CYTOLOGICAL AND BACTERIOLOGICAL ASPECTS OF REFRIGERATOR SLIME

BY

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CONTENTS

INTRODUCTION: Food storage and preservation is a problem, which has challanged the most able minds of men from the earliest times. It was solved after a fashion by the use of several methods, one of which was cooling or the reduction of temperature. The earliest method was cooling in porous earthenware vessels. Food was also preserved in caves or in flowing water sheltered from the sun. The ancient Egyptians cooled water by placing it in shallow trays of porous material on beds of straw, left exposed to the night winds. Simonides of Keos, early greek poet about 500 B.C., mentioned in one of his odes,"Guests sipping wines cooled with snow". Alexander, King of Macedon (323-336 B.C.) cooled wines for his soldiers in long trenches filled with snow. Tancrelus mentioned the freezing of water by a mixture of salt petre and snow in 1607. Reference is made in 1799 of natural ice sent from New York to Charleston. Natural ice was first used in America in 1802. Jacob Perkin invented a compression refrigerator in 1834 and in 1866 the first fruit and in 1872 the first beef and fish were successfully shipped.

The early ice chests were crude boxes having double walls, the space between being filled with sawdust or straw. The ice was obtained from natural sources in the winter and stored until the summer months when it was used. After the development of practical methods of producing artifical ice, a practical refrigerator became

necessary. The commercial production of household refrigerators was begun late in the nineteenth century. Through a steady process of improvement, the modern refrigerator is the result. The contents of this thesis deals with the composition and causative agents of the slime which may be found in the drain pipes of any refrigerator using ice.

MACROSCOPIC DESCRIPTION: The macroscopic discription must be general because of several factors which influence its appearance and consistancy. The source of the ice used, method of storing and delivery of the ice, cleanliness of the ice chamber, and the material and age of the drain pipes all contribute materially to the aspect of the growth. In general, it is soft, slick and fibrinous. The amount of both organic and inorganic extraneous material present depends upon the source and upon the storing and delivery of the ice. There is always present some dirt, sticks, straw, string or gravel, the percentage of which is variable with the purity of the water used in the production of the ice, and the care asserted during its handling and delivery. If natural ice is used, the extraneous materials present are much more pronounced. Their presence is also influenced by the placing in the ice chamber of food, which may be easily spilled or overturned. The color is usually light grey but may vary from white through all

shades of red and orange to black. The red and orange colors are usually imparted to the growth by a deposit of rust or other metallic salts from the material constituting the drain pipes. The growth ordinarily does not exhibit a strong odor, but in case of persistant placing of foods in the ice chamber more especially if occasionally spilled it may have a very disagreeable smell.

MICROSCOPIC DESCRIPTION: Microscopically the growth is a weird and interesting sight. One sees a dense network of fungus growth. The mycelial threads interweave so as to form the body of the slime, with countless numbers of bacteria and yeasts lying free in the open spaces formed by the crossing of the mycelia. Coccoid and bacillary, motile and non-motile, sporulating and non-sporulating bacteria are distinguishable under the 4 m.m. objective. The yeasts seem to be distributed at randon among the mycelial often times forming a line close to them. Lying entrapped can be seen numerous particles of dirt, small gravel, often times small pieces of wood, straw, burlap, rust scales, and decomposing food.

ANALYSIS OF THE GROWTH: Samples from 15 ice boxes were collected and analyzed upon two occasions which were six weeks apart. A sample could not be obtained from one ice box for the second analysis so that 14 specimens

were included in the second series.

The ice boxes were distributed as follows:

1 from the zoology department
1 " a cafe
1 from a soda fountain
1 from a candy case
5 from grocery stores
2 from meat markets
1 from a fraternity house
3 from the bacteriology department

Fungii and bacteria were found in all the samples during both the examinations. Yeasts were found in 13 samples the first analysis and 11 samples the second analysis. However only one speciman was negative for yeasts in both series. This was found to be true of the growth taken from a candy case in a drug store. Repeated capsule stains by Rosenow's method failed to demonstrate any capsulated organisms in the original growths taken from the ice boxes for either series of examinations. After analysis and isolation of the bacteria no capsulated organisms were discovered. By the Ziehl-Neelsen method the original materials did not show the presence of acid fast organisms, neither were any acid fast stains isolated from the materials during analysis. Smears of the original material were made on clean glass slides, and fixed with Carnoy's

solution (6parts absolute alcohol, 3 parts chloroform and 1 part glacial acetic acid) for from 2 to 3 hours. A part of the smears was washed in water and a part in 95% alcohol. Some of the smears were stained with eosin, some with dilute fuchsin and some were stained overnight in haematoxylin, washed and counterstained with eosin. After staining, those washed in water were mounted in glycerin and those washed in 95% alcohol were mounted in balsam. Neither method of washing and mounting showed the presence of Myxobacteriales nor Myxomycetes.

