THE EFFECT OF DIFFERENT CARBOHYDRATES ON THE MOLD, <u>ALTERNARIA SOLANI</u> (E. AND M.) JONES AND GROUT

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is hereby approved as counting toward the completion of the Master's degree in the amount of 8 hours' credit.

Committee on thesis:

William P. Allyn

B H Smith

Paul D. Wilkinson, Chairman

Representative of English Department:

Lohan C. Smith

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CHAPTER I

THE PROBLEM AND REVIEW OF LITERATURE

I. THE PROBLEM

Statement of the problem. The purpose of this study was to observe the effect different carbohydrates have on the growth and appearance of a common mold. The mold selected was grown on a solid synthetic medium in which the carbon source was varied. The carbon compounds used were twelve carbohydrates that are available in any biological or chemical laboratory.

During the growing period of twenty days the following observations were made: (1) daily radial growth; (2) changes in appearance of the giant colonies; (3) change of pH of the medium. Photographs were taken at intervals to supplement (1) and (2).

Mold selected. The mold, *Alternaria solani* (E. and M.) Jones and Grout, which was used in this study was selected after a preliminary experiment of the growth of twelve different molds. The reasons for selecting this organism were; (1) it is a common weed-type mold; (2) the genus, *Alternaria*, contains both parasites and saprophytes; (3) it grows almost everywhere; (4) it grows well on the synthetic medium selected for this experiment; and (5) the giant colonies have a regular margin of growth on solid media.

II. THE REVIEW OF LITERATURE

Carbon metabolism. The writings in the field of nutrition of the fungi are rather extensive, but most are on mineral requirements and accessory growth factors. Steinberg[1] states that the trend of physiological investigations during the past few years seems, on the whole, to center on studies of accessory growth factors and mineral nutrition, and that little has appeared concerning the physiology of reproduction or of carbon requirements.

Tamiya, as reported by Steinberg,[2] made an elaborate study of the relation between structure and assimilability of carbon compounds, using the mold, *Aspergillus oryzae*, as the test organism. He states that some compounds can serve for respiration but not for growth, and that no constant relation exists between respiration and growth since respiration was found to vary with the carbon source. In the testing of different carbon compounds, growth on sucrose (100%) was used as a standard of comparison. The percentages of

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[1] R. A. Steinberg, "Growth of Fungi in Synthetic Nutrient Solutions," *Botanical Revues*, 5:327-350, 1939.

[2] *Ibid.*

yields and materials listed were: Less than 120% with inulin, dextrin, gluconic acid, mannose, starch, and glycogen; 90 to 100% with maltose, raffinose, fructose, and sucrose; 60 to 90% with galactose, trehalose, dioxyacetone, adonitol, culcitol, glucose, sorbitol, and quinic acid; 30 to 60% with protocatechuic acid, arabinose, saccharic acid, monoacetin, and inositol; 10 to 30% with malic acid, tartaric acid, citric acid, succinic acid, lactic acid, salicylic acid, mannitol, ethyl alcohol, and glycerol.

Tamiya, according to Steinberg,[3] reported that every compound suitable for the growth of <u>Aspergillus</u> <u>oryzae</u> contained one or more of the following fifteen groups:

CH_3 – CHOH	C- – COH	CH_3 – CO	CH_2OH – CH_2	CHOH – CH_2	CH_2OH – CHOH	CHOH – CHOH	CH_2OH – CO
CH_2OH – CO-	CHO – CHOH	COOH – CO	COOH – C-	COOH – COOH	COOH – CHO	COOH – CO-	

Mosher,[4] in a study of the nutritional requirements of the pathogenic mold, <u>Trichophyton</u> <u>interdigitale</u>, checked the following sugars: d-glucose, sucrose, d-fructose,

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[3] <u>Loc</u>. <u>cit</u>.

[4] William A. Mosher, Donald H. Saunders, Lyle B. Kingery, and Roger J. Williams, "Nutritional Requirements of the Pathogenic Mold, <u>Trichophyton</u> <u>interdigitale</u>," <u>Plant Physiology</u>, 11:795-806, 1936.

d-mannose, lactose, maltose, d-galactose, and a blank. Growth ratings showed that this mold could grow without carbohydrates if other nutrients are present. It had good growth on all of the sugars except lactose which was no better than the growth on the blank in which sugar was omitted.

Horr,[5] in his study on the utilization of galactose by Aspergillus niger and Penicillium glaucum, makes the following summary:

1. Galactose and mannose are poor sources of carbon for Aspergillus niger and Penicillium glaucum.
2. Galactose, when used alone as a source of carbon decreases the rate of spore germination and mycelium development and causes abnormal mycelium development.
3. Galactose, when mixed with dextrose, levulose, or mannose, causes an acceleration in growth. This is not caused by mannose, which is also a poor source of carbon for this organism.
4. The addition of lactose to the culture solution containing sucrose has little, if any, effect upon the growth rate.
5. Preliminary correlations on the amount of dry matter formed and acidity developed do not indicate that acidity of the solution plays any important part in the acceleration of growth.
6. The data hold for the strains of Aspergillus niger and Penicillium glaucum used: other organisms or other strains of these organisms may react differently.

Bannon[6] states that microrganisms are able to use

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[5] W. H. Horr, "Utilization of Galactose by Aspergillus niger and Penicillium glaucum," Plant Physiology, 11:81-99, 1936.

[6] J. M. Bannon, "Influence of Glucose and Fructose on the Growth of Fungi," Botanical Gazette, 76:257-273, 1923.

disaccharides, hexoses, pentoses, alcohols, organic acids, and other organic compounds, but intermediary metabolism remains a mystery.

Accessory growth factors. In a study of this kind on carbon usage, another factor must be considered, that of mineral requirements of the organism being studied. To have more accurate control on the amount of growth on the different carbohydrates, it was deemed advisable to grow the organism in a synthetic medium, if possible, in which the nitrogen is supplied by an inorganic salt. Leonian's work on nutrition of fungi makes this an important factor in selecting an organism for study. Leonian[7] states:

> Fungi other than obligate parasites may be divided into two groups: those that manufacture their own auxithals (growth-promoting substances) and those that must secure these from extraneous sources. The first group grows readily on media composed of the essential inorganic salts and of dextrose, while the second group fails to grow on such media, regardless of time or environment, but must have, in addition, other substances.

Nitrogen requirements. Organisms, according to Robbins,[8] can be classified on the basis of their nitrogen requirements. He established these four groups on their

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[7] Leon H. Leonian, and Virgil G. Lilly, "Studies on the Nutrition of Funge: I. Thiamin, its Constituents and Source of Nitrogen," *Phytopathology*, 28:531-548, 1948.

[8] W. J. Robbins, "The Assimilation by Plants of Various Forms of Nitrogen," *American Journal of Botany*, 24:243-250, 1937.

ability to utilize nitrogen in different chemical forms:

Group I	Atmospheric N,	No_3, NH_4, or organic N.
Group II		NO_3, NH_4, or organic N.
Group III		NH_4, or organic N.
Group IV		(only) organic N.

For the work in this experiment, the mold Alternaria solani (E. and M.) Jones and Grout, was used. This mold has the ability to grow well in a medium containing a nitrate or an ammonium salt.

Trace elements. Trace elements are needed for growth in addition to the common elements used in any basic media. These are supplied by the normal contaminants found in most C. P. reagents and sugars used in making biological media. Wolf[9] summarized the results of Steinberg in which, by spectoscopic methods, he identified the following contaminants in C. P. reagents. The results are shown by the following table:

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[9] Frederick A. Wolf and Frederick T. Wolf, "The Fungi" Vol. II, pp. 11. John Wiley and Sons, New York, 1947.

Table 4

Elements shown to be present by the use of Spectroscopic Methods of Analysis.

Chemical Reagents	Contaminants
NH_4NO_3	Ca, K, Mg, Na
K_2HPO_4	Al, Ag, Cu, Mg, Na, Pb
$MgSO_4 \cdot 7\ H_2O$	Cu, Na
$ZnSO_4 \cdot 7\ H_2O$	As, B(?), Cu, Fe, Mg, Mn, Si, Sn (?)
$CuSO_4 \cdot 5\ H_2O$	Cu, Fe, Mg, Mn, Pb, Si
$MnSO_4 \cdot 2\ H_2O$	Al, Ca, Cu, Cr, Fe, Mg, Na, Si, V
Na_2MoO_4	Al, Ca, Cu, Fe, K, Li, Mg, Na, Ni, Si, V(?)
Dextrose	Al, Ag, B, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Rb, Rh, Si, Sn, Sr

For this experiment the mold Alternaria solani (E. and M.) Jones and Group, that grows well in a synthetic medium was used. This factor eliminates the need of accessory growth factors or the effect that the carbon in the organic nitrogen compound would have on growth.

