BACTERIOSIS of CULTIVATED MUSHROOMS.

A thesis submitted to the Faculty of The Graduate School of the University of Minnesota by A. G. Tolaas in partial fulfillment of the requirements for the degree of Master of Science, April 26th, 1912.

Introduction.

In commercial mushroom growing an unsightly spot of the cap and stem is very prevalent, especially in certain varieties. The spot, although not serious as far as the health of the mushroom is concerned, does have a direct bearing upon the market value.

The spot, which has been shown to be caused by a bacterial organism, discolors the surface of the caps. At first these discolorations are yellowish brown in color but become considerably darker as the mushroom grows older.

M. J. Costantin described a bacterial spot of mushrooms which is very similar to the one in question.

M. L. Matruchot studied the disease in 1909 describing it and assigning the manure used in the preparation of the beds as the probable source of infection.

The present work has been devoted to the study

of the nature of the disease, the conditions favorable to its presence, and to the cultural characteristics of the organism causing the trouble.

The work has been carried on at the caves of the Minnesota Mushroom Company at St. Paul. Repeated attempts have shown that the organism described in the paper is constantly found in the spots. It has been frequently isolated from the spots and when applied in pure culture to the healthy mushroom produces the characteristic discolorations.

NATURE of the DISEASE.

The "Bacterial Spot" of the cultivated mushroom, although not its worst enemy as far as the health and yield of the crop are concerned, affects the salability quite seriously. Constantin[1] in 1892 described a somewhat similar bacterial disease of mushrooms cultivated in the caves in the vicinity of Paris, to which he ascribed the name La Goutte. This name is explained by the appearance, on both cap and stem, during the first stages of the disease, of globules of a colorless to yellowish gray liquid varying in size from that of a pinhead to that of a small pea.

Some time after the mushrooms have been picked, brown to greenish brown spots appear where the globules have dried, forming a sort of clammy surface. After having been under a bell jar in the laboratory for

(1) M. Julien Costantin - La Goutte, Maladie Du Champignon De Couche. Extrait des Comptes rendus des se'-ances de la Societe' de Biologie. (Seance du 5 Mars, 1892)

a short time this clammy surface gradually becomes slimy. The bacteria are to be found in this slimy matter.

L. Matruchot[2], in studying the disease described by Costantin, mentions a peculiar viscosity of the cap at the place of the stains. This he ascribes to what he calls the active perspiration of the mushrooms, small drops or colorless or yellowish grey liquid exuding, in time darkening, and eventually producing the characteristic brown discolorations.

I have not noticed any peculiar viscosity of the caps or stems of the affected mushrooms, but this fact is probably due to the comparatively dry conditions in the caves where the study of the disease was made. It is rather difficult to maintain a desirable humidity in the caves during the winter time since articifial heating is necessary for the production of the crop. This, together with a continuous draft, which is rather

(2) M.L.Matruchot - Des Maladies Du Champignon De Couche La Goutte. La Culture Des Champignon Comestibles No. 24 June, 1909.

difficult to control under the existing conditions, has a tendency to lower the humidity. Hence the liquid exuding from the cap of the mushroom evaporates so rapidly as to prevent the presence of the droplets as noted by Costantin and Matruchot.

EXTERNAL CHARACTERISTICS.

According to observations made at the local caves, the disease first manifests itself by the appearance of faint yellowish spots on the cap and stem, especially on the cap. As the fruiting body develops these spots become considerably darker in color, some instances being observed where the color had turned to a rich chocolate brown. The appearance of this color is apparently due to a secretion formed by the infecting organism.

The degree of infection varies from a few isolated spots scattered about the surface of the cap to the characteristic brown discoloration of the entire cap. In a number of severe cases not only the entire

cap but also the gills showed the characteristic brown spots. Obviously the disease is of superficial origin, working from the epidermis in toward the center of the mushroom.

INTERNAL CHARACTERISTICS.

