Tolerance of Soil Micro Organisms to Media Changes.

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Our text books all give space to the discussion of the food requirements of bacteria. The discussion, although general, is liable to lead us to believe that most organisms may not grow if we change the composition of media slightly. Just what is the minimum ration for most bacteria is not known. Our knowledge of the effects of modifying the composition of culture media is meager, especially when environmental factors are considered.

The Horticultural Research Chemistry and Bacteriology Laboratories, of the Purdue Agricultural Experiment Station have been investigating media for the platings and subsequent culturing of soil bacteria. This paper reports a part of this investigation.

Soil Used.

Two types of soil were used in this work, silty clay from the Experimental orchard at Laurel, Indiana, and brown loam from the Station orchard where a cover crop investigation is under way. All samples reported on in this paper contained from 16 to 20 per cent. of moisture at time of sampling. The method of sampling was by means of Noyes' sampler for soil bacteriologists. Samples were taken of the upper nine inches of soil.

Media Used.

Lipman and Brown "synthetic" agar.

15 gms. *best* agar.
10 gms. Dextrose.
.05 gms. Witte Peptone.
.2 gms. Magnesium sulphate.
.5 gms. Di potassium hydrogen phosphate.
Trace Ferrous sulphate.
1,000 cc. Distilled water.

H. J. Conn's sodium asparaginate agar.

15 gms. *best* agar (used instead of 12).

1 gm. Sodium asparaginate.
1 gm. Dextrose.
.2 gm. Magnesium sulphate.
1.5 gm. ($NH_4H_2PO_4$) ammonium biphosphate.
.1 gm. Calcium chloride.
.1 gm. Potassium chloride.
Trace Ferrous chloride.
1,000 cc. Distilled water.

Soil Extract (Unheated).

15 grams of *best* agar dissolved in 1,000 cc. of solution made as follows: Two kilos of the brown loam soil were placed in a glass bottle, and 5 liters of distilled water added, the bottle was shaken at intervals and at end of 16 hours the mixture was filtered. One thousand cc. of the filtrate was used in place of distilled water in making up this media.

Soil Extract (Autoclaved).

Fifteen grams of *best* agar dissolved in 1,000 cc. of solution made the same as the soil extract (unheated), except that the two kilos of soil were wet well and heated under 25 lbs. pressure in the autoclave for three hours.

Soil and agar, leaf extract and agar, and wheat straw extract.

These three media were made as follows: To 15 gms. of the *best* agar were added 10 gms. of the material desired and 1,000 cc. distilled water. The mixture was heated in a double boiler until the agar was dissolved. After making up to volume the media was filtered and tubed.

Other Media.

To 15 gms. of *best* agar was added 1 gm. per liter of chemicals appearing as part of the name of the media and 1,000 cc. of distilled water.

Figure 1 expresses graphically the acidity of the various media. The procedure in titrating was as follows: To about 125 cc. of distilled water that has been boiling about 3 minutes in a Jena erlenmeyer flask was added 50 cc. of the media by means of a tall 50 cc. graduate (of small cross-section). Two drops of phenolpthalein solution was added and titration made with tenth normal sodium hydroxide. The only media neutralized at all was H. J. Conn's sodium asparaginate agar, and this was done with half normal soda, using a pipette graduated to one-twentieth of a cc.

Tube Media Test I.

Sample of 6/14, 1915.

Sample from Tree XIII—13 Plot F.

Laurel.

One cc. portions of the 1–400,000 dilution of the sample were plated on the following media:

Lipman and Brown agar.

Conn's sodium asparaginate agar.

Agar alone.

Soil and agar (Purdue soil).

Soil extract (autoclaved) and agar.

Soil extract (unheated) and agar.

(15 gms. agar in all media.)

Transfers were made from best colonies on each media to slants of other media. Tables give results of growth on these agar slants at end of 5 and 14 days' incubation at 22° C.

8 Colonies from L and B agar to

	5 Days.	14 Days.
Na. asp. agar	8 g.*	8 g.
	0 —	0 —
Soil ext. (unheated)	6 g.	7 g.
	2 —	1 —
Soil ext. (autoclaved)	5 g.	5 g.
	3 —	3 —
Agar alone	5 g.	5 g.
	3 —	3 —
Agar and soil	4 g.	5 g.
	4 —	3 —

*8 = growth. — = no growth unless otherwise specified.

8 Colonies from Na. asp. agar to

	5 Days.	14 Days.
L and B agar	7 g. 1 —	7 g. 1 —
Soil ext. (unheated)	5 g. 3 —	7 g. 1 —
Soil ext. (autoclaved)	5 g. 3 —	7 g. 1 —
Agar alone	7 g. 1 —	7 g. 1 —
Agar and soil	6 g. 2 —	7 g. 1 —

3 Colonies from Soil Extract (unheated) to

	5 Days.	14 Days.
L and B agar	3 g.	3 g.
Na. asp. agar	2 g. 1 —	3 g.
Soil ext. (autoclaved)	3 g.	3 g.
Agar alone	3 g.	3 g.
Agar and soil	3 g.	3 g.

3 Colonies from Soil Extract (autoclaved) to

	5 Days.	5 Days.
L and B agar	3 g.	3 g.
Na. asp. agar	3 g.	3 g.
Soil ext. (unheated)	3 g.	3 g.
Agar alone	3 g.	3 g.
Agar and soil	2 g. 1 —	2 g. 1 —

3 Colonies from Agar Alone to

	5 Days.	14 Days.
L and B agar	3 g.	3 g.
Na. asp. agar	3 g.	3 g.
Soil ext. (unheated)	2 g.	2 g.
	1 —	1 —
Soil ext. (autoclaved)	3 g.	3 g.
Agar and soil	2 g.	2 g.
	1 —	1 —

3 Colonies from Agar and Soil to

	5 Days.	14 Days.
L and B agar	2 g.	2 g.
	1 —	1 —
Na. asp. agar	2 g.	2 g.
	1 —	1 —
Soil ext. (unheated)	2 g.	2 g.
	1 —	1 —
Soil ext. (autoclaved)	2 g.	2 g.
	1 —	1 —
Agar alone	2 g.	2 g.
	1 —	1 —

Summary 5 Day Results.

20 transfers to L and B agar.....18 made growth.
20 transfers to Na. asp. agar.....18 made growth.
25 transfers to Soil ext. (unheated).....18 made growth.
25 transfers to Soil ext. (autoclaved).....18 made growth.
25 transfers to Agar alone.....20 made growth.
25 transfers to Agar and soil.....17 made growth.

Summary 14 Days.