CHAPTER II

THE EXPERIMENT

Before starting the experiment on growth with different carbohydrates, a preliminary test was set up to check different species of molds on the synthetic medium to be used in the final experiment. The medium used was a modification of one used in a course on Comparative Metabolism of the Fungi at the University of Chicago.[1]

Basic medium used at the University of Chicago:

Glucose	2%
Peptone	0.2%
(PO_4) mixture	to M/50*
$MgSO_4 \cdot 7H_2O$	0.05%
$FeCl_3$	trace
Agar, washed Difco	2%

*Equimolar solutions of KH_2PO_4 and K_2HPO_4 in Pyrex-distilled water in the ratio of 3.7/6.3 gives a pH value of 7.0.

I. PRELIMINARY TESTS

Media used. The basic medium used in all of the following experiments is a modification of the above medium.

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[1] J. R. Raper, Laboratory Outline for Botany 423, Chicago University, Spring 1949.

It was made up as follows:

Carbohydrate	20	grams
$NaNO_3$ or NH_4NO_3	2	grams
KH_2PO_4	1.0	grams
K_2HPO_4	1.0	grams
$MgSO_4 \cdot 7H_2O$	0.5	grams
$FeCl_3$	0.01	grams
Agar, washed Difco	20	grams
Water, distilled	1	liter

This medium after sterilization has a pH of 6.0.

For the preliminary work the solution was made without the nitrate salts and divided into two equal portions. To one portion 1 gram of $NaNO_3$ was added and to the other portion 1 gram of NH_4NO_3 was added. Dextrose was used as the carbon source.

Organisms tested. After sterilization, petri plates were poured with each type of the nitrate media and inoculated with the following organisms: *Fusarium culmorum*, *Fusarium moneliforme*, *Verticillium albo-atrum*, *Helminthosporium carbonum*, *Helminthosporium turcicum*, *Zycorynchus moelleri*, *Botrytis cineri*, *Alternaria solani*, *Macrosporium solani*, *Cephalothecium roseum*, *Helminthosporium maydis*, and *Monascus purpureus*.

Organism selected. The plates were incubated for two weeks at room temperature and, based on observations during this period, the mold, Alternaria solani (E. and M.) Jones and Grout, was selected to be used in study on the different carbohydrates. The reasons for using this mold were: (1) It is a common weed-type mold: (2) the genus contains both parasites and saprophytes; (3) it grows almost everywhere; (4) it has a regular margin of growth on solid media, making it possible to check its average radial growth increment per unit of time as an index of carbon availability; (5) it grows well in the synthetic medium selected for this experiment; and (6) nothing of this type experiment on this mold was found in the review of literature.

Smith[2] gives the following description of the genus:

> Alternaria: (Dem) Some species are of common occurence on numerous kinds of organic material under damp conditions, whilst others parasitic on clutivated plants.
>
> In culture on rich media most forms spread rapidly, with densely floccuse, dirty green mycelium. On poor media growth is less floccuse and darker in colour. The mycelium is very richly septate and often forms chains of short, swollen cells not unlike oikiospores. The conidia vary from roughly ovate to inverted club-shaped, with a more or less pronounced beak at the apex. They are produced in short chains, often branched, are greenish brown to dark brown in colour and have both cross and longitudinal septa, the degree of septation increasing with age. On rich culture media spore production is

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[2] George Smith, An Introduction to Industrial Mycology, (London: Edward Arnold and Co. Ltd., 1946), pp. 89-90.

usually sparse, but the individual spores are large and elaborately septate.

II. EXPERIMENT ON DIFFERENT CARBOHYDRATES

<u>Medium and carbohydrates used</u>. The stock solution of salts, which was used for the main experiment, was made double strength so that when the solutions of carbohydrates were added, the medium was in the same proportion as the medium used in the preliminary experiment.

Basic solution:

$NaNO_3$	4.0	grams
KH_2PO_4	2.0	grams
K_2HPO_4	2.0	grams
$MgSO_4.7H_2O$	1.0	grams
$FeCl_3$	0.02	grams
Water (distilled)	1	liter

The carbohydrate solutions were made as follows: Twelve flasks were used. To the flasks containing seventy-five milliliters of distilled water and three grams agar (difco), three grams of one of the following were added: d-xylose, dextrose, d-mannose, d-galactose, d-(-)-levulose, sucrose, lactose, maltose, starch (soluble), dextrin, and inulin. The twelfth was left as a blank (agar only). After sterilization, an equal amount of the salt solution was added to an equal amount of each carbohydrate mixture. A pH reading of each mixture was taken at this time.

Inoculation and incubation. Four petri dishes were used for each of the twelve different carbohydrate solutions. After setting for two days to check for possible contamination, the plates of media were inoculated by taking a portion of mycelia from the culture, *Alternaria solani* (E. and M.) Jones and Grout, growing on the medium containing $NaNO_3$, of the preliminary experiment. These portions, about one millimeter in diameter, were placed in the center of each plate. Incubation was at room temperature for a period of twenty days. The temperatures were fairly uniform as shown in Table I.

Data collected. All cultures showed signs of growth after twenty-four hours except those on plates containing agar only. Average measurements of radial growth were taken from the second day through the fifteenth day, and final measurements were taken on the twentieth day.

The different carbohydrate media were checked for pH at the end of the fifteenth and twentieth days of growth.

Changes and differences in appearance of *Alternaria solani* (E. and M.) Jones and Grout, were recorded by photographing a typical colony from each group on the fourth, fifth, seventh, ninth, tenth, fifteenth, and twentieth days. Color changes were recorded to accompany each set of photographs.

TABLE I

TEMPERATURE IN DEGREES FAHRENHEIT
DURING THE EXPERIMENT

Date	Maximum	Minimum	Average
June 25, 1949	90	72	81
June 26, 1949	89	70	80
June 27, 1949	88	69	78
June 28, 1949	93	70	82
June 29, 1949	89	70	80
June 30, 1949	95	71	83
July 1, 1949	96	72	84
July 2, 1949	95	74	84
July 3, 1949	93	74	84
July 4, 1949	94	72	83
July 5, 1949	92	76	84
July 6, 1949	89	74	82
July 7, 1949	88	72	80
July 8, 1949	92	73	82
July 9, 1949	88	74	81
July 10, 1949	89	66	78
July 11, 1949	87	60	74
July 12, 1949	90	61	76
July 13, 1949	90	66	78
July 14, 1949	91	67	79
July 15, 1949	89	68	78

These temperatures were obtained from the U. S. Department of Commerce Weather Bureau Monthly Climatological Summary, Hulman Field, Terre Haute, Indiana, June and July, 1949.

CHAPTER III

OBSERVATIONS, SUMMARY, AND CONCLUSIONS

I. OBSERVATIONS

<u>Change in pH</u>. During growth of the mold, the pH value of the media became alkaline except that medium containing agar only which showed no variation at all. Johnson[1] says that the tendency of molds is to change the reaction of the media toward neutrality or beyond. He also states that a greater growth tends to bring about a greater change in pH. In this experiment, the colonies on the medium containing inulin had a large radial growth by the fifteenth day, but was not as heavy or compact. At this time this medium showed less change in pH than the other media (except agar). By the twentieth day the pH of all the media (except agar) was approximately the same, and the growth on the medium containing inulin equaled the other colonies. (See Table II).

Johnson[2] explains the chemical change as follows:

> The reasons, or rather the mechanisms, of this change are not explained. It is accepted that the carbohydrates may be converted into acids and thus cause an acid reaction.

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[1] Harlan W. Johnson, "Relationships Between Hydrogen Ion, Hydroxyl Ion and Salt Concentrations and the Growth of Seven Soil Molds," Research Bulletin No. 76, January, 1923, Ames, Iowa.