The brown discoloration does not penetrate into the fruiting body to any great extent, the most severe cases observed showing the brown color but 3 or 4 mm. below the surface. The spots were rarely this deep, being observed only on a mushroom which had been infected six or seven days. The area in the immediate vicinity of this dark discoloration presents a sort of yellowish white appearance. In cases where the attack is not so severe the flesh is not discolored at all but remains perfectly white. As a matter of fact the flesh is not in the least injured for culinary purposes.

Matruchot states that the discolored flesh below the deeply stained surface may extend through to the gills.

There seems to be no exact stage in the development of the fruiting body when the conditions for an

attack of the disease are most favorable. The first appearance of the spots has been observed when the mushroom was but a quarter of an inch in diameter, that is in the early button stage, while in other instances the fruiting body had grown considerably before the first symptoms of the disease were evident. This fact would seem to indicate that insects which abound in great numbers in the caves play a part in the distribution of the infecting organisms. These insects are chiefly those that live in manure piles, and of course are brought into the caves with the manure used in making the beds, either in the egg, maggot or adult stage. Another noticeable fact is that the mushrooms show a greater percentage of discolored specimens during the first part of the bearing period of the bed. At this time the number of insects present is much greater than toward the end of the bearing period.

HUMIDITY and MOISTURE.

That humidity and moisture are important factors in determining the prevalence of the disease seems

evident. The best crops are obtained in that part of the caves where the humidity is greatest, but the spotting is also most serious, indicating that conditions favorable to the production of large crops are also favorable to the growth of the organism causing the spots.

The amount of infection varies considerably with the varieties of mushrooms under cultivation. The kinds most susceptible to the disease are the large white varieties, while the smaller and medium sized varieties are very seldom spotted. This fact is probably due to the longer time required for the larger varieties to reach maturity, thus giving the organism more time in which to develop.

ORIGIN of the DISEASE.

The disease probably originates in the manure used in the preparation of the beds. The presence of large numbers of micro-organisms in manure is a well known fact. One would naturally think that the process of curing the manure, especially if well done, would

inhibit the growth of bacteria, especially non-spore bearing organisms, as this one appears to be. Attempts to isolate the organism from the cured manure have not as yet been carried on satisfactorily, but work along this line will be continued.

INOCULATION EXPERIMENTS.

The general method of obtaining pure cultures for the purpose of performing inoculation experiments and for the determination of the cultural characteristics of the organisms obtained as the results of these inoculations, is as follows:-

The mushroom from which a culture was to be made was first washed free of all dirt with ordinary tap water and then with distilled water. The skin was then peeled off with a scalpel sterilized with alcohol and burned in a bunsen flame. Small pieces of tissue were then cut from the discolored portion beneath the skin and placed on beef agar slants. When a good growth had taken place on these slants pure cultures

were obtained by plating out in the usual method on beef agar and picking individual colonies and again transferring to beef agar slants. Inoculations were made from these tubes, although some inoculations were made from transfers obtained directly from the original tube without plating out.

Inoculation was made with an ordinary platinum loop using all the necessary precautions to insure perfect sterilization. The bacteria were merely smeared in a straight line on the surface without injuring the mushroom.

The first culture taken from a diseased specimen is designated as culture I, the second culture obtained from another diseased mushroom is designated as culture II, etc. Pure cultures obtained as the result of inoculation with strains of culture I are designated as culture IA; those obtained as the result of inoculations with strains of culture II are designated as culture IIA. The culture obtained from an inoculation made by a strain of culture IIA is then designated as

culture II B, etc.

The number of original cultures with which the inoculation experiments were carried on totalled seven, all being obtained from mushrooms that were in a more or less spotted condition. If more than one culture was made from a single mushroom every tube was designated by a number, as tube 1, 2, 3, etc., of culture I, II, etc.

INOCULATIONS, Feb. 5, 1912.

The first culture of the organism, culture I, was obtained on Jan. 22nd, 1912, from a badly affected mushroom, the entire cap of which was deeply stained. The culture was kept at ordinary room temperature and in forty-eight hours a greyish white growth, rather viscous in nature and accompanied by a very strong disagreeable odor, like that of decaying matter, had commenced round the edges of the piece of tissue. The growth continued to increase and eventually spread over the entire surface of the slant. A light

greenish color was observed diffusing through the medium.

