting chiefly of cocci before they have changed, is washed into sterile distilled water and allowed to stand for 72 hours it is found to have changed completely. The masses of round cocci are no longer found in any considerable number and single round cocci are also rare. Ovoid cocci, single and in lanceolated pairs, of the general size of the original cocci, are now quite common and there are numerous forms of the same length (1.5μ) but thinner, so that they appear as definite bacilli (Fig. 7), and even more numerous are still smaller bacillary forms definitely grading down to the smallest visible ones (Fig. 10). Especially interesting are numerous instances in which the thinner bacillary forms appear as branches or buds from the ovoid cocci or the lanceolated pairs (Figs. 8, 9, 11, 12). Many of these definitely bacillary forms are very actively motile. They may exhibit this motility while attached to the larger forms and impart to the latter a very active movement although less rapid than that of the detached bacilli. With the dark-field microscope the very smallest bacillary forms can be recognized as frequently motile. The round or ovoid cocci are never motile in the absence of one of these recognizable buds or branches. The bacillary forms stain not at all with methylene blue and much less readily than do the coccoid forms with carbolfuchsin or Giemsa’s method.

This complex result following the suspension of the coccoid culture type in distilled water is an essential restoration of the picture presented by the original samples after they had acquired a marked turbidity, as described in previous paragraphs. The same result is also spontaneously developed in the cultures, being characteristic of those colonies or masses which develop pronounced mucoid characters as likewise described above. There is a difference in that in the cultures the coccoid type is never so completely submerged as is often the case in the water suspension. A further difference is that the suspension of the culture in distilled water usually presents appreciable numbers of the larger yeast-like forms (Figs. 1 and 2) seen in the original material, while under cultural conditions favoring the mucoid transformation and the development of the motile bacillary forms the larger yeast forms are seldom or never seen.

The yeast-like forms appear in abundance under certain conditions which have not been brought under control. They have been some-
times, but are not usually, abundant in the condensation water of blood agar transplants during the first day or two before there is any visible growth. They have also been abundant and persistent in some of the glycerol agar cultures. Examined in fresh preparations when abundant these forms present the widest variety of shape. They are all resolved, however, into a coccus-like body, or clump of such, with a projected mycelial-like structure of variable length, width, and conformation. The mycelial projection frequently exhibits irregular contractions in the fresh condition, and usually does so when fixed and stained. The illustrations (Figs. 1 and 2) are from preparations left overstained. If differentiated the irregularities are emphasized and the projections are often coarsely granular, suggesting a disintegration into coccoid bodies. In the fresh, the mycelia are not granular and there is no suggestion of such a break-up. In the condensation water of blood agar cultures before visible growth appears there are frequently found clumps of coccoid bodies with a fringe of bacillary projections (Fig. 4).

As wholly aberrant there are rarely seen perfectly round bodies of larger size (5–6μ diameter). These in fresh preparation are homogeneous and thin walled, presenting no suggestion of a double contour or any heavier membrane. There are also seen rarely in stained preparations homogeneous, rather densely staining bodies of similar size and conformation. In a very few instances these have shown a suggestion of division into four ovoid bodies corresponding in size to the more usual coccoid forms.

The extreme pleomorphism presented by the original watery suspension and the earliest cultures naturally suggested a mixture. It was hoped to recover the smaller forms by filtration. The following experiment was done as a repetition of imperfectly executed preliminary trials.

Eight new Berkefeld filters, grade N, were prepared in the usual way. A number of cultures on blood agar 2 weeks old were suspended in distilled water and stood at room temperature for 5 days with frequent light shaking. The total quantity of suspension was about 100 cc. There was then added to this an equal amount of a heavy suspension of Bacillus abortus (Bang) made from an old laboratory culture capable of vigorous growth. This mixed suspension was immediately passed through the filters, 20 cc. through
each of the eight. The filtration was forced by the house vacuum and occupied about 10 minutes each. Each filtrate was divided immediately into two equal portions. One portion was planted on plain agar, 1 cc. per tube, the tubes sealed with wax, and incubated at 37.5°C. for 10 days as a test for B. abortus. The other portion was planted, 1 cc. per tube, on horse blood agar and kept at room temperature.

The experiment resulted as follows. Two of the filters passed B. abortus as well as the microorganism in question and are considered defective. Of the six shown to be efficient in removing B. abortus, the microorganism in question passed through three. Growth from the filtrates was slow, only developing after 2 to 3 weeks, and in only one case did all the tubes develop growth. In the other two cases about half the tubes developed. The growth which did develop was normal for the complete culture. All the forms which have been described were eventually observed in the first cultures from the filtrate. The small colonies consisting of cocci chiefly were the first to appear, but the mucoid transformation was prompt in all cases and was associated with the appearance of the motile bacillary forms. The yeast-like forms appeared when these first filtrate cultures were suspended in distilled water. This experiment was carried out when the microorganism had been under active cultivation for about 4 months.

In view of the possible origin of the culture in the virus blood of hog cholera its virulence for swine has been repeatedly tested. It does not produce cholera nor did the experiments develop any other evidence of pathogenicity.

DISCUSSION.

The culture described fulfills the condition postulated in the introduction. That is, we have to do with a pleomorphic microorganism certain forms of which are within the range of ordinary microscopic visibility, other forms at the limits of visibility, would, taken alone, be essentially invisible. Under certain conditions forms capable of regenerating the complete culture are filterable through Berkefeld N filters.