[2] *Loc. cit.*

TABLE II

CHANGE IN pH OF THE MEDIA DURING THE EXPERIMENT

Carbohydrate	pH at start	pH after 15 days	pH after 20 days
Xylose	5.9	7.8	8.0
Dextrose	6.0	7.9	8.0
Mannose	6.2	8.0	8.0
Galactose	6.1	8.0	8.0
Levulose	5.9	7.9	8.0
Sucrose	6.0	7.9	8.0
Lactose	6.0	8.0	8.0
Maltose	5.9	7.8	8.0
Starch	6.0	7.9	8.0
Dextrin	6.0	8.0	8.0
Inulin	5.9	7.4	8.0
Blank (agar)	6.0	6.0	6.0

Indicators used were Phenol red, Chlorophenal red, and Bogens Universal Indicator.

The use of the basic ions in such salts as KCl and K_2HPO_4 may cause some more acidity and the taking up of the acid radicles of the $NaNo_3$, $MgSO_4$ and K_2HPO_4 may give rise to alkaline reactions as pointed out by Hoagland (6, (2)). That the use of the nitrogen from $NaNO_3$ may cause alkalinity was demonstrated in a series by the author wherein the reaction of solutions originally containing 2 gms. and 4 gms. of $NaNO_3$ per liter were determined after Mucor glomerula had grown one week in them. At the start the reaction of both solutions was pH = 4.18. At the end the solution with 2 gms. gave a pH of 6.46 and the one with 4 gms. pH 6.85. It is evident that this can explain some of the alkalinity produced. It is difficult to explain, however, why an acid solution is changed toward alkaline while an alkaline solution is decreased in alkalinity.

Radial growth. All cultures showed growth at the end of twenty-four hours except agar. The cultures using agar as the only carbon source can be eliminated in further discussions since growth was so meager. By holding the plates up to the light, very faint growth could be observed. The mycelia spread out, but were scant, thin, and colorless. With growth on agar so meager, the influence of agar in the basic media with the other carbohydrate sources was regarded as megligible.

All cultures showed steady growth increases throughout the experiment as shown in Table III. The greatest growth took place during the first five days and the least during the last five days of the experiment as shown in Figure 1. Although this figure shows a good radial growth on inulin, the growth of the mycelial mat was thinner than the other cultures until it approached its peak of radial growth.

TABLE III

AVERAGE RADIAL GROWTH IN MILLIMETERS OF ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT ON DIFFERENT CARBOHYDRATE SOURCES

	Day of reading														
Carbohydrate	2	3	4	5	6	7	8	9	10	11	12	13	14	15	20
Xylose	4.6	7.5	10.6	14.5	17.3	20.0	22.5	25.1	26.9	28.8	30.1	31.8	32.5	36.0	38.9
Dextrose	8.3	11.5	15.8	19.0	21.5	24.4	27.0	29.4	30.9	32.6	34.0	35.8	37.0	38.5	41.8
Mannose	7.5	10.6	14.3	18.0	20.3	23.0	25.5	28.4	30.1	32.5	33.5	36.0	38.8	41.5	43.0
Galactose	7.5	10.3	14.4	18.0	20.5	23.4	25.8	27.8	29.1	31.4	32.9	34.1	35.4	36.3	37.5
Levulose	7.2	10.0	13.5	16.9	18.8	22.3	25.0	27.4	29.0	30.9	32.5	33.6	35.8	36.5	38.9
Sucrose	9.5	12.5	16.6	19.9	22.3	24.9	27.3	30.0	31.5	34.0	35.6	35.9	36.1	37.0	40.0
Lactose	8.4	11.0	14.5	19.1	22.3	25.0	27.9	30.5	31.4	34.0	34.9	36.1	37.1	37.9	40.7
Maltose	8.9	12.4	15.5	19.5	22.3	25.5	28.5	31.5	33.0	34.9	36.8	38.5	40.3	42.1	43.1
Starch	8.5	12.7	16.5	20.5	24.1	27.0	30.0	32.1	33.8	35.6	36.9	38.9	40.6	42.3	45.0
Dextrin	8.3	12.3	15.9	20.7	24.1	27.1	30.5	33.1	34.8	36.6	37.9	39.9	41.1	43.5	45.0
Inulin	8.7	13.5	15.5	20.5	25.0	29.0	33.0	35.3	36.8	37.3	37.8	38.5	39.3	39.3	42.0
Blank (agar)*	.0	10.0	15.0	20.0	22.5	29.0	31.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0

*The growth on agar was very scant, only one layer of colorless mycelia.

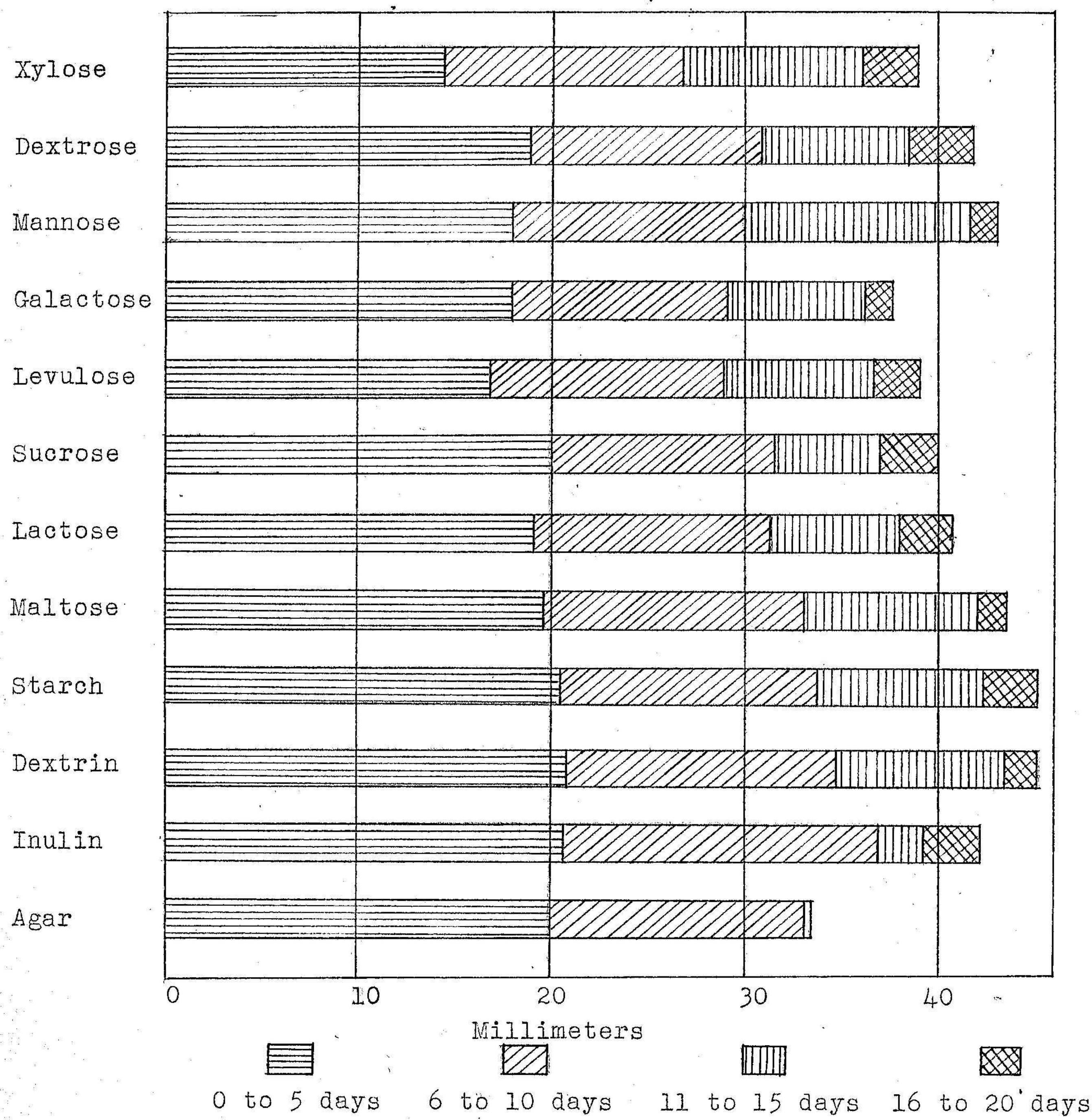

Figure 1

AVERAGE RADIAL GROWTH OF <u>ALTERNARIA SOLANI</u> (E. AND M.) JONES AND GROUT IN MILLIMETERS ON EACH TYPE OF CARBOHYDRATE FOR FIVE-DAY PERIODS

The variations in growth on both the different classes of carbohydrates and the compounds within the classes was slight as shown in Table IV.