Inoculation was made with a direct transfer obtained from tube 1 in the form of a straight line as explained under general methods. The next morning a brownish discoloration similar in appearance to the early stages of natural infection appeared along the line of inoculation. The mushroom was allowed to remain in the cave for three days before it was picked. From this inoculation culture I A was made.

The control mushroom over which a sterile needle had been drawn showed no mark whatever.

INOCULATIONS, Feb. 13, 1912.

A mushroom about ¾ of an inch in diameter was inoculated with a direct transfer obtained from culture III which was made Feb. 8, 1912. The next morning a faint brown discoloration showed along the line of inoculation. Forty-eight hours after the inoculation was made the color had become considerably darker. The area of discoloration did not extend any further in the next two days, and the mushroom having completely e-

volved, was picked and culture III A was made.

A small mushroom was inoculated with a direct transfer from culture IV which was obtained Feb. 8, 1912. A light brown discoloration was present along the line of inoculation on the following morning. The color had increased a little in width. Four days after making the inoculation the mushroom was picked and two tubes of culture IV A was made.

A young mushroom was inoculated with a direct transfer from tube 1 of culture V made on Feb. 8, 1912, from a very badly affected mushroom. Within twenty-four hours growth of the organisms had taken place as evidenced by the appearance of a narrow yellowish brown line along the path of inoculation. The line had increased in extent and deepness of color within the following twenty-four hours. Four days after the inoculation was made no perceptible growth had taken place over that shown within the forty-eight hour period. The mushroom was picked and two tubes of Culture V A were made.

Control inoculations showed no discoloration.

INOCULATIONS, Feb. 17th, 1912.

Inoculation was made on a young mushroom with a strain of culture I A obtained on Feb. 9th, as a result of the inoculation with culture I on Feb. 5. Infection did not take place along the entire line of inoculation but only in three or four spots. However, these spots were distinct enough to distinguish them from spots caused by natural infection. The mushroom was picked and culture I B was made.

Control inoculation showed no mark whatever.

INOCULATIONS, FEB. 20th, 1912.

A mushroom was inoculated with tube 1 from culture II, which was obtained from tissue just beneath the skin on the stem. No infection occurred.

Inoculation made from direct transfer from culture III A, obtained from the inoculation made with culture III on Feb. 13th. No infection occurred.

Inoculation was made on a mushroom ¾ of an inch in diameter with a direct transfer from culture IV A obtained from inoculation with culture IV on Feb. 13, 1912. In twenty-four hours a brown line along the path of inoculation was evident, extending clear across the cap of the mushroom. Within forty-eight hours the line was fully ¼ of an inch wide. A cross section made through the line of inoculation showed the brown discoloration extending about 4 mm. into the flesh. On the second day after inoculation the mushroom was picked and culture IV B was made.

Inoculation was made on a mushroom similar in size to the one above with culture V A. The presence of the organisms along the line of inoculation was evident the next morning, as shown by the appearance of the characteristic brown discoloration. Forty-eight hours after the inoculation the discolored area was about ¼ of an inch wide, and penetrated the tissue for about 4 mm. The mushroom was picked and culture V B was made. No evidence of discoloration could be seen on the

control mushroom.

INOCULATIONS, Feb. 26th, 1912.

Inoculation was again made with a strain of culture I obtained by plating out. No infection occurred.

A mushroom was inoculated with a strain of culture II obtained in the same manner as the strain from culture I. No infection occurred.

A mushroom was inoculated with a strain of culture III obtained as the above. No infection occurred.

Inoculation was made with a strain of culture IV obtained as the above. No infection was the result.

Inoculation was made with a strain of culture V obtained in the same manner as the above. The result of the inoculation was negative.