20 transfers to L and B agar.....18 made growth.
20 transfers to Na. asp. agar.....19 made growth.
25 transfers to Soil ext. (unheated).....21 made growth.

25 transfers to Soil ext. (autoclaved)..... ...20 made growth.
25 transfers to Agar alone..... ...20 made growth.
25 transfers to Agar and soil..... ...19 made growth.

Notes.

When tubes of organisms grown originally on same media were put side by side the following differences were noted.

(1) Agar alone supported very poor growths.

(2) Agar and soil supported fully as poor growths as agar alone.

(3) The two extracts acted about the same, although the heated extract grew the organisms originally grown on Na. asp. agar a little the best.

(4) L. and B. agar and Na. asp. agar supported good growths.

(5) From any macroscopic test the growths on the L. and B. agar were far superior to those on the Na. asp. agar.

TUBE MEDIA TEST II.

Samples of 6/14, 1915.

Samples from Tree VI—24. Plot C.

Laurel.

One cc. portions of the 1–400,000 dilution of the sample were plated on the following media:

Lipman and Brown agar.
Conn's sodium asparaginate agar.
Agar alone.
Soil and agar (Purdue soil).
Soil extract (unheated) and agar.
Soil extract (autoclaved) and agar.
(15 gms. agar in all media.)

Transfers were made from best colonies on each media to slants of other media. Tables give results of growth on these agar slants at end of 5 and 14 days' incubation at 22° C.

8 Colonies from L and B agar to

	5 Days.	14 Days.
Na. asp. agar	7 g. 1 —	7 g. 1 —
Soil ext. (unheated)	6 g. 2 —	5 g. 3 —
Soil ext. (autoclaved)	5 g. 3 —	6 g. 2 —
Agar alone	4 g. 4 —	6 g. 2 —
Agar and soil	4 g. 4 —	4 g. 4 —

8 Colonies from Na. asp. agar to

	5 Days.	14 Days.
L and B agar	8 g.	8 g.
Soil ext. (unheated)	7 g. 1 —	6 g. 2 —
Soil ext. (autoclaved)	8 g.	8 g.
Agar alone	8 g.	8 g.
Agar and soil	8 g.	6 g. 2 —

Summary 5 Days.

8 transfers to L and B agar..... 8 made growth.
8 transfers to Na. asp. agar..... 7 made growth.
16 transfers to Soil ext. (unheated)..... 13 made growth.
16 transfers to Soil ext. (autoclaved)..... 13 made growth.
16 transfers to Agar alone..... 12 made growth.
16 transfers to Agar and soil..... 12 made growth.

Summary 14 Days.

8 transfers to L and B agar..... 8 made growth.
8 transfers to Na. asp. agar..... 7 made growth.
16 transfers to Soil ext. (unheated)..... 11 made growth.
16 transfers to Soil ext. (autoclaved)..... 14 made growth.
16 transfers to Agar alone..... 14 made growth.
16 transfers to Agar and soil..... 10 made growth.

Notes.

When tubes of organisms grown originally on same media were put side by side the following differences were noted:

(1) Agar alone supported very poor growths.

(2) Agar and soil supported fully as poor growths as agar alone.

(3) The two extracts acted about the same, although the heated extract grew the organisms originally grown on Na. asp. agar a little the best.

(4) L. and B. agar and Na. asp. agar supported good growths.

(5) From any macroscopic test the growths on the L. and B. agar were far superior to those on the Na. asp. agar.

Tube Media Test III.

Samples of 6/25, 1915.

Sample No. 6. Rye Plot.

Cover Crop Investigations.

One cc. portions of the 1 to 400,000 dilution of this sample were plated on the following media:

Lipman and Brown agar.

Conn's sodium asparaginate agar.

Agar alone.

Soil and agar (Purdue soil).

Soil extract (unheated) and agar.

Soil extract (autoclaved) and agar.

(15 gms. agar in all media.)

Colonies developing well on first two media listed were put on other media and growth noted at end of 5, 11, and 15 days' incubation at 22° C.

From 4 Colonies on L and B agar to

	5 Days.	11 Days.	15 Days.
Na. asp. agar	3 g.	1 g.	4 g.
	1 —		
Soil ext. (unheated)	2 g.	2 g.	3 g.
	2 —	2 —	1 —
Plain agar	3 g.	3 g.	3 g.
	1 —	1 —	1 —

From 4 Colonies on Na. asp. agar to

	5 Days.	11 Days.	15 Days.
L and B agar	4 g.	4 g.	4 g.
Soil ext. (unheated)	2 g. 2 —	3 g. 1 —	4 g.
Plain agar	3 g. 1 —	3 g. 1 —	3 g. 1 —

Summary 5 Days.

4 transfers to L and B agar.....4 made growth.
4 transfers to Na. asp. agar.....3 made growth.
8 transfers to Soil ext. (unheated).....4 made growth.
8 transfers to Agar alone.....6 made growth.

Summary 15 Days.

4 transfers to L and B agar.....4 made growth.
4 transfers to Na. asp. agar.....4 made growth.
8 transfers to Soil ext. (unheated).....7 made growth.
8 transfers to Agar alone.....6 made growth.

Notes.

When tubes of different media containing the same organism from the same original colony were put side by side, the following was noted:

(1) The growth on agar alone, soil and agar or on soil extract (unheated) was small.

(2) The soil extract carried a better growth than the soil alone.

(3) L. and B. agar and Na. asp. agar carried a good growth.

(4) There was more development of distinguishing characteristics as to form of streaks and chromogenisis present, with the L and B agar.

Tube Media Test IV.

Samples of 6/25, 1915.

Sample No. 7. Clean Culture Plot.

Cover Crop Investigation.

One cc. portions of the 1 to 400,000 dilution of this sample were plated on the following media:

Lipman and Brown agar.

Conn's sodium asparaginate agar.

Agar alone.
Soil and agar (Purdue soil).
Soil extract (unheated) and agar.
Soil extract (autoclaved) and agar.
(15 gms. agar in all media.)

Colonies developing well on each media were transferred to slants of other media. Tables give results of growth on these agar slants at end of 5. 11, and 15 days. Incubation at 22° C.

From 4 Colonies on L and B agar to

	5 Days.	11 Days.	15 Days.	Shown in Plate
Na. asp. agar	2 gr. 2 —	2 gr. 2 —	3 gr. 1 —	I
Agar alone	3 gr. 1 —	3 gr. 1 —	3 gr. 1 —	
Soil ext. (unheated)	2 gr. 2 —	3 gr. 1 —	3 gr. 1 —	

From 4 Colonies on Na. asp. agar to

	5 Days.	11 Days.	15 Days.	Plate.
L and B agar	4 g.	4 g.	4 g.	II
Agar alone	3 g. 1 —	3 g. 1 —	3 g. 1 —	
Soil ext. (unheated)	3 g. 1 —	4 g.	4 g.	