The growths were streaked on plates which were poured from three kinds of agar namely, plain agar, Dextrose potato agar, and eosin-methylene blue agar. In each case the materials were streaked on two plates of each kind of medium. The composition of the media used were as follows:

Plain agar: 1 litre of distilled water
.3 grams of meat extract
15 grams of agar
10 grams of peptone
5 grams of salt(nacl)
Ph of 7.2 - 7.4

Dextrose potato agar:
Distilled water up to 1 litre
Broth from 150 grams of potatoes
10 grams of dextrose
15 grams of agar
5 grams of salt
Ph adjusted to 5.4 - 5.6

Eosin-Methylene blue agar:
100 C.C. of plain agar as in first media
5 C.C. of 20% lactose
5 C.C. of 20% saccharose
1½C.C. of 3% aqueous eosin solution

2 C.C. of .5% aqueous Methylene blue
Ph of 7.2 - 7.4

One plate from each medium was incubated at 37 degrees C. and one plate from each was incubated at 18 degrees C. The bacterial colonies were picked and the plates examined for the presence of yeasts or molds at 48 hours. The bacterial colonies were picked onto plain agar slants and later inoculated into dextrose, lactose, saccharose, mannite and peptone broths, litmus milk and gelatin. A gram stain and motilities were done on the cultures at 24 hours.

The analysis yielded a large group of organisms which were evidently members of the large and heterogenous group known as the water bacteria. It also yielded B. Mesentericus, B. Subtilis, Members of the proteus group, Coli-Aerogenes and an unidentified organism which will be designated as "C".

The organism designated as "C" does not produce capsules, is non-motile and loses the stain when stained by Gram's method. It is a large chained organism ranging from 3.5μ to 5μ in length and from 1.5μ to 2.25μ in breadth. After about 72 hours it produces shadow forms, but does not produce spores. It does not ferment dextrose, lactose, saccharose or mannite, but shows a very heavy floculant growth in all the broths mentioned. It neither produces indole nor

liquifies gelatine. There is no perceptible change produced in litmus milk. It exhibits a thick, white abundant growth on a potato slant and grows in large, round, convex, smooth, viscid and almost transparent colonies on plain agar at 48 hours.

Table 1 shows the incidents of each of the bacteria found in the two preceding analyses.

TABLE 1: INCIDENTS OF BACTERIA FOUND IN THE ANALYSES.

1st analysis 15 samples		2nd analysis 14 samples
15	Water Bacteria	14
10	B.Mesentericus	8
7	"C"	6
8	B.Subtilis	5
3	Proteus Group	2
1	Coli-Aerogenes	2

A typical strain of bacteria was selected from the water bacteria group, B. Mesentericus, B. Subtilis, "C", because these four types were the most commonly occuring bacteria in the samples analyzed. They were incubated on plain agar at 4, 16, 18, 25, 37, and 42 degrees centigrade in order to determine their temperature range of growth. The results show that all the organisms concerned are able to grow over a wide range of temperature.

TABLE 2: TEMPERATURE RANGE OF BACTERIA ISOLATED FROM REFRIGERATOR SLIME

ORGAN.	4°C.	16°C.	18°C.	25°C.	37°C.	42°C.
B.Mesen.	-7 Dys.	+48 Hr.	+48 Hr.	+24 Hr.	+24 Hr.	+24 Hr.
Wat.Bact.	-7 "	+48 "	+48 "	+24 "	+24 "	+24 "
"C"	-7 "	+48 "	+48 "	+24 "	+24 "	+24 "
B.Sub.	-7 "	+48 "	+48 "	+24 "	+24 "	+24 "

The same four strains of bacteria were incubated at 37 degrees centigrade on plain agar with varying Ph values. This experiment was carried out to determine the limiting Ph values for the bacteria under consideration. The Ph values used were; 3.6, 4, 4.6, 5, 5.6, 8, 8.6, 9, 9.6 and 10. The apparent break in the center was because I felt sure that the bacteria would grow over that range. The 37°C. incubator was used because all the strains were known to grow at 37°C. and produce a visible growth quicker than if incubated at 16 or 18°C. This experiment showed the widderange of possible growth for these organisms and that they were able to reproduce at the Ph of the waste water from the ice chamber of the refrigerator. The average Ph of the waste water was found to be from 5.8 to 6 for the refrigerators under observation. Table 3 gives the results of the experiment determining the Ph range of the bacteria.

TABLE 3: Ph RANGE OF BACTERIA ISOLATED FROM REFRIGERATOR SLIME

Ph VAL.	WATER BACTERIA	B.MESEN.	"C"	B.SUBTILIS
3.6	—96 Hrs.	—96 Hrs.	—96 Hrs.	—96 Hrs.
4.0	—96 "	—96 "	—96 "	—96 "
4.6	—96 "	—96 "	—96 "	—96 "
5.0	—96 "	—96 "	+72 "	+48 "
5.6	+72 "	+48 "	+48 "	+48 "
8.0	+48 "	+48 "	+48 "	+48 "
8.6	+48 "	+48 "	+48 "	+43 "
9.0	+48 "	+48 "	+48 "	+48 "
9.6	+48 "	+48 "	—96 "	—96 "
10.0	+48 "	+48 "	—96 "	—96 "

The yeasts were found quite consistantly throughout the analyses. However only one attempt was made to cultivate them. The reason will be brought out later in this paper. Red, white and green yeasts were observed, however the white yeasts were by far the most often inhabitants of the slime. Table 4 gives their incidents of occurrence in the sample examined.