All of the cultures used for inoculation on this day were obtained by plating out from the original cultures. The plates were poured on Feb. 20th, and placed in the incubator at 37° C. The colonies in all cases developed rather slowly and did not show a sufficient

growth for transferring until the fourth day after incubation. Now the majority of the colonies, in fact all of them, were similar in size, shape, and general appearance, the only difference being between the surface and subsurface colonies, the former being round while the latter were lenticular in shape. When looked at with the microscope under low power both surface and subsurface colonies appeared granular in structure. Pure cultures were obtained from the plates on Feb. 24th, and also kept in the incubator at 37° C. until they were taken out for the purpose of inoculation on the 26th. A luxuriant greyish white, glistening growth was obtained in all cases. A noticeable feature in regard to the plates poured and the subsequent cultures obtained from them was the lack of the greenish pigment which in every case was present in all the original cultures and subsequent cultures made from them and incubated at ordinary room temperature, which is nearly that of the caves where the temperature ranged from 15° C. to 20° C. The characteristic odor present

in all of the cultures, however, had not disappeared.

From the results obtained with these inoculations it seems that when grown at a temperature considerably higher than that in which they thrive on the mushrooms their infecting power is entirely lost when transferred to the mushrooms at the ordinary cave temperature. This interpretation may not be the true one, as other factors may have been responsible for the negative results, but to me it seems very probable.

INOCULATIONS, Mch. 4th, 1912.

Inoculation was made on a half grown mushroom with a strain of culture II which was plated out and kept in the incubator at 37° C. The subsequent cultures obtained from plates were not kept at incubator temperature.

Result of inoculation - negative.

On the same day a mushroom was inoculated with the original culture but no infection resulted.

A mushroom was inoculated with a strain of culture III obtained in the same manner as the above. No

infection occurred.

Inoculation of another mushroom with the original culture resulted negatively.

Inoculation with a strain of culture V obtained in the same manner produced negative results. A mushroom inoculated with the original culture also produced negative results.

The same negative results were obtained with original culture and with the strain obtained by plating out.

Here again we have the same results as those obtained with the inoculations made on Feb. 6th. The original cultures were five weeks old at this time, and it seems that the organism had lost its viability.

INOCULATIONS, Mch. 9th, 1912.

Made with tube 1, third transfer of culture VII A obtained by the plating out method.

No infection occurred.

Control inoculation showed no mark whatever.

INOCULATIONS, Mch. 12th, 1912.

Made with the second transfer of culture VI and with a third transfer obtained from culture VI by plating out.

No infection occurred.

Neither of these cultures were very old, the first being six days old and the latter being but one day old. The plates were kept in the incubator at 37° C. as was the subsequent pure culture with which the inoculation was made.

Inoculation was made with a second transfer of culture VII obtained directly, and with a third transfer obtained from the same original culture by the plating out method. In both cases negative results were obtained.

Controls showed no mark.

INOCULATIONS, Mch. 18th, 1912.

A mushroom inoculated with a third transfer of culture VI produced no infection.

Inoculation was made with tube 1, second trans-

fer of culture VII. The following morning a yellowish brown line was observed along the path of inoculation. The area of discoloration did not increase very much within the next forty-eight hours but became a dark brown in color. Width of line was about ¼ of an inch, and the discoloration extended about 1/8 of an inch below the surface of the mushroom.

Another mushroom was inoculated with a direct transfer of culture VIII. No infection took place.

Still another mushroom was inoculated with a third transfer of culture VIII obtained by plating out. Brown line showed up the following morning. The discoloration was ¼ inch wide and extended into the tissue for 1/8 inch. The flesh in the immediate vicinity of the discolored area was slightly yellowish in color.

Control showed no discoloration.

Attempts were made to obtain pure cultures from these discolored areas, but in washing the mushrooms they were allowed to remain in a 1:1000 $HgCl_2$ solution for five minutes, thus killing the organisms

that were present.

INOCULATIONS, Mch. 28th, 1912.

A half grown mushroom was inoculated from tube 2 of a second transfer from culture IV B, which was obtained as a result of inoculation with culture IV A on Feb. 20th. Characteristic brown discoloration showed along the path of inoculation the following morning. Within forty-eight hours the discolored area had spread and was ¼ of an inch wide. The mushroom was picked and culture IV C was obtained.