From 3 Colonies on Plain Agar to

	5 Days.	11 Days.	15 Days.
L and B agar	2 g. 1 —	2 g. 1 —	2 g. 1 —
Na. asp. agar	2 g. 1 —	2 g. 1 —	2 g. 1 —
Soil ext. (unheated)	2 g. 1 —	2 g. 1 —	2 g. 1 —

General Notes.

When tubes of different media containing the same organism from the same original colony are put side by side, the following is noted:

(1) The growth on agar alone, soil and agar or on soil extract (unheated) is small.

(2) The soil extract carries a better growth than the soil alone.

(3) L. and B. agar and Na. asp. agar carry a good growth.

(4) There is more development of distinguishing characteristics as to form of streaks and chromogensis present, with the L. and B. agar.

TUBE MEDIA TEST V.

Sample of 7 16, 1915.

Sample No. 8. Millet Plot.

Cover Crop Investigations.

One cc. portions of the 1 to 400,000 dilution of this sample were plated on the following media:

A. Wheat straw extract.
B. Leaf extract.
C. Starch.
D. Agar alone.
E. Ammonium nitrate.
F. Conn's sodium asparaginate.
G. Soil.
H. Soil and starch.
I. Lipman and Brown agar.
J. Ammonium nitrate and starch.
(15 gms. agar is basis of all media.)

Colonies developing well on each media, plates III and IV, were transferred to slants of other media. Tables give results of growth on these slants at end of 6, 10 and 14 days' incubation at 22° Centigrade.

From 3 Colonies on Soil and Agar to

	5 Days.	11 Days.	15 Days.
L and B agar	2 g. 1 —	2 g. 1 —	2 g. 1 —
Na. asp. agar	2 g. 1 —	3 g.	3 g.
Soil ext. (unheated)	3 g.	3 g.	3 g.
Agar alone	2 g. 1 —	3 g.	3 g.

From 3 Colonies on Soil Extract (unheated) to

	5 Days.	11 Days.	15 Days.
L and B agar	3 g.	3 g.	3 g.
Na. asp. agar	3 g.	3 g.	3 g.
Agar alone	3 g.	3 g.	3 g.

From 3 Colonies on Soil Extract (autoclaved) to

	5 Days.	11 Days.	15 Days.
L and B agar	3 g.	3 g.	3 g.
Na. asp. agar	3 g.	3 g.	3 g.
Agar alone	2 g. 1 —	2 g. 1 —	2 g. 1 —
Soil ext. (unheated)	3 g.	3 g.	3 g.

Summary (5 Days Results).

16 transfers to L and B agar.....14 made growth.
16 transfers to Na. asp. agar.....12 made growth.
17 transfers to Plain agar.....13 made growth.
17 transfers to Soil Ext. (unheated).....13 made growth.

Summary (15 Day Results).

16 transfers to L and B agar.....14 made growth.
16 transfers to Na. asp. agar.....13 made growth.
17 transfers to Plain agar.....14 made growth.
17 transfers to Soil ext. (unheated).....15 made growth.

4 Colonies from L and B agar to

	6 Days.	10 Days.	14 Days.	Shown in Plate
Wheat Straw Ext.	3 g.	3 g.	3 g.	V
	1 —	1 —	1 —	
Leaf Ext.	1 g.	1 g.	1 g.	
	3 —	3 —	3 —	
Starch	4 g.	4 g.	4 g.	VI
Agar alone	3 g.	4 g.	4 g.	VII
	1 —			
Ammonium Nitrate	4 g.	4 g.	4 g.	VIII
Na. asp. agar	3 g.	3 g.	4 g.	IX
	1 —	1 —		
Soil	3 g.	3 g.	3 g.	X
	1 —	1 —	1 —	
Soil and Starch	2 g.	2 g.	2 g.	
	2 —	2 —	2 —	
L and B agar	4 g.	4 g.	4 g.	XI
Ammonium Nitrate and Starch	3 g.	3 g.	4 g.	
	1 —	1 —		
Soil and Ammonium Nitrate	2 g.	2 g.	3 g.	
	2 —	2 —	1 —	
Soil Extract (unheated)	2 g.	3 g.	4 g.	
	2 —	1 —		

4 Colonies from Na. asp. agar to

	6 Days.	10 Days.	14 Days.	Plate.
Wheat Straw Ext.	4 g.	4 g.	4 g.	V
Leaf Ext.	3 g. 1 —	3 g. 1 —	3 g. 1 —	
Starch	4 g.	4 g.	4 g.	VI
Agar alone.	4 g.	4 g.	4 g.	VII
Ammonium Nitrate	4 g.	4 g.	4 g.	VIII
Na. asp. agar.	4 g.	4 g.	4 g.	IX
Soil.	4 g.	4 g.	4 g.	X
Soil and Starch.	4 g.	4 g.	4 g.	
L and B agar.	4 g.	4 g.	4 g.	XI
Ammonium Nitrate and Starch	4 g.	4 g.	4 g.	
Soil and Ammonium Nitrate	3 g. 1 —	3 g. 1 —	1 g.	
Soil Ext. (unheated).	4 g.	4 g.	4 g.	

4 Colonies from Starch to

	6 Days.	10 Days.	14 Days.
Wheat Straw Ext.	2 g. 2 —	2 g. 2 —	2 g. 2 —
Leaf Ext.	1 g. 3 —	1 g. 3 —	1 g. 3 —
Starch	3 g. 1 —	4 g.	4 g.
Agar alone	3 g. 1 —	3 g. 1 —	3 g. 1 —
Ammonium Nitrate	4 g.	4 g.	4 g.
Na. asp. agar	4 g.	4 g.	4 g.
Soil	4 g.	4 g.	3 g. 1 —
Soil and Starch	2 g. 2 —	2 g. 2 —	2 g. 2 —
L and B agar	4 g.	4 g.	4 g.
Ammonium Nitrate and Starch	3 g. 1 —	3 g. 1 —	3 g. 1 —
Soil and Ammonium Nitrate	4 g.	4 g.	4 g.
Soil Ext. (unheated)	3 g. 1 —	3 g. 1 —	4 g.