TABLE 4: INCIDENTS OF YEASTS FOUND IN THE ANALYSES.

1st series of 15 samples		2nd series of 14 samples
12	White Yeasts	9
1	Red Yeasts	7
1	Green Yeasts	0

It will be readily seen that there is no close correl-

ation between the results of the two series during analyses.

The molds were grown on acid dextrose potato agar as well as being identified directly from the incubated plates at 48 hours. As previously stated, the molds were found in every sample in each series of analysis of the slime.

TABLE 5: INCIDENTS OF MOLDS FOUND IN THE ANALYSES

1st series 15 samples		2nd series 14 samples
15	Penicillium	14
10	Rhizapus	10
2	Fusarium	2
4	Aspergillus	0
0	Sporodina	3

In only one case was a single mold obtained from a sample. All the other samples showed the presence of from two to five fungii. Some molds were observed but were not identified because of their uncommon occurrence. Plates poured at the same time and from the same media were incubated along with the inoculated plates in each incubator. They did not show the presence of molds so that my results in the analyses were not laboratory contaminations. The three most commonly occurring molds namely; Penicillium, Rhizapus, and Aspergillus were grown in pure culture on plain agar and on acid dextrose potato agar at varying temperatures.

The growths on the two media were identical as to time of appearance in the several temperatures under observation. The temperature observed were 16, 18, 25, 37, and 42 degrees centigrade. All three molds grew at all the temperatures and Table 6 shows the time of appearance of a visible growth upon the media at the respective temperatures under observation.

TABLE 6: TEMPERATURE RANGE OF MOLDS ISOLATED FROM REFRIGERATOR SLIME

ORGAN.	MEDIA	16°C.	18°C.	24°C.	37°C.	42°C.
Penic.	Pl.agar	36 Hrs.	36 Hrs.	24 Hrs.	24 Hrs.	24 Hrs.
Penic.	Pot.agar	36 "	36 "	24 "	24 "	24 "
Rhiz.	Pl.agar	36 "	36 "	24 "	24 "	24 "
Rhiz.	Pot.agar	36 "	36 "	24 "	24 "	24 "
Asper.	Pl.agar	36 "	36 "	24 "	24 "	24 "
Asper.	Pot.agar	36 "	36 "	24 "	24 "	24 "

In the original analysis of the specimens from the refrigerators it was found that the fungii grew better on the acid dextrose potato agar, however in pure culture no advantage was shown in the preceding experiment. It is quite easily seen from this experiment that the fungii will grow under a wide variation of temperature conditions thereby fitting them for inhabitation of the ice box slime. The same three fungii were plated on plain agar plates of varying Ph values in order to fix approximately the extremes for the Ph under which they would produce a visable growth. The same values were

used as for the bacteria when the analogous experiment was carried out to determine their range of growth. The values used were a Ph of 3.6, 4, 4.6, 5, 5.6, 8, 8.6, 9, 9.6, and 10. The results show that the fungii are able to grow in a more acid medium than the bacteria. Table 7 will give the results in tabular form.

TABLE 7: Ph RANGE OF MOLDS ISOLATED FROM REFRIGERATOR SLIME

Ph VALUES	PENICILLIUM	ASPERGILLUS	RHIZAPUS
3.6	-96 Hrs.	-96 Hrs.	-96 Hrs.
4.0	+48 "	+48 "	-96 "
4.6	+48 "	+48 "	+48 "
5.0	+48 "	+48 "	+48 "
5.6	+48 "	+48 "	+48 "
8.0	+72 "	+48 "	+48 "
8.6	-96 "	-96 "	-96 "
9.0	-96 "	-96 "	-96 "
9.6	-96 "	-96 "	-96 "
10.0	-96 "	-96 "	-96 "

From the results of the analysis of the slime from 15 ice boxes upon two examinations, it would follow that the growth was composed of four classes of substances namely; fungii, bacteria, yeasts and debris. Probably the fungii are responsible for the production of the slime. The fungii seem to form the skeleton for the growth thereby straining out of the waste water any organic debris

together with bacteria and yeasts. Since the average temperature of the waste water in the boxes examined was 10° C., the growth must necessarily be slow. However the temperature is not low enough to completely inhibit the growth of the organisms constituting the body of the slime.

SYNTHYSIS: All attempts to reproduce the slime from the original growth or from 2 or more of its components obtained from it during analysis failed, when they were attempted to be grown on solid or in liquid media. Plain agar, acid dextrose agar, ferrous thiosulphate agar, plain broth, potato broth and ice water all failed to produce any growth which resembled the original in the least. On the solid media the fungii always overgrew the bacteria and in the liquid media the reverse was true.