Pictorial record. A pictorial record of appearance and growth, as shown in Figures 2-9 inclusive, was obtained by photographs taken at intervals when marked changes in appearances were visible. Ridgeway's[3] color charts were used as a standard for color in the accompanying descriptions. These photographs are enlarged to approximately one-half natural size.

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[3] Robert Ridgeway, "Color Standards and Color Nomenclature," published by the author, Washington, D. C., 1912.

TABLE IV

RANK IN GROWTH AT END OF EACH FIVE DAYS PERIOD

	Period			
Carbohydrate	5th day	10th day	15th day	20th day
Pentose				
Xylose	11	11	11	10
Hexoses				
Dextrose	7	7	6	6
Mannose	8 & 9	8	4	4
Galactose	8 & 9	9	10	11
Levulose	10	10	9	9
Disaccharides				
Sucrose	4	5	8	8
Lactose	6	6	7	7
Maltose	5	4	3	3
Polysaccharides				
Starch	2 & 3	3	2	1 & 2
Dextrin	1	2	1	1 & 2
Inulin	2 & 3	1	5	5
Agar	12	12	12	12

Description of the colonies of Alternaria solani (E. and M.) Jones and Grout after four days growth on the different carbohydrate media. Photographs are on opposite page (Figure 2). Read left to right in each row.

Row 1

Xylose: Center dark olive green, margin white
Dextrose: Center light lavender, then a darker ring, margin a dirty white
Mannose: Black center shading into brown, margin white

Row 2

Galactose: Dark center then pale tan fading out to a white margin
Levulose: Center a raised gray brown mat, narrow white margin
Sucrose: Ring pattern of light and dark shades of gray brown, margin dirty white

Row 3

Lactose: Dark to light olive green, margin light gray
Maltose: Brown mat, white margin
Starch: Dark brown fading to gray brown, margin white

Row 4

Dextrin: Dark greenish brown, wide white margin
Inulin: Thin spreading colony, gray brown at center fading out to a dirty white margin
Agar: Very scant growth, colorless

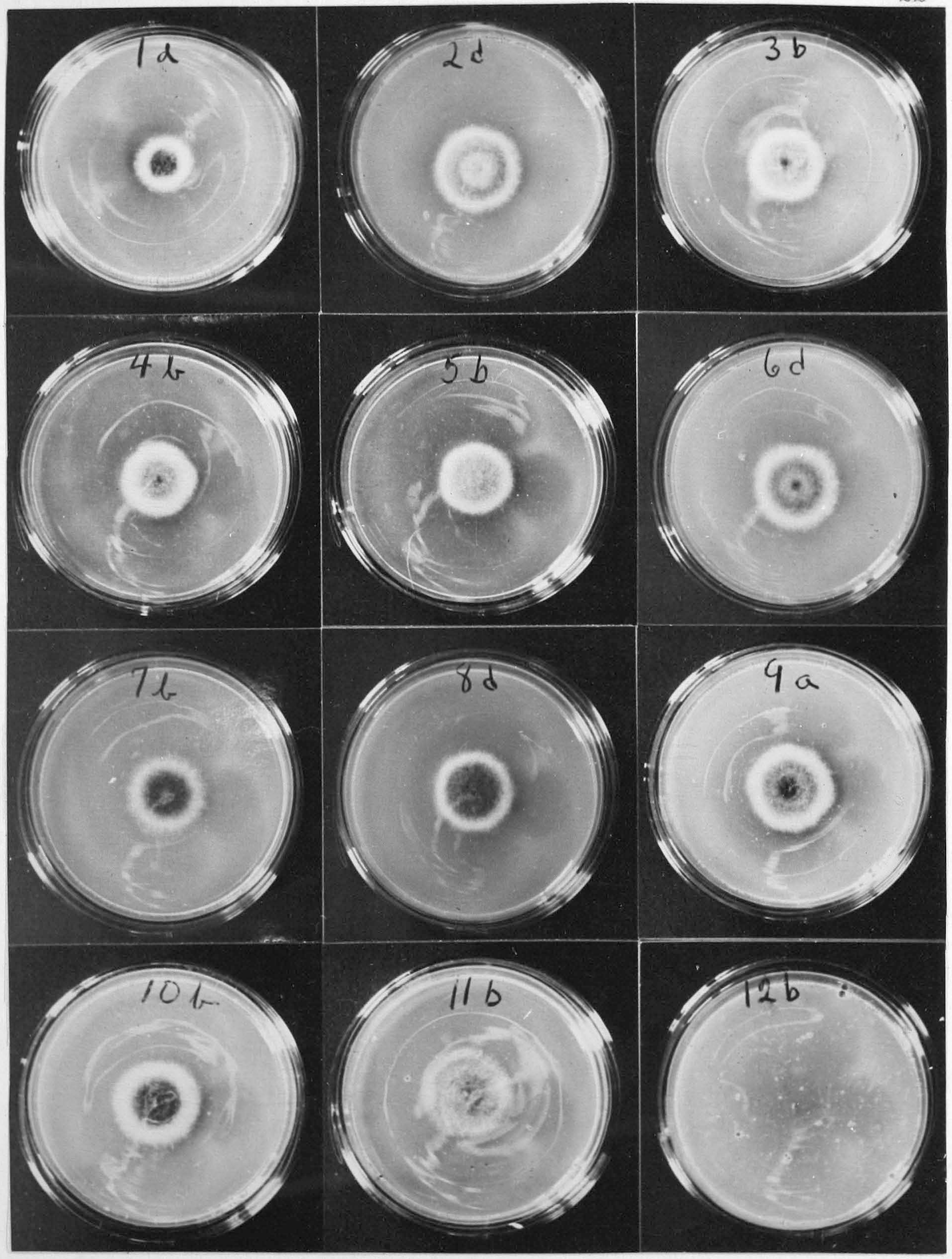

FIGURE 2

PLATE I

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.

FOUR-DAY OLD COLONIES. ($x\frac{1}{2}$)

Description of the colonies of <u>Alternaria</u> <u>solani</u> (E. and M.) Jones and Grout after five days growth on the different carbohydrate media. Photographs are on opposite page, (Figure 3). Read left to right in each row.

<u>Row 1</u>

Xylose:	Center olivaceous black, margin white
Dextrose:	Center pale purplish gray, then grayish olive, margin white
Mannose:	Dark center, then purplish gray, margin white

<u>Row 2</u>

Galactose:	Center gray brown, then mouse gray, margin white
Levulose:	Raised purplish gray mat, margin white
Sucrose:	Rings of gray browns, outermost ring an olive gray, margin white

<u>Row 3</u>

Lactose:	Neutral gray, margin light gray
Maltose:	Purplish gray fading to a slate olive, margin white
Starch:	Rings of purplish gray and gray green, margin white

<u>Row 4</u>

Dextrin:	Drab greenish olive fading to a light gray, margin white
Inulin:	Dark to light olive brown, margin gray, growth thinner than in the above colonies
Agar:	Very scanty colorless mycelia