4 Colonies from Agar alone to

	6 Days.	10 Days.	14 Days.	Shown in Plate.
Starch	2 g. 2 —	2 g. 2 —	2 g. 2 —	
Na. asp. agar	2 g. 2 —	3 g. 1 —	3 g. 1 —	XII
L and B agar	3 g. 1 —	4 g.	4 g.	XII

4 Colonies from Ammonium Nitrate to

	6 Days.	10 Days.	14 Days.	Shown in Plate
L and B agar	4 g.	4 g.	4 g.	XII
Na. asp. agar	4 g.	4 g.	4 g.	XII
Starch	4 g.	4 g.	4 g.	

4 Colonies from Soil and Starch to

	6 Days.	10 Days.	14 Days.
L and B agar	4 g.	4 g.	4 g.
Na. asp. agar	4 g.	4 g.	4 g.
Starch	2 g. 2 —	2 g. 2 —	2 g. 2 —

4 Colonies from Soil alone to

	6 Days.	10 Days.	14 Days.
L and B agar	3 g. 1 —	3 g. 1 —	4 g.
Na. asp. agar	4 g.	4 g.	4 g.
Starch	4 g.	4 g.	4 g.
Soil and starch	4 g.	4 g.	4 g.

4 Colonies from Ammonium Nitrate and Starch to

	6 Days.	10 Days.	14 Days.
L and B agar	1 g.	4 g.	4 g.
Na. asp. agar	3 g. 1 —	4 g.	4 g.
Starch	4 g.	4 g.	4 g.
Soil and starch	3 g. 1 —	3 g. 1 —	3 g. 1 —

Summary 6 Days.

12 transfers to Wheat Straw Ext.	9 made growth.
12 transfers to Leaf Ext.	5 made growth.
32 transfers to Starch.	27 made growth.
12 transfers to Agar alone.	10 made growth.
12 transfers to Ammonium Nitrate.	12 made growth.
32 transfers to Na. asp. agar.	28 made growth.
12 transfers to Soil.	10 made growth.
20 transfers to Soil and Starch.	15 made growth.
32 transfers to L and B agar.	30 made growth.
12 transfers to NH_4NO_3 and St.	10 made growth.
12 transfers to Soil and NH_4NO_3.	9 made growth.
12 transfers to Soil Ext.	12 made growth.
212 transfers.	177 made growth.

Summary 14 Days.

12 transfers to Wheat Straw Ext.	9 made growth.
12 transfers to Leaf Ext.	5 made growth.
32 transfers to Starch.	28 made growth.
12 transfers to Agar alone.	11 made growth.
12 transfers to Ammonium Nitrate.	12 made growth.
32 transfers to Na. asp. agar.	31 made growth.
12 transfers to Soil.	10 made growth.
20 transfers to Soil and Starch.	15 made growth.
32 transfers to L and B agar.	32 made growth.
12 transfers to NH_4NO_3 and St.	11 made growth.
12 transfers to Soil and NH_4NO_3.	11 made growth.
12 transfers to Soil Ext.	12 made growth.
212 transfers.	187 made growth.

Notes.

(1) In this set of tests, as in those run previously, there was very little growth on the agar alone, the soil, and the soil extract slants. Practically all the organisms tested made some growth on these media.

(2) Ammonium nitrate furnishing nitrogen both in NH_4 and NO_3 did not grow better cultures than agar alone. This latter is from observations made after fourteen days' incubation.

(3) Wheat straw extract grew but little better cultures than the soil extract, while leaf extract was a total failure as a media.

(4) Starch furnishing sources of energy, and being capable of being

split in many ways by enzymatic action, grew good cultures both alone and in combination with other materials.

(5) As noted in all other tests the Lipman and Brown agar grew the best cultures and apparently developed their distinguishing chromogenic characteristics much better than the sodium asparaginate agar.

(6) From macroscopic comparisons the starch media seemed to be the real competitor of the Lipman and Brown agar.

Tube Media Test VI.

Testing Organisms from Laurel Soils.

Plated on Lipman and Brown Agar.

When transferred to slants of different media.

Samples taken 7/27/1915.

Description of colonies from which transfers were made:

No. 1. Round, curled edge, wrinkled in structure, green in color, a mold 1.5 cm. in diameter.

No. 2. Elliptical, curled edge, wrinkled in structure, green in color, a mold 1.5 cm. long.

No. 3. Round, lobate edge, wrinkled structure, brown (pale) in color, a mold 1 cm. in diameter.

No. 4. Round, entire edge, granular structure. White raised center with brown ring outside, apparently a mold about .5 cm. in diameter.

No. 5. Discoid, crenate edge, smooth structure, milk white in color, .5 cm. in diameter, a mold.

No. 6. Round, entire edge, smooth structure, salmon red in color, 3 mm. in diameter.

No. 7. Round, ciliate edge, granular structure. Yellow in color, deep yellow at center, about 1 cm. in diameter.

No. 8. Round, ciliate edge, granular center and fibrant outer portion describes structure. Center dark green, border light green, about 4 mm. in diameter.

No. 9. Round, plain edge, smooth in structure, salmon red with yellowish outside ring, produces yellow pigment soluble in media, about 4 mm. in diameter.

No. 10. Round though dented, crenate edge, spotted structure, white in color, about 8 mm. in diameter.

No. 11. Discoid, lobate edge, spotted structure, white in color with heavy black center, about 6 mm. in diameter.

No. 12. Round, entire edge, granular structure, heavy center, milk white in color, about 1 cm. in diameter.

No. 13. Round, entire edge, smooth structure, yellow in color, about 3 mm. in diameter.

No. 14. Round, entire edge, smooth structure, dark red in color, about 4 mm. in diameter.

No. 15. Round, entire edge, spotted structure, white with brown center, about 8 mm. in diameter.

No. 16. Discoid, lobate edge, wrinkled structure, yellowish white in color, about 8 mm. in diameter.

Observations of Growth and Relative Growth were made at end of 5th, 7th, and 15th days. Temperature of incubation, 22° to 23° C. on following media:

Lipman and Brown agar.
Conn's sodium asparaginate agar.
Ammonium nitrate agar.
Starch agar.
Ammonium nitrate and starch agar.

Observations of Growth and Ranking 5 Days.