With this in mind an apparatur was manufactured which almost duplicated the conditions which are ordinarily found in the drain pipes of a refrigerator. Figure 1 shows its construction. The bottles containing the media had a capacity of from 4 to 5 litres. The pinchcocks were adjusted so that 3 to 4 drops fell each minute. Two media were used, one of which was plain tap water. The other media was tap water containing 1% meat infusion and the broth from 150 grams of potatoes per litre. The entire apparatus was sterilized in the autoclave at 15 pounds pressure for one half hour before

each experiment.

The first experiment consisted of the seeding of the apparatus with B.Mesentericus using the potatox meat infusion water. The experiment was allowed to continue at room temperature (23°C.) in the light for 96 hours at which time a heavy growth was obtained. The growth was slimy but did not resemble the original in being fibrinous or hard to pull apart.

After sterilization, the apparatus was seeded with Penicillium spores. The temperature conditions and the mediumused were the same as used in the preceding experiment. A visible growth had appeared in 6 hours, a heavy growth was present in 24 hours and the apparatus was stopped up in 48 hours. The growth was soft and feathery and was fibrinous much the same as the samples taken from the refrigerators. However, it was not slimy, but had a starchy feel which made it easily distinguishable from the original growth.

The apparatus was again sterilized and seeded with B.Mesentericus and Penicillium, duplicating both the medium and incubation conditions. A good growth was obtained in 96 hours which closely resembled the original in every respect with the exception that it contained no extraneous material. The growth was examined microscopically and except for the previously mentioned difference

it was just the same as the original growth.

The apparatus was then seeded with a small amount of the original again using the same conditions of incubation and medium. A good growth was obtained in 96 hours. The growth obtained was identical to the original sample as far as a microscopical examination could determine. By analysis the growth was found to contain the same constituents as the original sample with which the apparatus was seeded.

The apparatus was again seeded with a portion of the original material. Sterile tap water served as the medium and the temperature of incubation was 25 degrees centigrade. It required 7 days before a satisfactory growth was obtained. Upon examination it was found to contain the same constituents as the original material from which it was grown. When the incubation temperature was changed to 18° C., the time required to produce the growth and its components were the same.

When the apparatus was seeded with both B.Mesentericus and Penicillium using sterile tap water as medium and incubating at 18° C., an easily visible growth was obtained in 8 days. Again the microscopic examination showed the growth to resemble the original material very closely. When analyzed, the growth was found to contain only B.Mesentericus and Penicillium.

After these preliminary experiments using the original and combinations of its components in pure culture, grown under varied conditions of food supply, temperature and light, a series of experiments was carried out using various other combinations of the fungii and bacteria which were isolated from the original material during analysis. In this series of experiments, potato meaxt infusion water was used as a medium. The incubation temperature was 18° C. The light was excluded from the apparatus during growth. Table 8 gives the results of this series of experiments.

TABLE 8: TIME REQUIRED TO PRODUCE GROWTH OF VARIOUS COMBINATIONS OF FUNGII AND BACTERIA

COMBINATIONS	MEDIUM	TEMP.	APP. OF GROWTH
Aspergillus & Water Bacteria	Pot.meat inf. water	16° C.	7 days
Aspergillus & B.Mesentericus	"	"	7 "
Aspergillus & "C"	"	"	7 "
Aspergillus & B.Subtilis	"	"	7 "
Penicillium & Water Bacteria	"	"	5 "
Penicillium & B.Mesentericus	"	"	5 "
Penicillium & "C"	"	"	6 "
Penicillium & B.Subtilis	"	"	5 "
Rhizapus & Water Bacteria	"	"	6 "
Rhizapus & B.Mesentericus	"	"	6 "
Rhizapus & "C"	"	"	7 "
Rhizapus & B.Subtilis	"	"	6 "

In each case a microscopic examination was carried out and each was found to resemble very closely the original material obtained from the various ice boxes. However, there was no extraneous material present in the synthetic growths, being the only noticeable difference from the microscopic appearance of the original samples. Analysis of the synthetic growths yielded the same organisms with which the apparatus was seeded in the various experiments in the series.

No further mention has been made of the yeasts because, when grown either alone or in combination with one of the fungii the species isolated failed to produce a growth which resembled the slime found in the drain pipes of the refrigerators. This fact led me to believe that the yeasts were not one of the essential organisms in the slime, but were merely there because they had been strained out of the waste water by the fungii. They probably multiply and add to the body and volume of the slime, but I consider them as non-essential elements in the growth of the slime.

DISCUSSION: The slime is a complex substance composed of fungii, bacteria, yeasts and debris which may be of varying nature. Probably the fungii and bacteria are the essential factors in its growth. Neither of these components alone can produce the slime. The fungii in pure

culture, or various combinations of fungii are capable of producing a troublesome growthin the drain pipes which would stop them up. However, the bacteria lend the slimy qualities and reduce the time required to produce the growth in sufficient quantities to cause trouble. Both the fungii and bacteria present in the slime are members of groups which are widely distributed in nature. Most of which are found as laboratory contaminants, in dust, hay, surface waters, and many other places. It may be easily seen that the population of the slime will be variable. This fact is due to the variation of methods and materials used in the production, handling and delivery of the ice consumed. The cleanliness of the ice chamber itself and the absence of food in it contributes materially to the components which may be found in the slime.