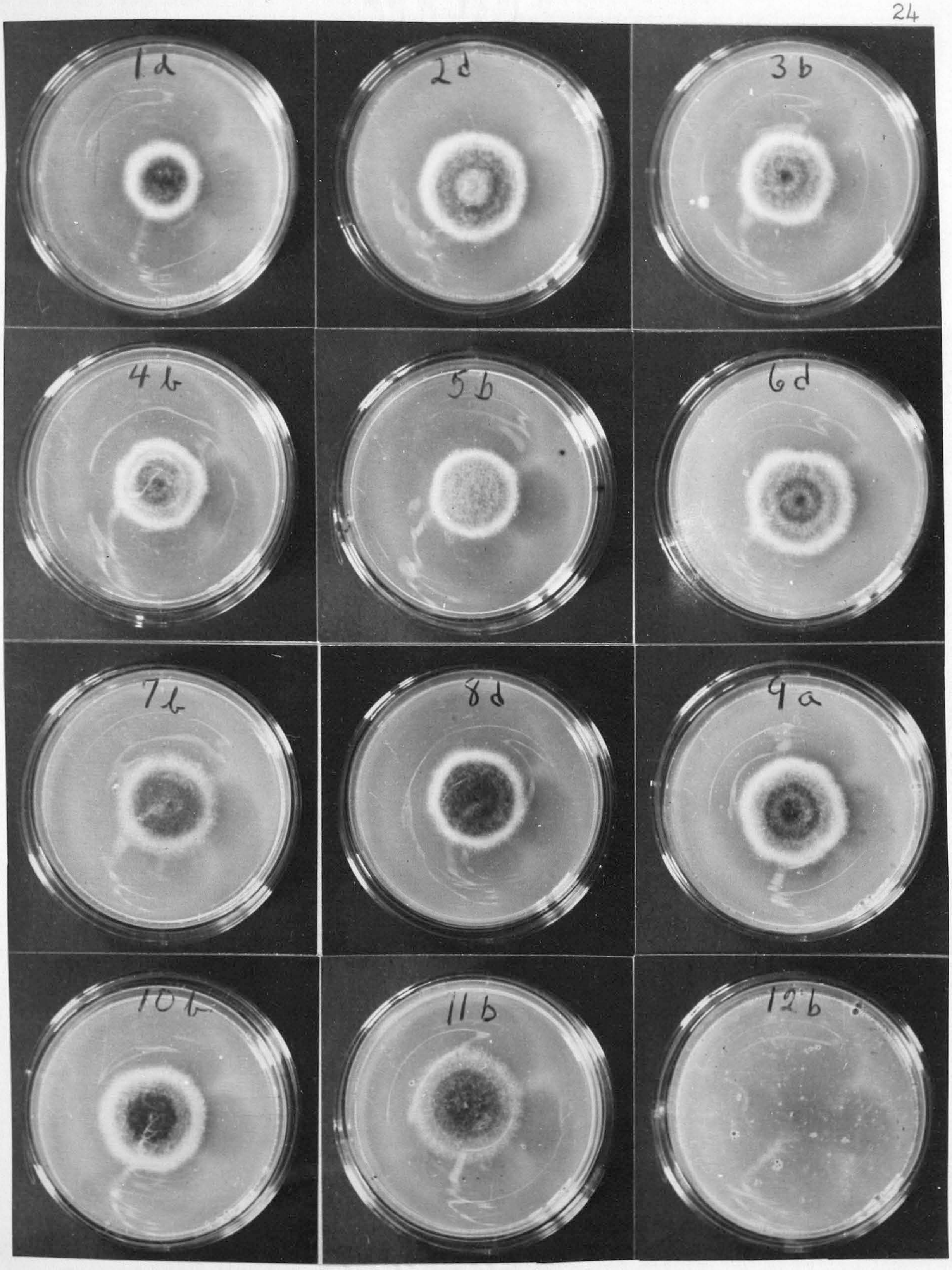

FIGURE 3

PLATE II

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.
FIVE-DAY OLD COLONIES. (x½)

Description of the colonies of <u>Alternaria solani</u> (E. and M.) Jones and Grout after seven days growth on the different carbohydrate media. Photographs are on opposite page (Figure 4). Read left to right in each row.

<u>Row 1</u>

Xylose:	Center olivaceous black, then greenish olive getting darker at outermost edge of mat, margin white
Dextrose:	Center pale purplish gray, then deep olive gray, margin light gray
Mannose:	Dark center, then pale purplish gray changing to deep olive gray, margin light gray

<u>Row 2.</u>

Galactose:	Center gray brown, then mouse gray to a gray with a green under tone, margin white
Levulose:	Center purplish gray, then greenish olive, margin white
Sucrose:	Concentric rings of dark and light shades of gray brown, margin white

<u>Row 3</u>

Lactose:	Neutral gray shading into an andover green, margin white
Maltose:	Purplish gray center, then slate olive, margin white
Starch:	Purplish gray fading to shades of gray greens, margin white

<u>Row 4</u>

Dextrin:	Drab greenish olive fading out to a margin of light gray
Inulin:	Dark to light olive brown, margin gray, Mat thinner than the other colonies
Agar:	Very scant colorless mycelia

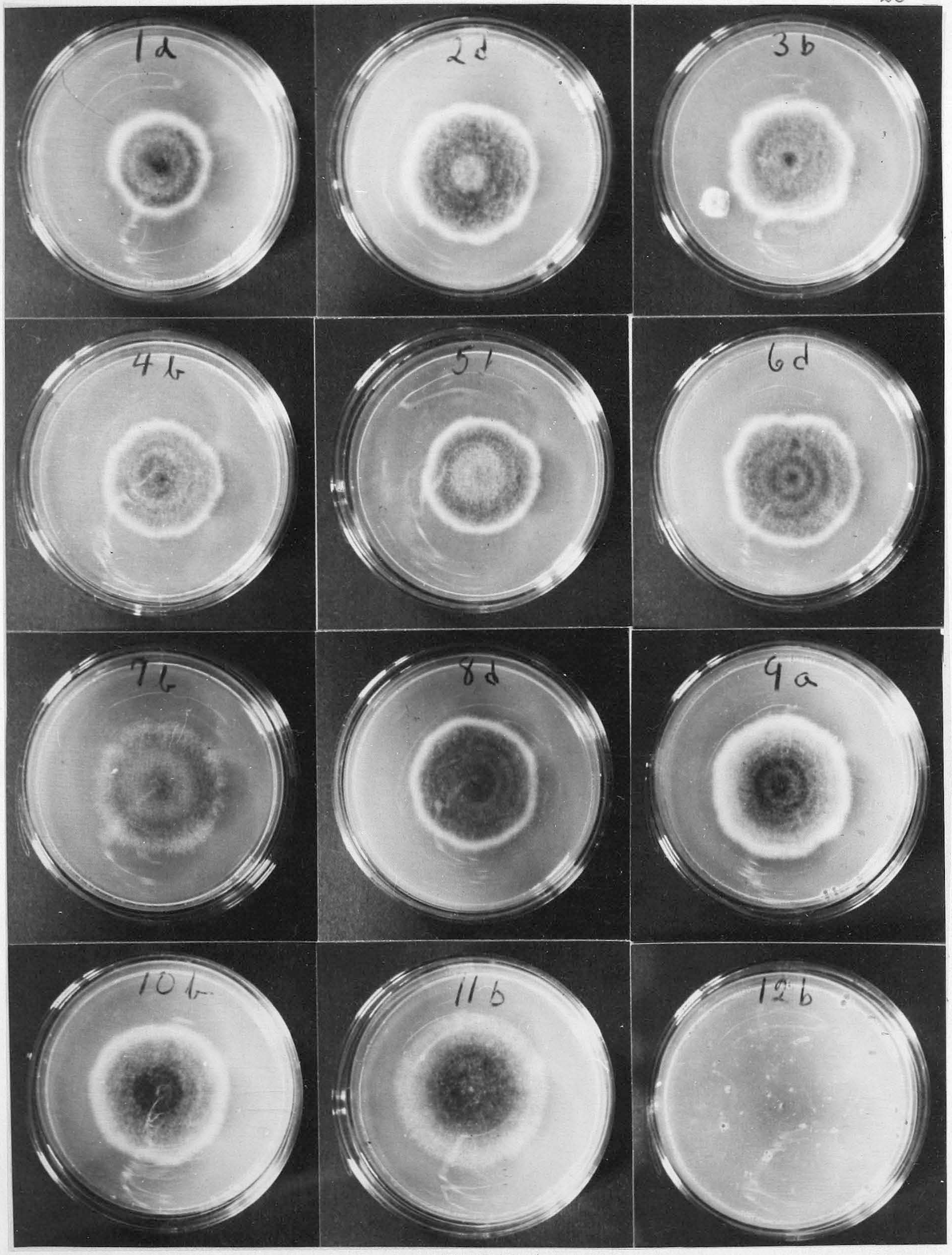

FIGURE 4

PLATE III

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.
SEVEN-DAY OLD COLONIES. (x½)

Description of the colonies of Alternaria solani (E. and M.) Jones and Grout after nine days growth on the different carbohydrate media. Photographs are on opposite page (Figure 5). Read left to right in each row.

Row 1

Xylose: Center dark gray brown with a purplish overcast, then taupe to olive gray, margin white

Dextrose: Light purplish in center then darker with a light purplish overcase next to a margin of light gray

Mannose: Center brownish, then taupe to a light gray margin. Contaminant changing the shape of colony in plate 3b

Row 2

Galactose: Center brown with a lavender overcast, then dark brown shading into tan, margin a light gray.

Levulose: Rings of light and dark taupe, margin light gray.