No.	L and B agar.	Na. asp. agar.	NH_4NO_3 agar.	Starch agar.	NH_4NO_3 and Starch.
1.	– 5(%)	* 1	* 4	* 3	* 2
2.	* 4	* 1	* 3	* 2	– 5
3.	– 5	* 1	– 5	* 2 or 3	* 2 or 3
4.	– 5	* 3	* 1	* 5	* 2
5.	* 5	* 1	* 3	– 5	* 2
6.	* 2	* 1	* 5	* 4	* 3
7.	* 3	* 1	– 5	* 2	* 4
8.	* 1	* 2	* 4	* 3	* 5
9.	* 2	* 1	* 1	* 5	* 3
10.	* 1	* 4	* 3	* 2	– 5
11.	* 1	* 3	– 5	* 2	– 5
12.	* 1	* 1	* 1	* 1	* 1
13.	* 5	* 2	* 3	* 4	* 1
14.	* 1	* 4	* 5	* 3	* 2
15.	* 4	* 3	* 5	* 2	* 1
16.	* 2	* 1	* 3	– 5	* 4
Av. all	2 94	1 87	3 69	3 16	2 97
Av. 6-16.	2 10	2 10	3 91	3 00	3 10

* = Growth.

– = No growth.

(%) No growth, ranked lowest so that a relative general average may be made.

Observations of Growth and Ranking 7 Days.

No.	L and B agar.	Na. asp. agar.	NH_4NO_3 agar.	Starch agar.	NH_4NO_3 and Starch Agar.
1	− 5 (%)	* 1	* 3	* 2	* 4
2	* 3	* 2	− 5	* 1	− 5
3	− 5	* 1	* 4	* 2	* 3
4	* 4	* 1	* 5	* 3	* 2
5	− 5	* 2	* 1	− 5	* 3
6	* 5	* 4	* 2	* 1	* 3
7	* 1	* 2	* 5	* 3	* 4
8	* 2	* 1	* 4	* 3	* 5
9	* 1	* 2	* 5	* 4	* 3
10	* 1	* 2	* 1	* 3	− 5
11	* 2	* 1	* 5	* 4	* 3
12	* 1	* 2	* 1	* 1	* 1
13	* 1	* 2	* 5	* 4	* 3
14	* 1	* 4	* 5	* 3	* 2
15	* 1 or 2	* 1 or 2	* 5	* 4	* 3
16	* 2	* 1	* 4	− 5	* 3
Av. all	2.50	1.81	3.76	3.00	3.25
Av. 6-16	1.64	2.00	3.72	3.18	3.18

* = Growth.

− = No Growth.

(%) No growth, ranked lowest so that a relative general average may be made.

Observations of Growth, Color of Growth and Ranking 15 Days.

No.	L and B agar.	Na. asp. agar.	NH_4NO_3 agar.	Starch agar.	NH_4NO_3 and Starch Agar.
1.	— 5	* 1 Bl. Br. Gr.	* 4 White	* 2 Li. Green	* 3 Bl. Gr.
2.	* 1 Bl. Gr.	* 3 Li. than 2	* 4	* 2 Li. than 1	* 5
3. . . .	— 5	* 1 Green	— 5	* 2 Green	* 3 Wh.-Gr.
4. . . .	* 4 Cream	* 1 White	* 3 White	* 5 Li.-Gr.	* 2 D.-Brown
5.	— 5	* 1 Heavy Wh.	* 3 White	* 4 White	* 2 White
6. . .	* 4 Red	* 3 Red	* 5 White	* 1 Red	* 2 White
7.	* 1 Y.-White	* 3 Green	* 5 White	* 4 Y.-Green	* 2 Yellow
8.	* 2 Green	* 1 Green	* 4 White	* 3 Y.-Green	* 5 White
9.	* 1 Y.-Red	* 4 R.-Yell.	* 5 White	* 3 Y.-White	* 2 Y.-White
10.	* 1 White	* 3 White	* 4 White	* 2 White	— 5
11.	* 3 Brown	* 1 Brown	* 5 White	* 4 White	* 2 White
12.	* 4 Br.-Wh.	* 5 White	* 3 Br.-Wh.	* 2 Br.-Wh.	* 1 Br.-Wh.
13.	* 1 P.-Gr.	* 4 P.-Gr.	* 5 P.-Gr.	* 3 P.-Gr.	* 2 P.-Gr.
14.	* 1 Red	* 4 Cream	* 5 D.-Wh.	* 3 Red	* 2 Red
15.	* 1 Br.-Wh.	* 2 D.-Wh.	* 5 D.-Wh.	* 4 Br.-Wh.	* 3 Br.-Wh.
16.	* 2 Br.-Wh.	* 3 Y.-Wh.	* 4 Br.-Wh.	— 5	* 1 Br.-Wh.
Av. all	2 56	2 50	4 31	3 06	2.56
Av. 6-16.	1 91	3.00	4.55	3.09	2.45

* = Growth.
— = No growth.

Summary.

Average All Sixteen Organisms.

	L and B agar.	Na. asp. agar.	NH_4NO_3 agar.	Starch agar.	NH_4NO_3 and Starch Agar.
5 days.....	2.94	1.87	3.69	3.16	2.97
7 days.....	2.50	1.81	3.76	3.00	3.25
15 days.....	2.56	2.50	4.31	3.06	2.56

Summary.

Average Organisms 6 to 16 Inc.

	L and B agar.	Na. asp. agar.	NH_4NO_3 agar.	Starch agar.	NH_4NO_3 and Starch Agar.
5 days.....	2.10	2.10	3.91	3.00	3.10
7 days.....	1.64	2.00	3.82	3.18	3.18
15 days.....	1.91	3.00	4.55	3.09	2.45

Notes.

(1) The comparisons between the growth of an organism on the different media were practically as marked at 5 days as they were at 15.

(2) The five molds Nos. 1, 2, 3, 4, 5, were more easily transferred to sodium asparaginate agar than to some of the media.

(3) Where molds are included the greatest number of failures of growth occurred on Lipman and Brown agar.

(4) Studying Nos. 6 to 16 inclusive, it was found that the Lipman and Brown and the Sodium asparaginate agar were about alike in amount of growths produced on slants, and that the ammonium nitrate agar was the poorest media considered.

(5) When chromogenesis is considered, Starch alone and in combination with the Ammonium nitrate brought out as much chromogenesis as the Lipman and Brown agar.

Summary of Investigation.

This paper gives the results of tests made on agar slants where the two media most commonly used for plating soils are compared. The results

of comparisons between these media, and comparisons of them—with agar alone, with soil, wheat and leaf extract media, with ammonium nitrate and starch media, both alone and in combination—showed that organisms once grown on media will generally grow when transferred to other media.

The rate of development seemed more important than the fact that the organism grew. Comparisons of growth at end of different periods of incubation were usually the same. Where growth was good it developed slowly enough so that it could not be termed a flash growth. Where growth was poor, distinguishing characteristics peculiar to the organism were rarely apparent.