Another mushroom was inoculated with a second transfer of culture V B obtained as the inoculation with V A on Feb. 20th. A faint brown colored line showed along the path of inoculation twenty-four hours later. In forty-eight hours the growth had increased and the color was considerably darker. The extent of brown colored portion, both in width and depth, was not as great as was the case in the inoculation produced with the culture from IV B. The specimen was picked and culture V C was made.

Another inoculation was made with tube 2, third transfer of culture VII, obtained by plating out. In this case the plates and subsequent pure cultures were incubated at ordinary room temperature and the characteristic greenish pigment diffusing through the medium was present. Twenty-four hours after the inoculation the characteristic discoloration was present in the form of a line 1/8 of an inch wide. The mushroom was picked forty-eight hours after the inoculation was made and culture VII A was made.

Inoculation was made with tube 2, third transfer of culture VIII. This culture was obtained by plating out and incubating at ordinary room temperature. The brown discoloration developed in a similar manner to the discoloration obtained by inoculation with the same culture on Mch. 18th. The mushroom was allowed to stand for two days when it was picked and culture VIII A was made. Control inoculation did not show any mark.

INOCULATIONS, Apr. 9th, 1912.

A small mushroom about ½ of an inch in diameter was inoculated with a culture obtained by plating out from tube 1 of culture IV C. Plates and subsequent cultures were incubated at room temperature. The morning following the day of inoculation the characteristic brown color of the disease had appeared. The discoloration extended across the cap of the mushroom, for the entire length of the line of inoculation, and was 1/8 of an inch wide, penetrating into the flesh 4 mm. Two days after the inoculation the specimen was picked and culture IV D was made.

Inoculation was made with a third transfer from culture V C obtained in the same manner as the above. The characteristic brown discoloration was present on the following morning. The mushroom was picked after two days and culture V D was made.

A mushroom was inoculated with tube 1, third transfer of culture VIII A obtained in the same manner as the above cultures. No infection occurred.

Control showed no infection.

INOCULATIONS, Apr. 16th, 1912.

Inoculation was made with tube 3, third transfer of culture VII A, which was obtained by plating out. Infection as evidenced by the appearance of brown discolored line along path of inoculation took place. The specimen was picked and culture VII B was made.

Another mushroom was inoculated with tube 3, third transfer of culture VIII A obtained by plating out. The characteristic markings developed in twenty-four hours. After two days the mushroom was picked and culture VIII B was made.

In both cases the plates and cultures were kept at room temperature.

The control did not show any discoloration.

DESCRIPTION of the ORGANISM.

The organism causing the spot on cultivated mushrooms is a very small rod like bacillus, rounded at both ends, appearing slightly oval in shape. The

size of the organism when stained directly from the tissue culture is very small, varying from 1.0 u. to 1.5 u. in length, and about 0.5 u. in width. When grown on beef agar and beef bouillon the size seems to increase somewhat, some organisms being observed that measured about 2.5 u. long by 1.0 u. wide.

It is easily stained by the ordinary basic anilin stains, carbol fuchsin giving the clearest result. The different stains tried were gentian violet, aqueous fuchsin, methylene blue, Loeffler's alkaline methylene blue, and Gram's method. The organism is Gram negative. Attempts were made to stain the organism in situ, in the mushroom, using Kuhn's methylene blue stain, carbol fuchsin and Flemming's triple stain, but no very satisfactory results have as yet been obtained.

Endospores - The organism does not produce spores as shown by staining and by making the heat test for spores.

Fresh bouillon cultures of the organism were

made from the various cultures used in making culture determinations. For control tubes fresh cultures of Bacillus subtilis and Bacillus coli were used.

The culture tubes were then placed in water heated to 80° C. and were kept there for twenty minutes. The cultures were then immediately cooled and placed in the incubator at 37° C. No growth was produced in any of the tubes except in that containing the culture of Bacillus subtilis.