Sucrose: Alternate shades of dark and light taupe, margin gray

Row 3

Lactose: Dark purplish gray to light purplish gray, ring of growth next to margin gray tan, margin gray

Maltose: Concentric rings light to dark gray browns, margin light gray

Starch: Dark purplish gray gradually fading out to a margin of light gray

Row 4

Dextrin: Gradual shading from dark brown to a light tan, margin light gray

Inulin: Deep olive brown shading toolive brown, wide gray margin. Thin spreading colony

Mannose: Colony growing on mannose that is not contaminated, colors same as 3b

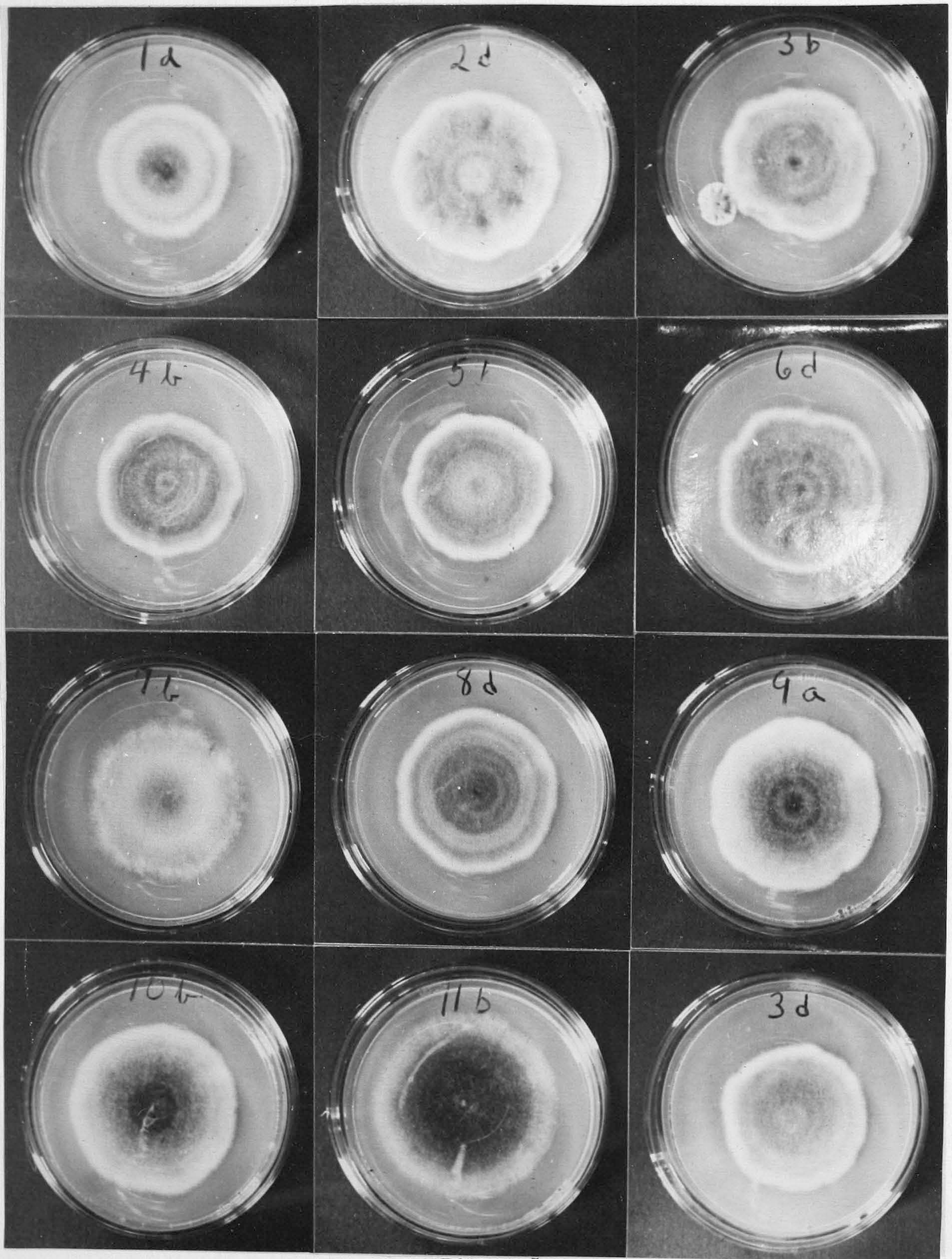

FIGURE 5

PLATE IV

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.
NINE-DAY OLD COLONIES. (X½)

Description of the colonies of Alternaria solani (E. and M.) Jones and Grout after ten days growth on the different carbohydrate media. Photographs are on opposite page (Figure 6). Read left to right in each row.

Row 1

Xylose: Dark brown center slightly sunken, then light to dark purplish gray, margin a dirty white
Dextrose: Rings light and dark shades of purplish gray, margin light gray
Mannose: Dark to light purplish gray, margin light gray

Row 2

Galactose: Shades of brown with a purplish gray overcast, margin light gray
Levulose: Rings of light and dark taupe, margin light gray
Sucrose: Concentric rings of shades of purplish gray with the last ring olive green fading into a gray margin

Row 3

Lactose: Dark to light purplish gray, margin light gray
Maltose: Rings of light and dark taupe, margin a light gray
Starch: Dark to light purplish gray, dark gray ring next to margin of light gray

Row 4

Dextrin: Shading of dark brown to light tan, margin gray
Inulin: Brown with a purplish gray overcast, margin gray
Mannose: Colony 3a developed a yellow growth on one edge, first noticed on the seventh day. By the tenth day when this picture was taken one half of the colony was covered with a loose raised growth, tawny olive to cinnomon buff in color

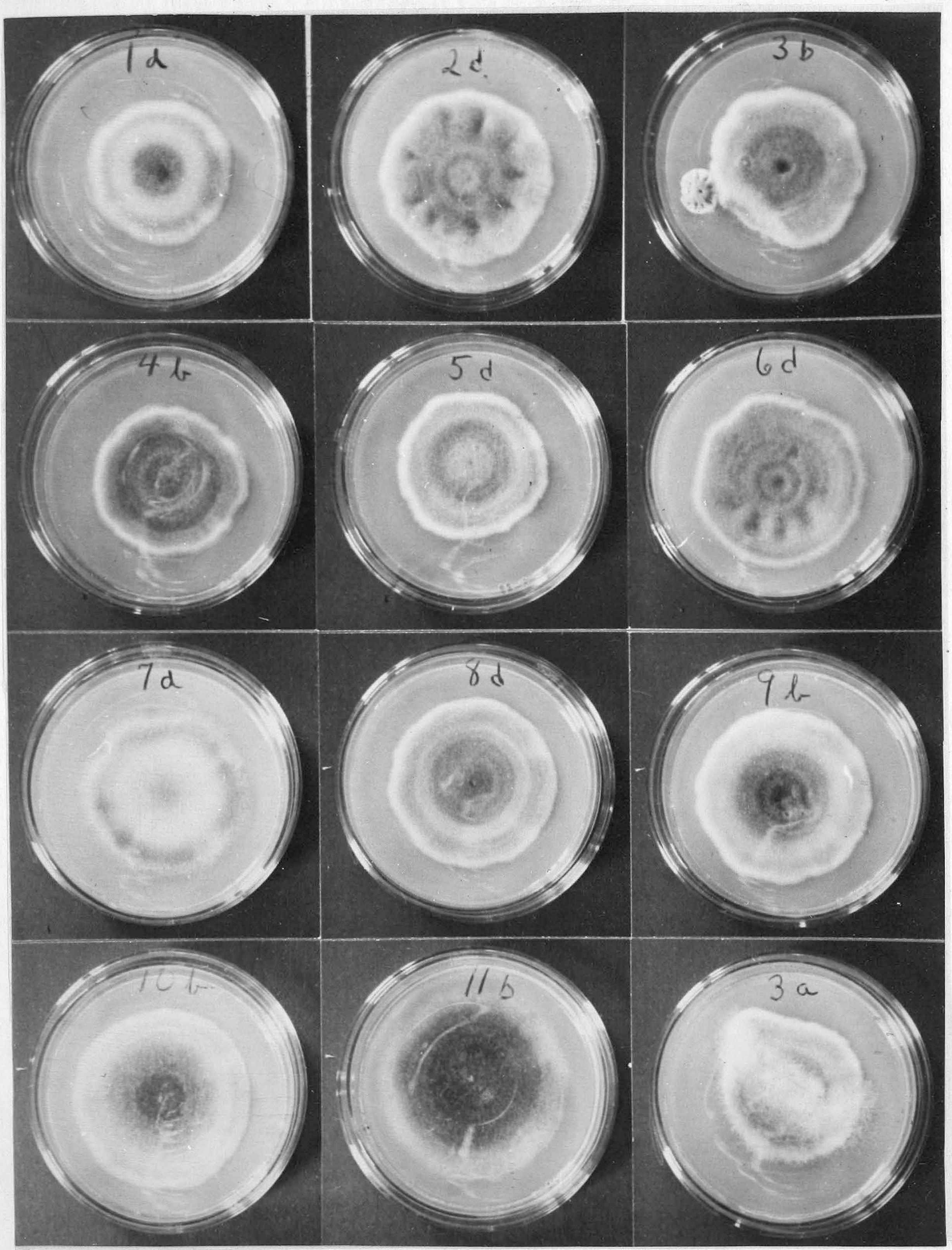

FIGURE 6

PLATE V

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.
TEN-DAY OLD COLONIES ($x\frac{1}{2}$)

Description of the colonies of <u>Alternaria solani</u> (E. and M.) Jones and Grout after fifteen days growth on the different carbohydrate media. Photographs are on opposite page (Figure 7). Read left to right in each row.