It follows as a practical consequence of this that in dealing with filterable virus material as the subject of cultivation experiments growths which are within the range of ordinary visibility may not be disregarded or discarded as inconsequential on this count alone.
The consideration of the systematic position of this microorganism is of considerable secondary interest. Because of the appearance of the larger forms referred to, the general character of the luxuriant growth, and the evident complexity of the cultural forms, I have considered that it was probably to be classified as a yeast or yeast-like fungus. The predominant type of multiplication in the earlier stages of all cultures and in all stages when cultural conditions are rather unfavorable is by fission. If, therefore, the assignment of the culture to the yeasts were accepted, it would fall naturally into the group of schizosaccharomyces.

The schizosaccharomyces as described by Guilliermond (15) seem to comprise two distinct groups at the present time. One of these is made up of a number of well known cultures closely related in fermentational activity, size, and general morphology to the yeasts whose direct multiplication is by budding. The other group comprises a number of microorganisms found in the organs of insects. They have been generally accepted as yeasts on morphological grounds but have not been cultivated. They are of smaller size on the whole than the first group and their systematic position would still seem to be open to some question. It is interesting that two of these, found in *Chermesis strobiophila* Kalt. and *Chermesis abietis* L., as described and classified by Sulc (16), are the smallest of the yeasts (length 1–2 μ) and in this respect are comparable to the fission forms of the culture in hand. Considered to be a yeast the culture in hand appears to be more closely allied to these insect forms than to any other.

As an alternative to this assignment the microorganism above described might be thought of as a pleomorphic bacterium. The curious phenomenon of a non-motile micrococcus with motile bacillary branches would be presented, together with extreme changes in size in both the morphological series. The larger forms which have been thought of as yeasts might be reconciled with this view as involution forms. On this alternative the culture would have many points of resemblance to the group *B. radiobacter* of the root nodules of legumes and to *B. azotobacter* of the soil. With these bacteria extreme pleomorphism prevails and very small forms occur. Löhnis' (17, 18) attempts to recover the culture from filtrates were apparently unsuccessful in the case of *B. azotobacter*. 
A second alternative has been constantly in mind, namely, that the culture may really be a mixture. This has seemed less probable as the properties of the culture have been developed. It involves the conception of at least two microorganisms both filterable and living in such close association that they are inseparable by plating methods. It need only be pointed out that even accepting this alternative it in no way diminishes the significance of the culture as a type of filterable microorganism presenting easily visible forms and readily cultivatable.

It may further be suggested that if this microorganism is a yeast the basic forms, small as they are, should show more evidence of definite internal structure than is the case.

While suggesting such restrictions and alternative interpretations as have arisen during the study of the culture, my inclination is to regard the microorganism as a fission yeast further characterized by its filterability and therefore designated provisionally as *Schizosaccharomyces filtrans*.

**SUMMARY.**

A microorganism cultivated in the course of experiments with the virus of hog cholera shows the following points of interest.

It is pleomorphic in extreme degree.

Among the forms are coccoid and bacillary bodies at the lower limits of visibility.

The culture can be completely regenerated after filtration through some Berkefeld N filters although these retain *Bacillus abortus* (Bang).

The culture is carried forward by multiplication of forms of easy visibility (1.5μ).

Associated with these at times are still larger forms bearing a striking general resemblance to those yeasts which develop abortive mycelia.

Motile bacillary forms appear to arise as buds or branches from round or ovoid coccii.

While cognizance is taken of possible alternatives the microorganism is provisionally classified with the yeasts and recognizing its filterability is named *Schizosaccharomyces filtrans*.

The culture is not pathogenic for swine.
BIBLIOGRAPHY.


EXPLANATION OF PLATE 7.

**Figs. 1, 2, and 3.** From 14 day culture on glycerol agar. Giemsa stain without differentiation.  × 1620.

**Fig. 4.** From blood agar culture suspended in distilled water. Giemsa stain without differentiation.  × 1620.

**Fig. 5.** From 2 day culture on blood agar. Carbol-fuchsin stain.  × 1620.

**Fig. 6.** Same culture as Fig. 5, stained with Giemsa’s method.  × 1620.

**Figs. 7, 8, 9, 10, 11, and 12.** From suspensions of blood agar cultures in distilled water. Stained by Giemsa’s method.  × 1620.

**Note.**

The bacillary and branched or budded forms as shown in Figs. 7 to 12 are apt to be very actively motile.

The independent division of the bacillary forms is strongly suggested in Fig. 7. The origin of the bacillary from the coccoid forms is strongly suggested in Figs. 11 and 12, where the suspensions were active and the cocci free, as well as in Fig. 4 from a suspension of an old culture where the cocci remained in masses.

The granular transformation of the coccoid forms is shown in Figs. 5 and 6.
The yeast-like forms are shown in Figs. 1 and 2, while Fig. 3 from the same preparation shows how close may be the relation between these and the coccoid forms of Figs. 11 and 12.

An attempt to construct a complete developmental cycle on the basis of these suggestions seems to be unwarranted because of an insufficient understanding of what happens among the smallest forms of Figs. 5 and following.
(Lewis: Filterable yeast-like microorganism.)