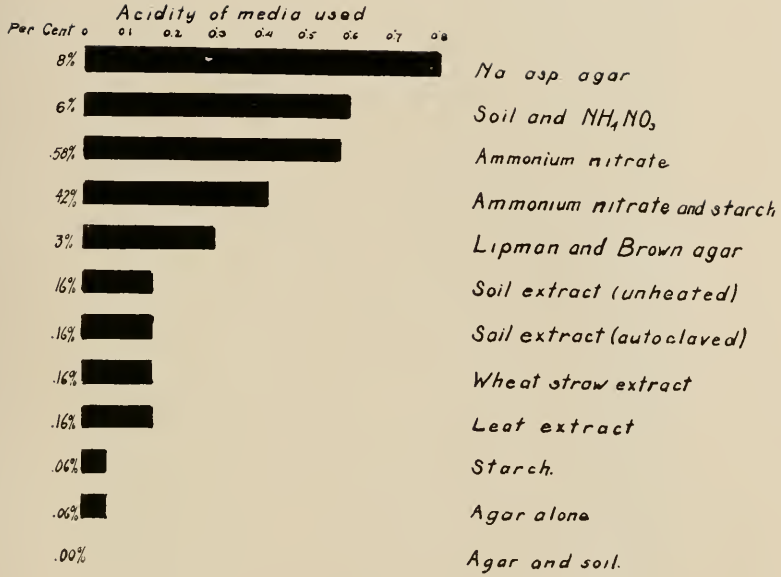
The explanation of the tolerance observed is not that those organisms growing when soil is plated on inferior media are probably the same organisms that yield the best colonies on better media. Picking out organisms plated on the best media and growing them on poorer media supports the above statement. Chromogenesis was augmented by the presence of carbohydrate in the media.

Comment.

Many expect that soil biology will explain results for which chemical and physical causes have not been found. Many look to the control of plant growth through the application of principles of microbiology.

Soils with their large or small amounts of decaying organic matter, of both plant and animal origin, must be a possible medium for the growth of all kinds of bacteria. One reason why the number of bacteria in our prairie soils has not been found to vary with the crop-producing power of the soil may be the tolerance of many kinds of bacteria to all present chemical and physical differences between types of prairie soil. In sandy and poor soils some believe that there is a relationship between the number of bacteria and the crop-producing power of the soil. The factors of temperature, aeration and moisture are more constant in the rich soil, and for this reason the changes in soil moisture, the variation in soil temperature, and the movement of soil gases must exert a more marked influence on the presence of and the activities of certain micro-organisms than the food factor does.

FIGURE 1


Acidity of media used
Per Cent 0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
8%
6%
.58%
42%
3%
.16%
.16%
.16%
.16%
.06%
.06%
.00%
Na asp agar
Soil and NH_4NO_3
Ammonium nitrate
Ammonium nitrate and starch
Lipman and Brown agar
Soil extract (unheated)
Soil extract (autoclaved)
Wheat straw extract
Leaf extract
Starch.
Agar alone
Agar and soil.

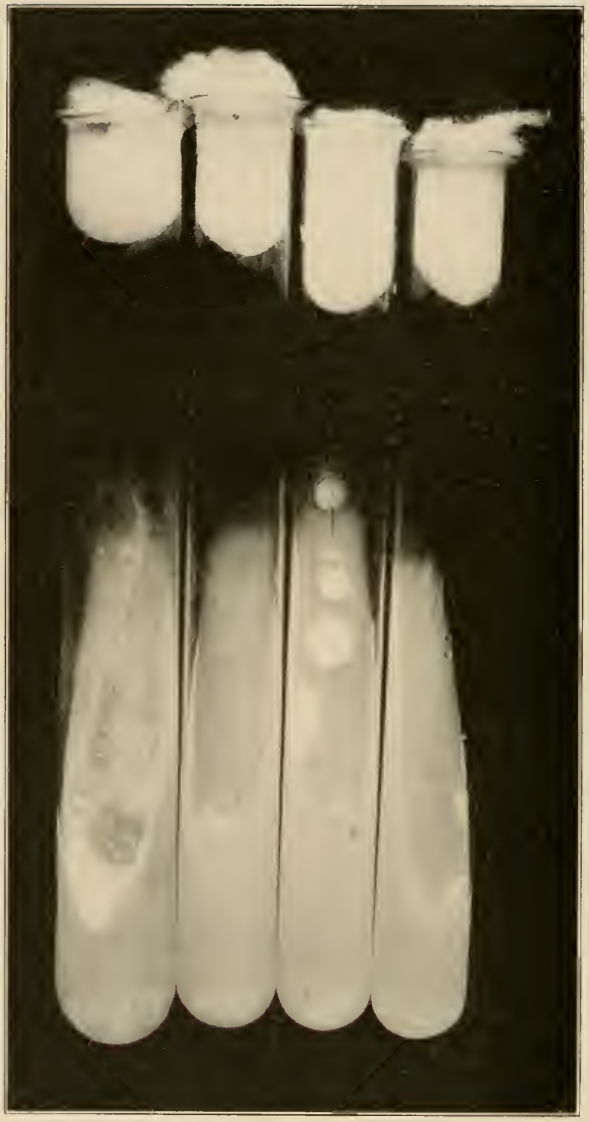


PLATE I.
4 Colonies from L. and B. agar on Na. asp. agar.

PLATE II.
4 Colonies from Na. Asp. agar on L. and B. agar

PLATE III.

Some of the plates from which organisms were obtained for tube media test V.

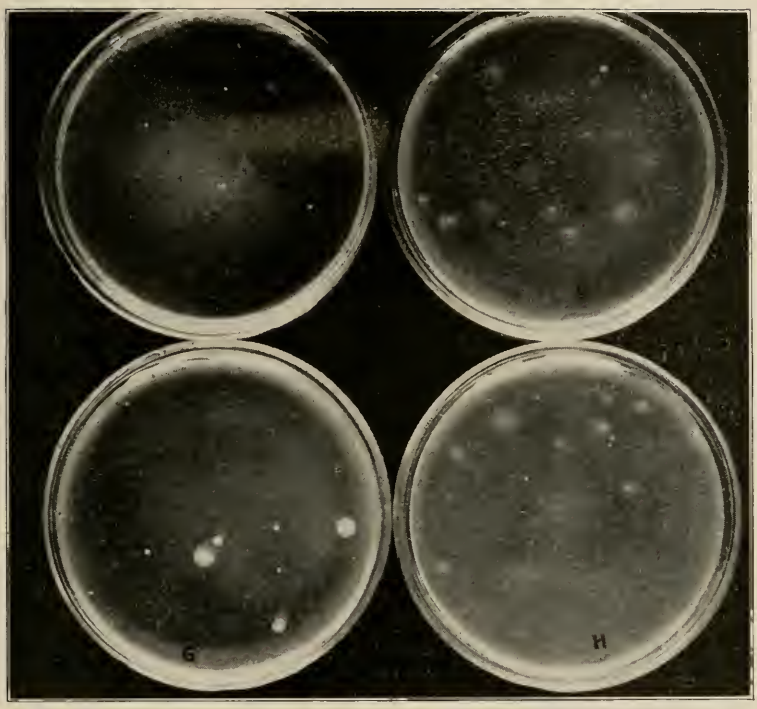


PLATE IV.