<u>Row 1</u>

Xylose: Dark sunken center of olivaceous black then mouse gray, pale mouse gray, light grayish olive, margin white
Dextrose: Brownish drab to light brownish drab, margin dark grayish olive
Mannose: Center mouse gray, then drab gray, mouse gray, margin light gray.

<u>Row 2</u>

Galactose: Center of colony fuscous, shading into hair brown, margin light gray
Levulose: Purplish gray tones, dark ring, olivaceous black, margin light gray
Sucrose: Purplish gray, outer ring olivaceous black

<u>Row 3</u>

Lactose: Center and outer ring deep grayish olive, balance of colony a pale drab gray
Maltose: Shades of hair brown and benzo brown, margin a dark grayish olive
Starch: Rings of mouse gray, pale mouse gray, light cinnamon drab, and a grayish olive margin

<u>Row 4</u>

Dextrin: Chaetura drab, hair brown, and ecru drab
Inulin: Dark brown with a mouse gray overtone; mat heavier than during earlier period of growth
Agar: Very scant colorless mycelia

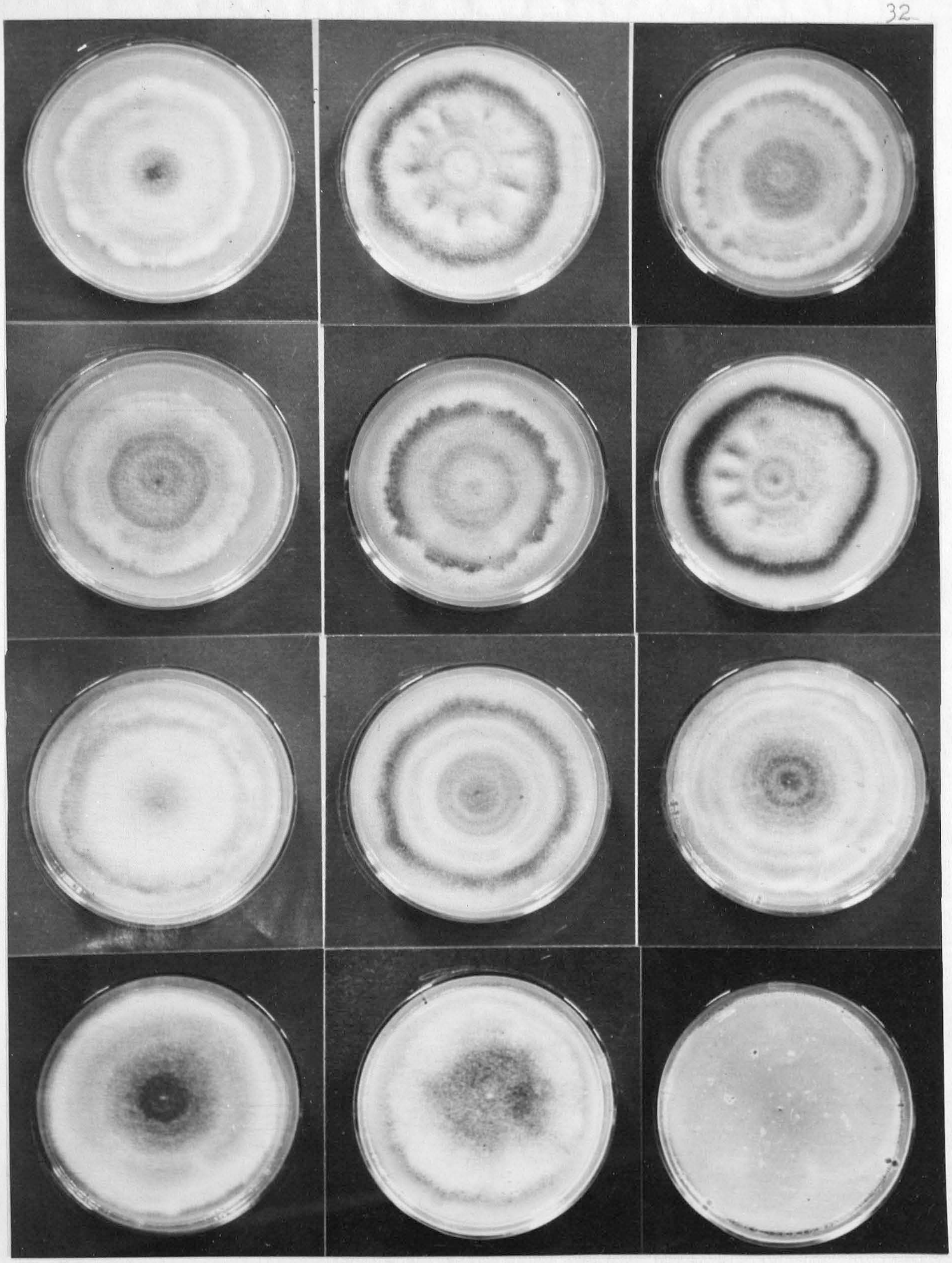

FIGURE 7

PLATE VI
ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.
FIFTEEN-DAY OLD COLONIES ($x\frac{1}{2}$)

Description of the reverse side of the colonies of Alternaria solani (E. and M.) Jones and Grout after fifteen days growth on the different carbohydrate media. Photographs are on opposite page (Figure 8). Read left to right in each row.

Row 1

Xylose: Fuscous to light drab
Dextrose: Bluish slate black, drab, and chaetura black
Mannose: Drab and bluish slate black

Row 2

Galactose: Drab, fuscous, and drab
Levulose: #4 slate to chaetura black
Sucrose: Drab, #4 slate, and black

Row 3

Lactose: #5 slate gray to #4 slate
Maltose: #5 slate gray, light drab, and #4 slate
Sucrose: Hair brown to #3 blackish slate

Row 4

Dextrin: Hair brown to blackish slate
Inulin: Black to fuscous black
Agar: Colorless