Motility - The organism is actively motile as observed in hanging drops made from twenty-four hours broth cultures. Attempts were made to stain the flagella using Loeffler's flagella stain, and a concentrated solution of magenta red, but no successful stains have as yet been obtained.

CULTURAL CHARACTERS.

Nutrient Agar.

When a piece of diseased tissue is placed on a beef agar slant and incubated at ordinary room tem-

perature, the bacterial growth can be seen in from thirty-six hours to three days, appearing around the edges of the piece of tissue. This growth has a sort of slimy appearance greyish white in color, spreading out from and over the piece of tissue, and eventually over the entire slant. A greenish pigment is diffused through the medium and a rather strong disagreeable odor, like that of decaying matter, is always present.

On plates obtained from a tissue culture the colonies very seldom show up earlier than the third day when incubated at 20° - 25° C. The colonies attain a size of two to 5 mm. in three or four days after their first appearance. The surface colonies are round, greyish white in color, shining, slightly raised. The subsurface colonies are lenticular in shape. When seen with the microscope under low power the colonies are granular in appearance.

When transfers are made directly from a tissue culture or from a plate colony, a considerable

amount of growth takes place in twenty-four hours, which in three days will spread over the entire surface of the medium. The growth, greyish white in appearance, is always accompanied by the presence, to a greater or lesser degree, of the green pigmentation, and by the rather putrescent odor. When spread over the entire surface the growth looks greenish, due to the greenish colored mdeium.

Potato Cylinders.

When grown on sterile potato cylinders the organism develops a rather moderate growth in twenty-four hours, sort of whitish, glistening and slimy. The growth develops rapidly, covering the cylinder in five to seven days, the color of the growth turning to a creamy white or yellowish white.

Potato Agar.

An abundant greyish white growth is produced on Potato agar in forty-eight hours.

Nutrient Beef Bouillon.

Clouding begins to appear in from twenty-four to forty-eight hours. A ring or pellicle, very thick in some cases, is always formed, together with increased clouding. The disagreeable odor is present. A sediment forms in the bottom of the tube in a week.

Nutrient Gelatine.

In gelatine stabs, the growth is best at the top; liquefaction begins in forty-eight to ninety-six hours, at first saccate, gradually liquefying to the sides of the tube and finally liquefying the entire medium. Entire liquefaction takes place in three weeks or more. The gelatine tubes were incubated at a temperature of 15° C. Medium is stained light green.

Litmus Milk.

In none of the cultures tried was there any perceptible change within twenty-four hours. Separation of the whey from the casein begins in from forty-eight to seventy-two hours, the color of the whey being blue.

Digestion begins soon after and is usually complete in about fifteen days. The litmus is gradually reduced until eventually no color remains except a greenish tinge, which is characteristic of media in which the organism grows. It is a purplish green by transmitted light.

Litmus Dextrose Agar.

No acidity is produced and no gas formation. Litmus is not reduced.

Nitrate Broth.

Prepared by adding to distilled H_2O 0.1% peptone and 2% KNO_3 (nitrite free).

The test for nitrites was made after five days incubation, the tubes at the end of this time being distinctly cloudy. To 3 cc. of the culture 2 cc. of a solution of Amidonaphthalene in acetic acid and a solution of sulphanilic acid in acetic acid were added. A pink color in every case indicated the presence of nitrites.

Dunham's Solution.

The test for indol production was tried with Dunham's solution. The tests were made from seven to ten day old cultures. In all cases clouding of the medium had taken place. After adding the concentrated H_2SO_4 and the $NaNO_2$ solutions the cultures were heated for a few minutes and in every case the pinking of the medium showed the presence of indol. In some instances the tests were rather faint, but enough strong tests were obtained to conclude that the production of indol takes place.

Fermentation Tubes.

Fermentation tubes filled with muscle-sugar-free-dextrose-broth were used for the determination of gas production. No gas is produced, but a clouding of the medium took place.

Methylene Blue Nutrient Broth.