Some of plates from which organisms were obtained for tube media test V.

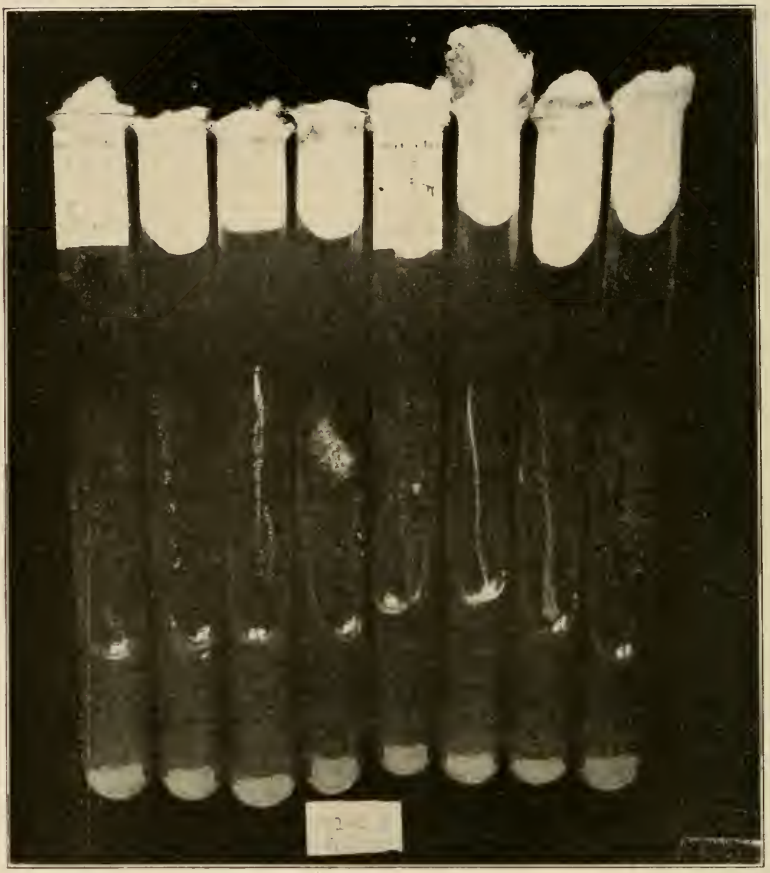


PLATE V.

At left: 4 organisms from L. and B. agar to wheat straw extract agar.
At right: 4 organisms from Na. asp. agar to wheat straw extract agar.

PLATE VI.

At left: 4 organisms from L. and B. agar to starch agar.
At right: 4 organisms from Na. asp. agar to starch agar.

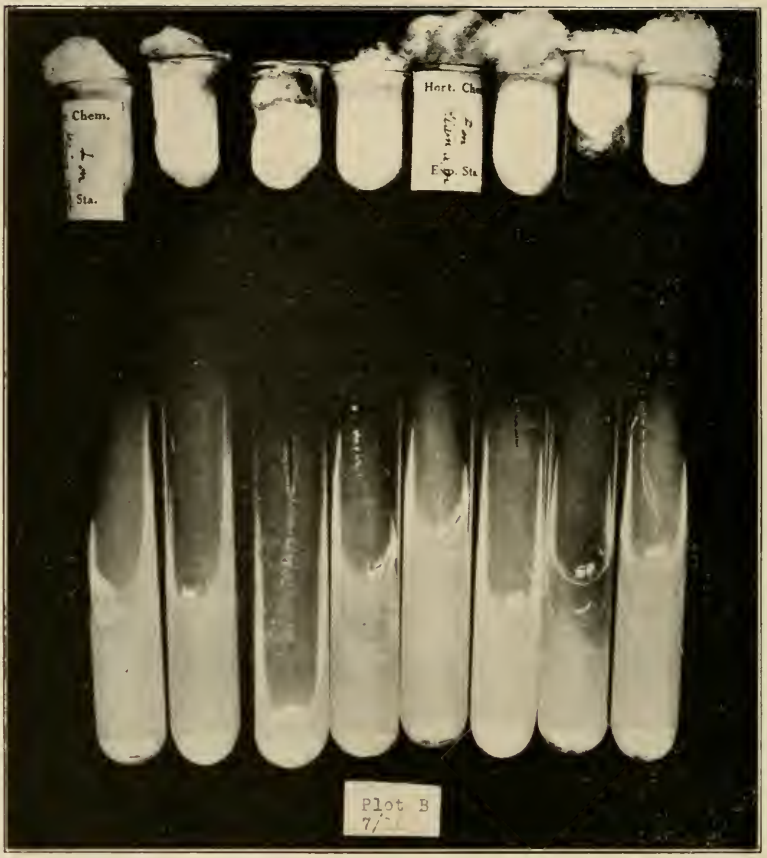


PLATE VII.

At left: 4 organisms from L. and B. agar to agar alone.
At right: 4 organisms from Na. asp. agar to agar alone.