The slimy character of the growth might be attributed to any one, or to various combinations of several causes: the presence of capsular material, or to Myxobacteriales, or to Myxomycetes, or to the slimy character of wet protein or to the water included among the mycelial threads of the common fungii. As has been stated in this paper, I was unable to demonstrate capsulated organisms either in the original material or after analysis. Rosenow's method was used when attempting to demonstrate the capsules

by stained smears. Dilute acetic acid also failed to show the presence of mucins in a filtrate from the slime. Had they been present, it would have been an indication that capsular material was present. Neither was I able to find Myxobacteriales nor Myxomycetes present in the growths taken from the refrigerators. As would be expected they did not appear during the process of analysis because the media used were unsuitable for their growth. However they did not grow when the slime was planted on rabbit dung agar as recommended by Bergey and incubated at both 37° and 18° centigrade. Nor were they found in stained smears of the original material. Haematoxylin counter-stained with eosin, dilute fuchsin and safranin were used to stain the smears. Since their optimum temperature is about 30°, one would hardly expect to find them growing at a temperature between 10° and 15° centigrade. These facts leave only two possibilities, and in my opinion a combination of the slimy character of the wet bacterial protein present, together with water held entrapped among the mycelial threads of the fungii explains the slimy character of the growth as commonly found in refrigerators.

The yeasts may play a small part by adding slightly to the volume of the slime, but in my opinion are non-essential factors in its growth. Likewise the debris, both organic and inorganic, is a purely passive constituent.

It affects the macroscopic appearance giving it color and sometimes a grandular consistancy. It is needless to add that it has no active part in the sense of growth.

In the preceding experiments, a much more nutrient medium and a slightly higher temperature were used than are found in the ice boxes in general usage. However the product was essentially the same as the original samples taken from the several refrigerators examined. The time required to produce the growth under normal conditions is several weeks. In the laboratory, this time was shortened to from 5 to 8 days. This fact together with the absence of debris were the only ones of variance from the originals which I observed. Finally the slime may be considered as a product of fungii and bacterial association with which every house wife is acquainted yet of which she has little knowledge.

CONCLUSIONS:

1. The slime is composed essentially of fungii and bacteria.
2. The fungii are members of the most commonly occuring species which may be found almost anyplace in nature.
3. The bacteria are of the water bacteria group, and of spore producers which enjoy wide distribution.

4. Members of the Coli-Aerogenes, Proteus and Ps. Pyocyaneus groups were occasionally found.
5. No pathogenic organisms were isolated in either of the two series of samples analyzed.
6. Possibly yeasts add something to the body of the slime.
7. Stained smears and attempts to grow them failed to show the presence of Myxobacteriales or Myxomycetes.
8. Tests with dilute acetic acid were negative when a filtrate from the slime was tested for mucins.
9. By the use of Rosenow's method of staining, no capsulated organisms were demonstrated in either the original material or after analysis.
10. The slimy growth is a product of bacterial and fungus associations whereby the natural state of both components is somewhat altered.
11. Perhaps the slimy character of the growth is due to the slimy character of wet bacterial protein present together with the water entrapped among the mycelial threads of the fungii.

KEY

Fig. 1 - Apparatus used to obtain synthetic growth of slime.

Fig. 2 - Original material

Fig. 3 - Also original material

Fig. 4 - Growth from original material

Fig. 5 - Rhizapus together with B.Mesentericus

Fig. 6 - Penicillium together with B.Mesentericus

Fig. 7 - Rhizapus together with "C"

Note - Figures 2 to 7 inclusive are photographs of camera-lucida drawings x 150.