Nutrient broth tubes to which had been added a sufficient amount of Methylene blue to color the medium

a deep greenish blue were inoculated with the various organisms under observation. In from twenty-four to forty-eight hours a slight change to a lighter green was noticed, the change of color going on for two or three days after the first change was observed. The final color of the medium is a yellowish green, thus showing the power of the organism to reduce methylene blue.

CONTROL.

An efficient method of control for the spot has not yet been found. Different sprays were tried after the mushrooms had already made their appearance, but in no case save on the plot sprayed with a solution of $CuSO_4$ was there a greater percent of healthy specimens than on the control plot. The $CuSO_4$ solution used was rather strong, 40 grams $CuSO_4$ to 1 gallon of water. However, the mushrooms on the plot sprayed with this mixture were affected by the spray, a sort of smoky grey discoloration taking place. The epidermal tissues were evidently killed, for a couple of days

after the spraying was done the mushrooms presented a cracked appearance. The different solutions tried besides $CuSO_4$ were Lysol 1 cc. in one gallon of water, Benetol 1 cc. in 1 gallon of water, Sodium carbonate 60 grams in one gallon of H_2O, and Potassium carbonate 75 grams in one gallon of H_2O.

Since the organism apparently originates in the manure it seems obvious that the best method of control is to prevent the development of bacterial growth by treating the manure with some proper disinfectant. Since the curing of the manure does not destroy the organisms present in it, the proper time to apply the disinfectant would obviously be just before the beds were to be made. The kind of disinfectant best suited to the purpose has yet to be determined by experiment, for several factors have to be considered, especially the action on the manure with regard to its fertilizing value and the action of the disinfectant on the spawn with which the bed is to be sown.

CONCLUSION.

A brown spot of mushrooms is a disease causing an unsightly appearance of the specimens affected.

The discoloration rarely extends very deep into the tissues.

Work at the Minnesota Mushroom Caves has resulted in the isolation of the organism. This has been done by repeated inoculations and re-isolations.

The inoculations with the various cultures obtained produced discolorations very similar to those caused by natural infection.

The organism causing the discolorations is a small rod like bacillus with rounded ends, somewhat oval in shape.

This organism is motile as seen in hanging drops from twenty-four hour cultures in Nutrient Broth.

Attempts to stain the flagella have so far been unsuccessful.

Attempts to stain the organisms in the tissues

have not been very satisfactory.

The organism is easily grown on various culture media. That it is a reducing organism is shown by the reduction of nitrates, the reduction of litmus in litmus milk, and by the reduction of methylene blue.

When grown in Dunham's solution it produces indol.

Apparently the manure used in the mushroom beds is the origin of the organism. This has not been definitely determined, but it is the intention to continue the work along this line. It is the belief that insects are to a certain degree responsible for the spread of the disease.

No effective method of control has as yet been obtained, but experiments are to be continued in an endeavor to secure a preventive of the disease.

EXPLANATION of PLATES.

Plate I. Three mushrooms showing different stages in the progress of the disease.

Plate II. Mushrooms inoculated with pure cultures obtained from cultures III, IV and V, showing the discoloration produced.

Plate III. Mushrooms inoculated with pure cultures obtained from cultures IV A and V A.

Plate IV. Petri capsule of Beef Agar on which a number of insects caught in the caves were allowed to walk over.