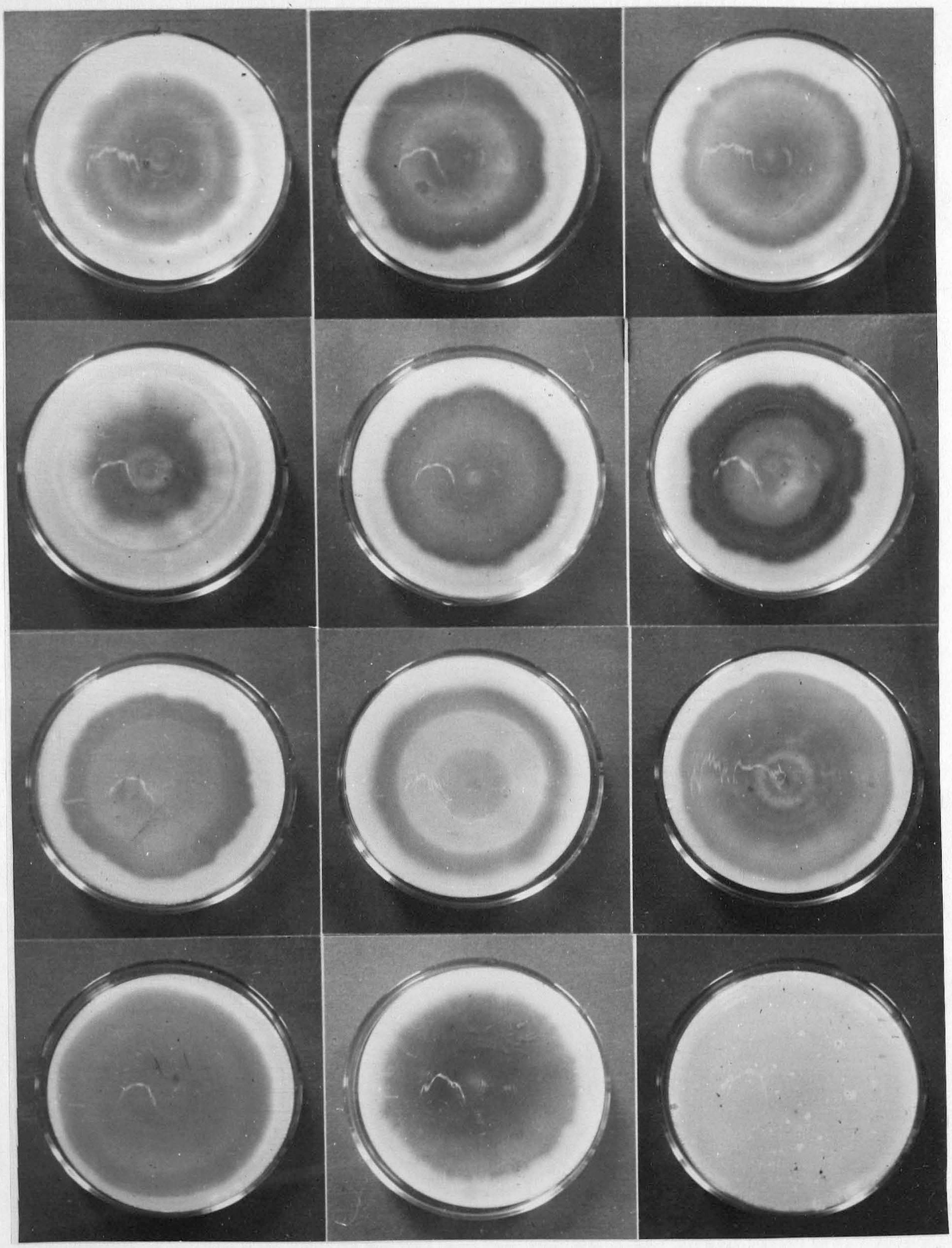

FIGURE 8

PLATE VII

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.

FIFTEEN-DAY OLD COLONIES ($x\frac{1}{2}$) REVERSE SIDE

Description of the colonies of <u>Alternaria solani</u> (E. and M.) Jones and Grout after twenty days growth on the different carbohydrate media. Photographs are on opposite page (Figure 9). Read left to right in each row.

<u>Row 1</u>

Xylose: Center olivaceous black, then pale mouse gray to mouse gray, margin white
Dextrose: Pale drab gray with an olivaceous black margin
Mannose: Deep mouse gray to pale mouse gray, margin gray

<u>Row 2</u>

Galactose: Color variation from fuscous to hair brown
Levulose: Color variation from light mouse gray to dark mouse gray
Sucrose: Pale mouse gray with an olivaceous black margin

<u>Row 3</u>

Lactose: Color variation from pale drab gray to deep mouse gray
Maltose: Rings of mouse gray, pale mouse gray, drab gray, and chaetura drab
Starch: Color variation from deep mouse gray to pale mouse gray with a margin of dark mouse gray

<u>Row 4</u>

Dextrin: Dark mouse gray, deep mouse gray, light mouse gray, and a dark mouse gray margin
Inulin: An overcast of dark and light mouse grays with a darker margin. Mat heavier than during its earlier stages of growth
Agar: No change

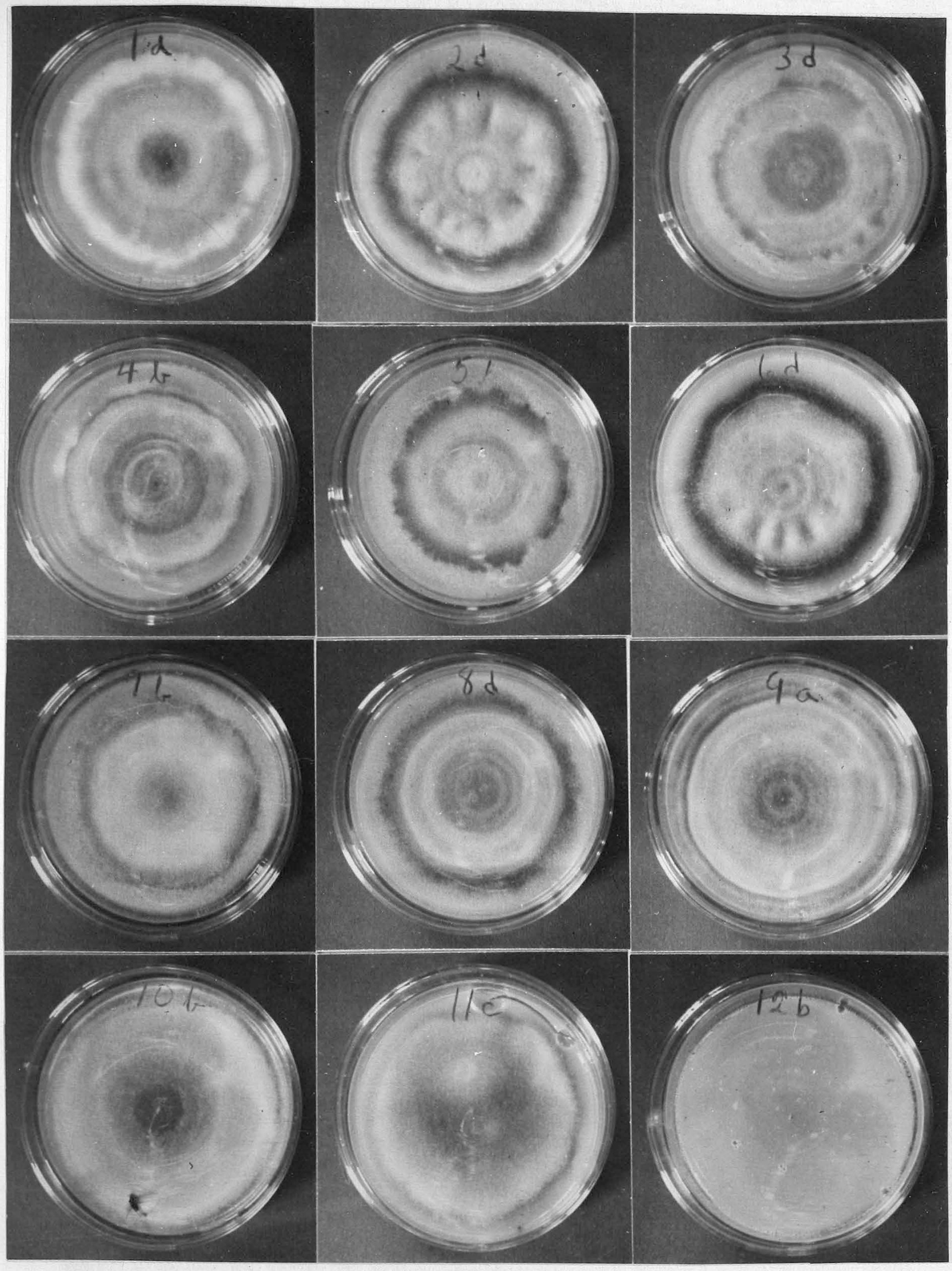

FIGURE 9

PLATE VIII

ALTERNARIA SOLANI (E. AND M.) JONES AND GROUT.

TWENTY-DAY OLD COLONIES (x½)

II. SUMMARY AND CONCLUSIONS

Summary. The results may be summarized as follows:

1. The pH of the media, except in the check plate, changed from slightly acid to slightly alkaline during the twenty-day period.

2. Growth was good on all carbohydrates used except that on galactan (agar).

3. Total growth was most marked on dextrin and starch and least on galactose except agar.

4. All cultures (except agar) had a heavy compact mat about one--two millimeters above the surface at the end of the growing period.

5. The rate of growth was greater during the first part of the growing period than during the last.

6. There was a marked difference in the appearance of the colonies growing on the different carbohydrates.

7. The influence of agar in the basic media with the other carbohydrate sources could be regarded as negligible.

Conclusions. The different carbohydrates seem to have very little effect on either the final growth of *Alternaria solani* (E. and M.) Jones and Grout or on the pH of the media in which it is grown. The only marked difference the various carbohydrates seemed to produce was a variation in the appearance and in the pattern of growth.

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