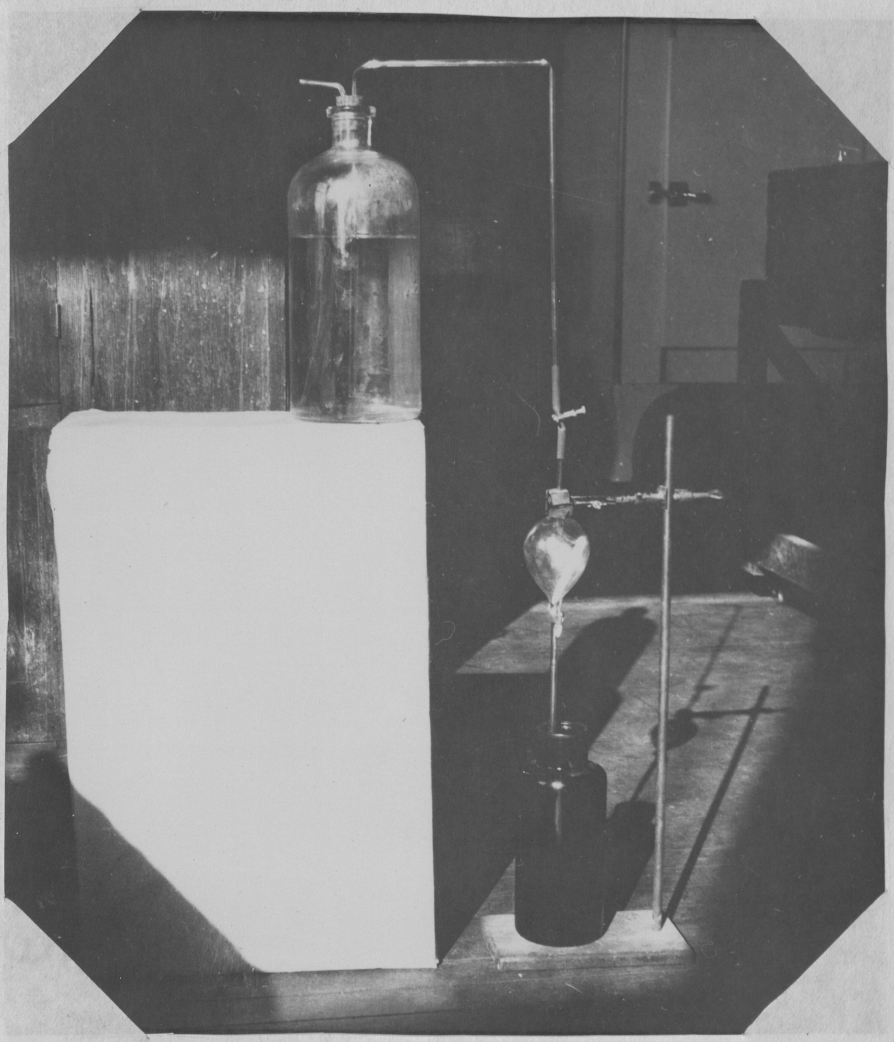

Fig. 1

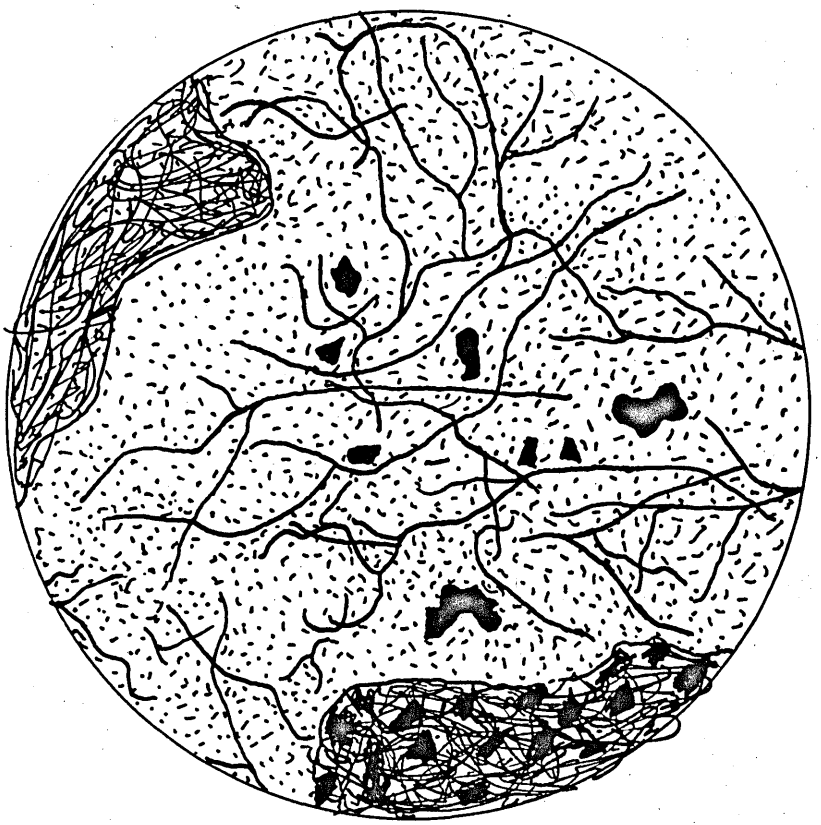

Fig. 2