Plate I

Plate II

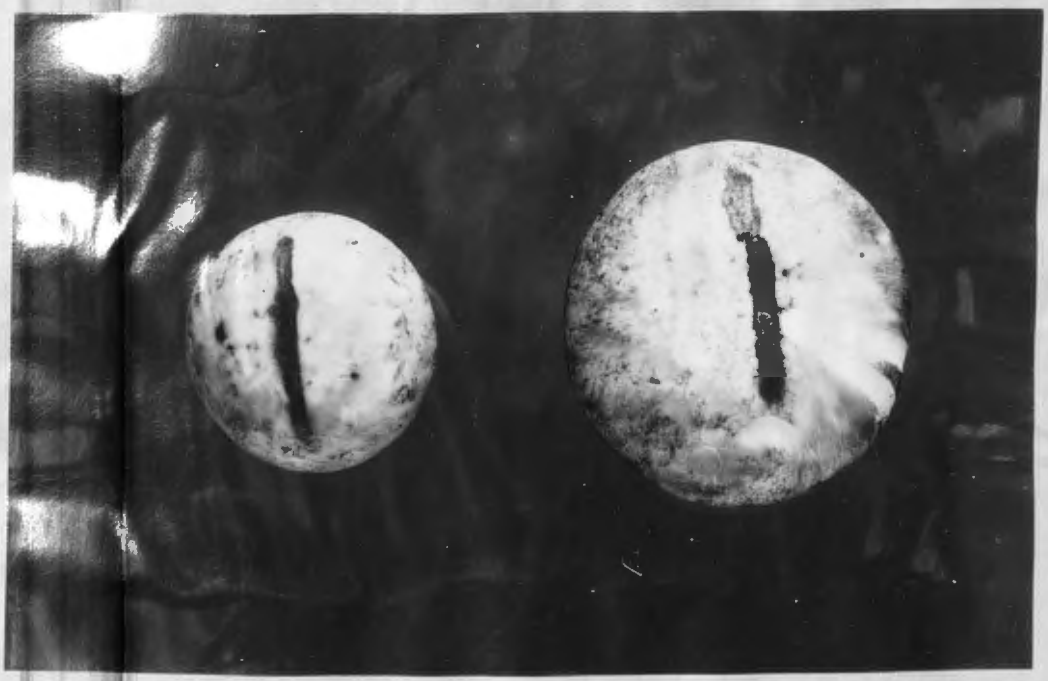

Plate III

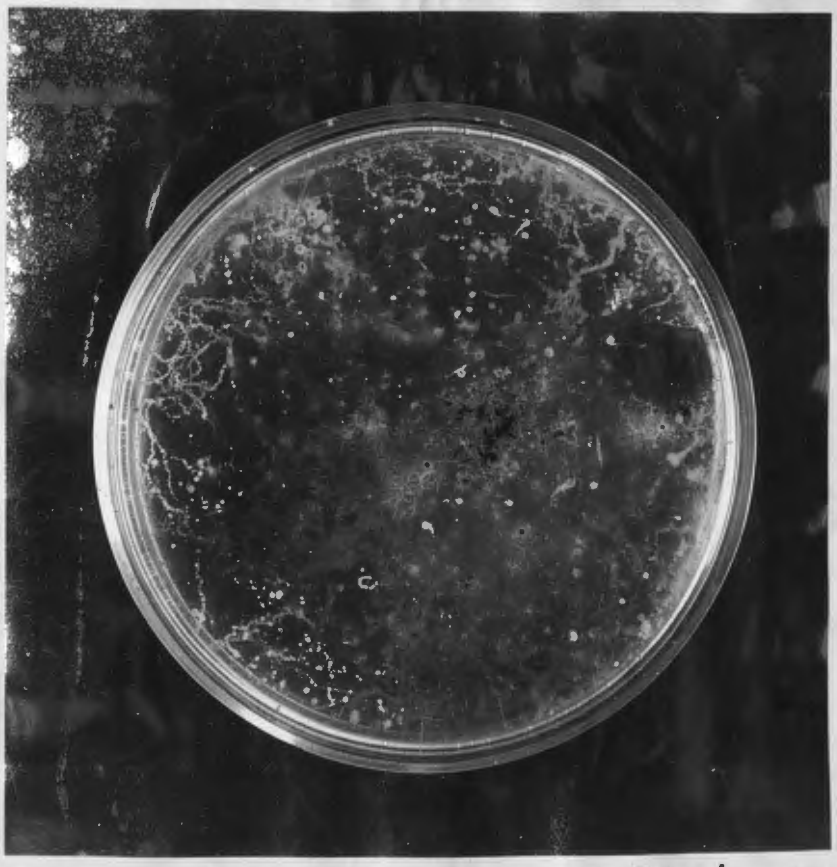

Plate IV