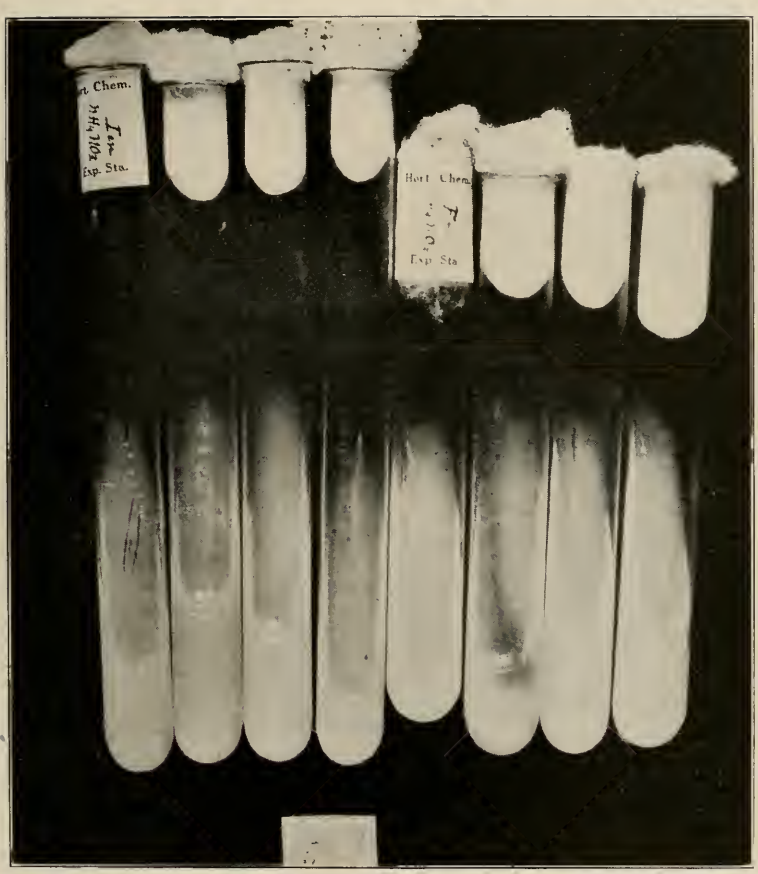


PLATE VIII.

At left: 4 organisms from L. and B. agar to ammonium nitrate agar.
At right: 4 organisms from Na. asp. agar to ammonium nitrate agar.

PLATE IX.

At left: 4 organisms from L. and B. agar to Na. asp. agar.
At right: 4 organisms from Na. asp. agar to Na. asp. agar.

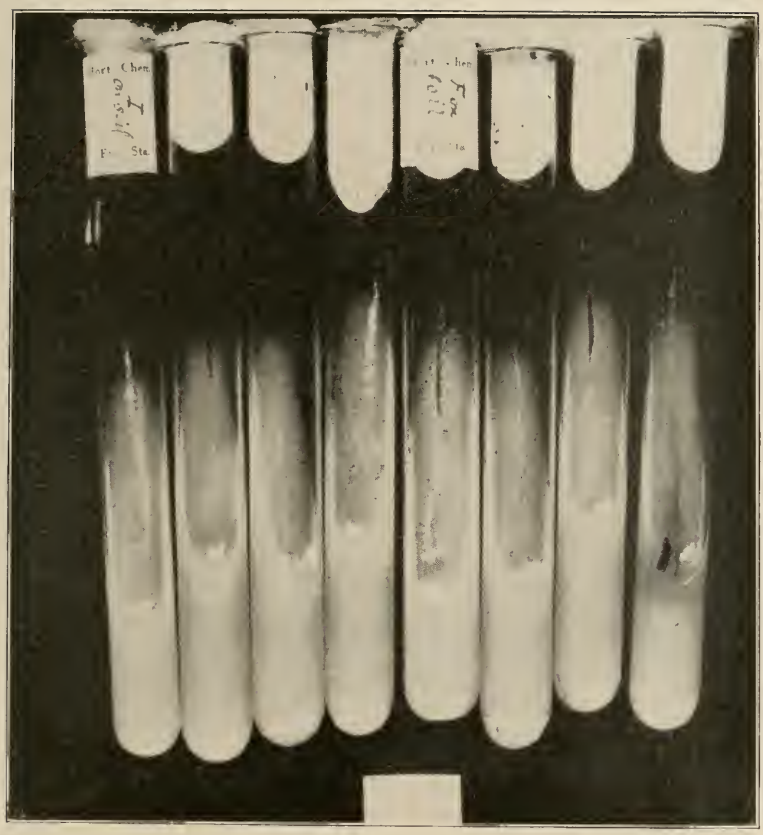


PLATE X.

At left: 4 organisms from L. and B. agar to soil and agar.
At right: 4 organisms from Na. asp. agar to soil and agar.

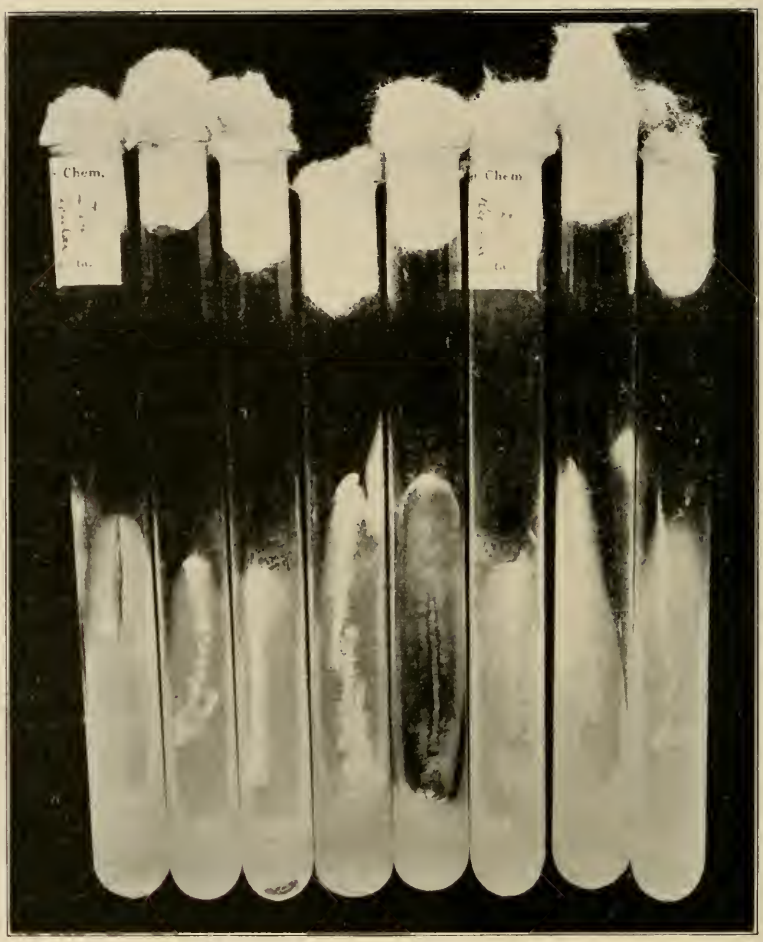


PLATE XI.

At left: 4 organisms from L. and B. agar to L. and B. agar.
At right: 4 organisms from Na. asp. agar to L. & B. agar.

PLATE XII..

Extreme left: 4 organisms from agar alone on Na. asp. agar.
Left center: 4 organisms from agar alone on L. and B. agar.
Right center: 4 organisms from Na. asp. agar on Na. asp. agar.
Extreme right: 4 organisms from Na asp. agar on L. & B. agar.