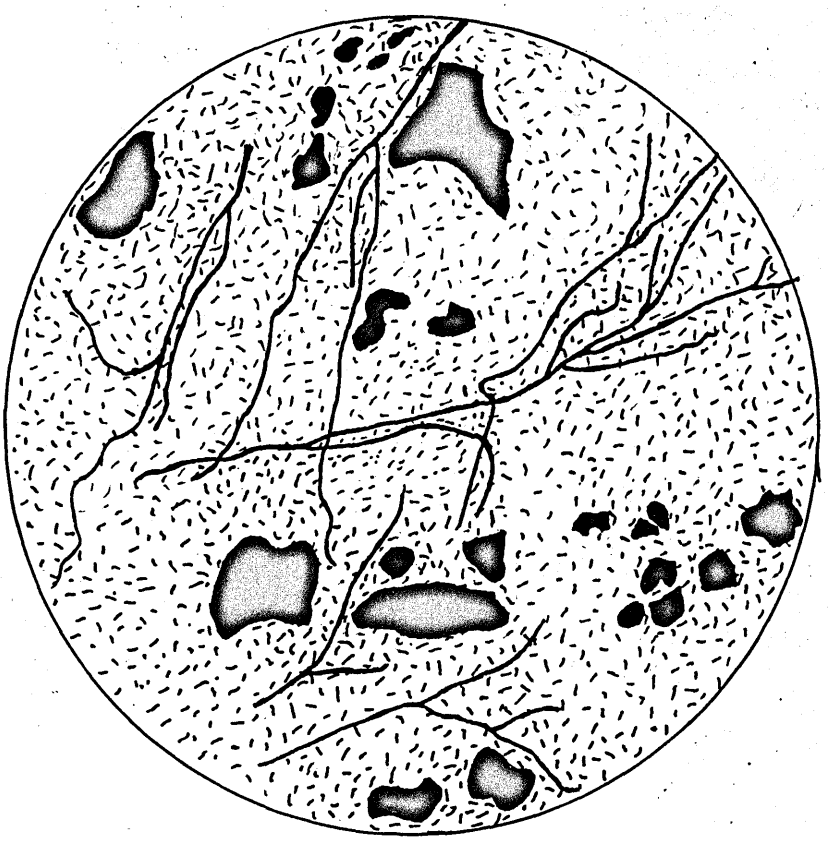

Fig. 3

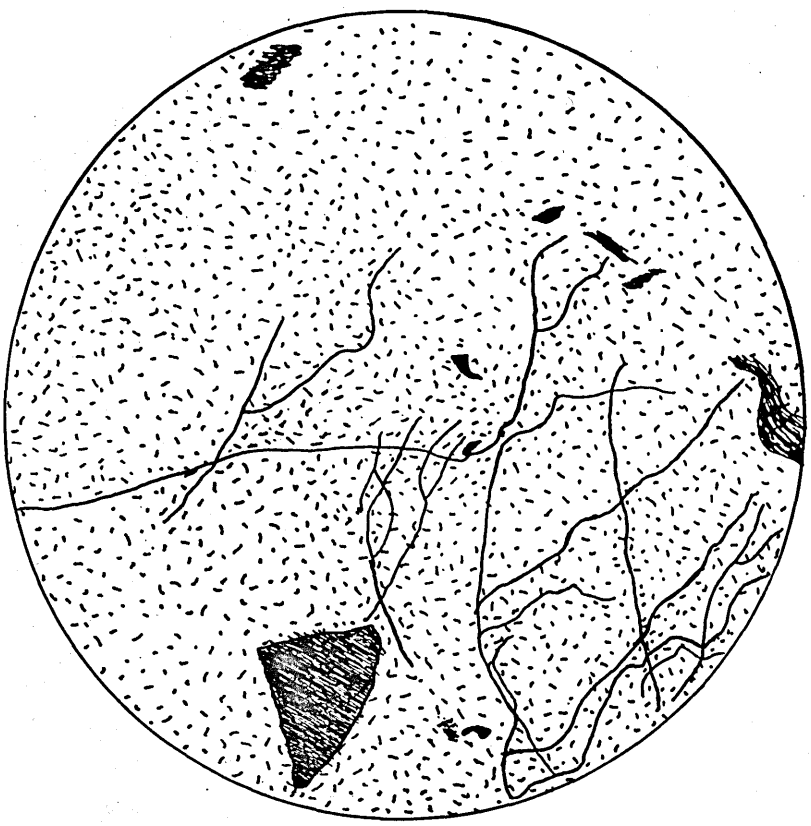

Fig. 4

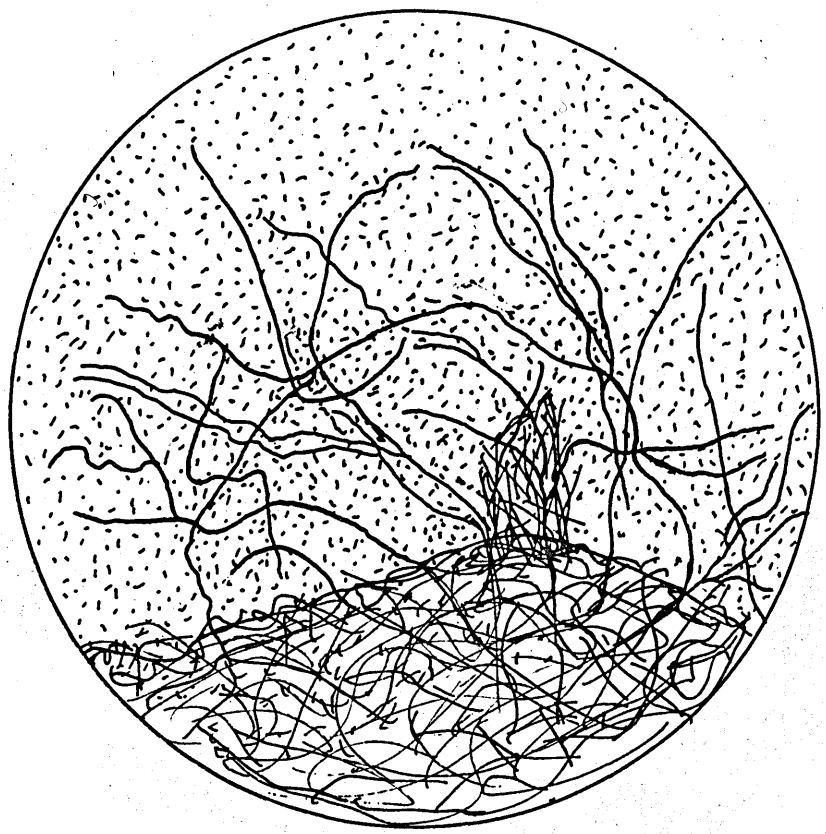

Fig. 5

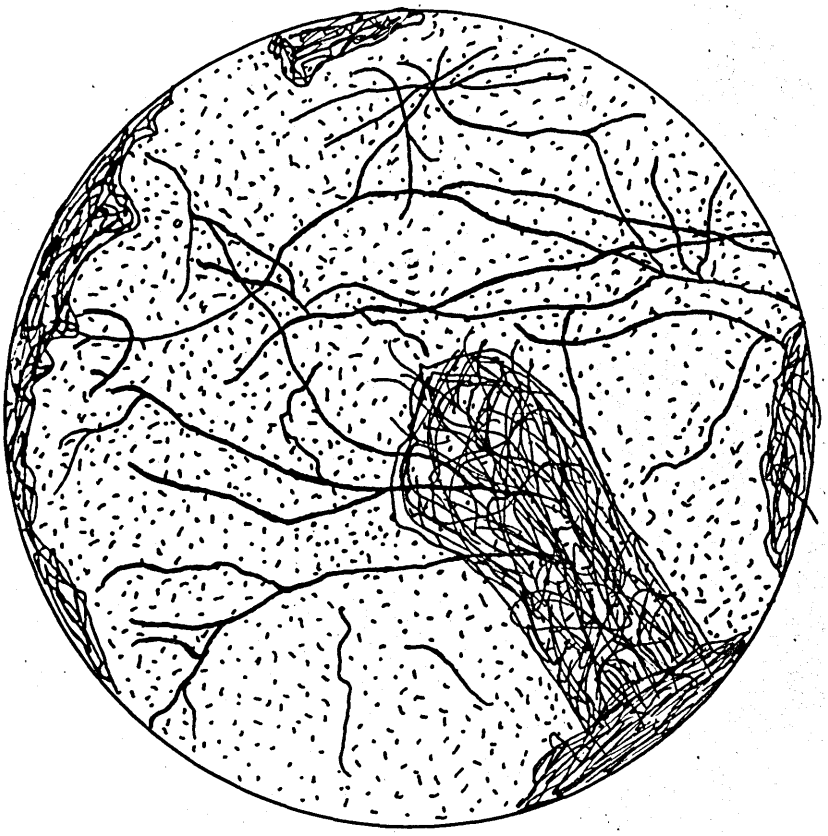

Fig. 6

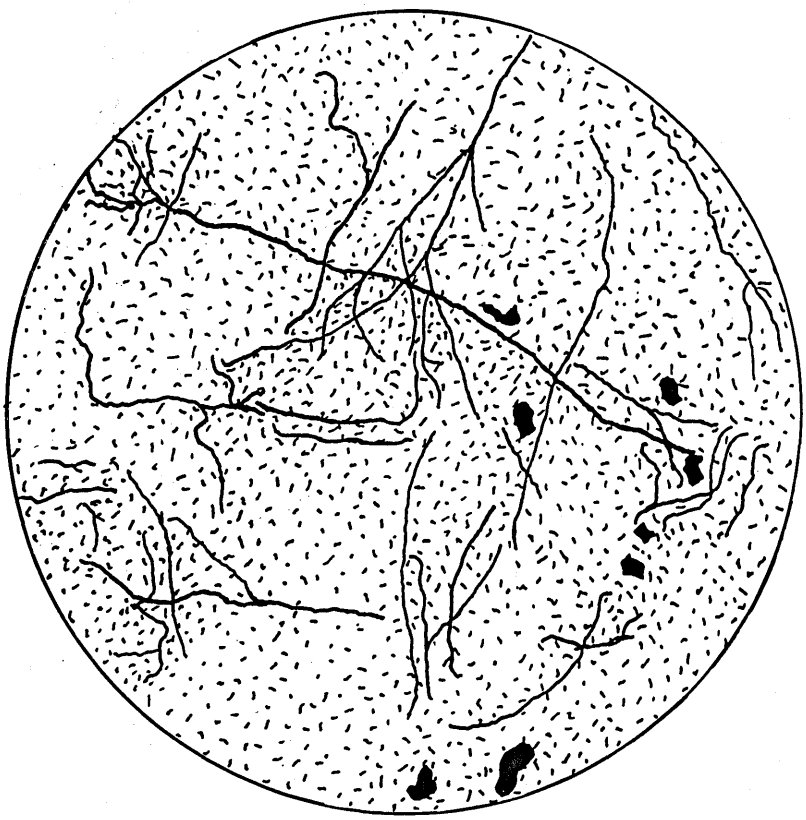

Fig. 7