

siderable tonic contraction and the increased tonus was usually maintained.

In the case of the esophagus and cardia, Carlson, Boyd and Percy<sup>3</sup> suggest that the inhibition resulting from direct or reflex stimulation of the vagus nerve is due to the existing state of tonus. While this hypothesis would explain some of our results, a great many of them are not consistent with it, and we are forced to conclude that another factor is involved. We are continuing these experiments to obtain further data.

Tables I and II summarize the results of the inhibitory effect of pilocarpine.

TABLE I.

Intestine Contracted by Acetyl choline	No. Trials	Effect of Pilocarpine		
		No. Relaxed	No. Contracted	No. No effect
Duodenum	19	17	1	1
Jejunum	21	16	0	5
Ileum	11	8	1	2
Distal colon	8	6	1	1
Proximal colon	4	3	0	1
Mesial colon	3	3	0	0

TABLE II.

Intestine Contracted by Physostigmine	No. Trials	Effect of Pilocarpine		
		No. Relaxed	No. Contracted	No. No effect
Duodenum	4	4	0	0
Jejunum	3	3	0	0
Ileum	4	3	0	1
Distal colon	3	2	0	1

### 7894 C

#### Use of Platform Method of Growth in Demonstrating Pigments of Certain Pathogenic Fungi.\*

JOHN W. WILLIAMS.

*From the Department of Biology and Public Health and the Homberg Memorial Infirmary, Massachusetts Institute of Technology, Cambridge, Mass.*

The platform method of growth has been described.<sup>1, 2</sup> A piece

<sup>3</sup> Carlson, A. J., Boyd, T. E., and Pearey, J. F., *Arch. Int. Med.*, 1922, **30**, 407.

\* Contribution No. 49 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

<sup>1</sup> Williams, John W., *Science*, 1934, **80**, 2064.

<sup>2</sup> Williams, John W., *Arch. Dermat. and Syph.*, in publication.

of blotting paper is bent so that there is a horizontal portion which will just fit into a test tube with 2 legs to act as supporting members. The liquid medium is added almost to the horizontal portion. The tubes are plugged with cotton and sterilized. The plant is made on the horizontal portion. The 2 legs keep the plant supplied with moisture and nutrient by absorption. Care must be taken that the blotting paper contains no substance inhibitory to the organism.

In these experiments the organisms were allowed to grow 6 weeks. The following tubes were planted: pulp tubes with 1% peptone, 4% dextrose and 1% asparagin, 4% dextrose; broth tubes with 1% peptone, 4% dextrose and 1% asparagin, 4% dextrose. The pH was adjusted to 5.6. Growth took place at room temperature in diffused light.

The organisms studied were: *Achorion schoenleinii*, *Epidermophyton cruris*, *Epidermophyton rubrum*, *Indiella americana*, *Microsporon felineum*, *Monosporon apiospermum*, *Trichophyton granulolum*, *Trichophyton gypseum asteroides*, *Trichophyton gypseum lacticolor*, *Trichophyton niveum*, *Trichophyton purpureum*, *Trichophyton sulfureum*.

*Achorion schoenleinii*. Asparagin medium. In broth there is a small mycelial, intact growth, continuous with the sunken plant; this growth is white and on its surface is a shredded appearance resembling poor grade cotton; all growth is submerged and there is slight milkiness of the broth. On the platform there is sparse growth.

Peptone medium. On broth there is a wrinkled, pellicle of light tan covered with sparse powder; the pellicle tends to form irregular small cones at the medium centrally; there is a small bit of fuzzy mycelium in the bottom of the tube; the medium is amber colored. On the platform is a low sparse growth, very little extending down the stilts and a brown color where the mycelia stick to the glass of the tube; there is no growth in the broth, but the medium is somewhat lighter than the control.

*Epidermophyton cruris*. Asparagin medium. On broth is a floater most of which is submerged, white with cottony bits hanging as roots in the broth. On the platform is a low, sparse white mycelial growth extending only a short way down the stilts.

Peptone medium. On broth is a white pellicle from which hangs a loose, slightly layered mycelium becoming looser and less as the sunken plant at the bottom of the tube is approached; there is a suggestion of amber at base and sunken mycelium. On the platform is a low mycelial growth extending down the stilts to the medium; where the growth touches the glass is a light caramel

tint; this tint is imparted slightly to the medium; there are no growths in the liquid. On peptone the medium with growth becomes lighter than the control.

*Epidermophyton rubrum*. Asparagin medium. There is a loose fuzzy, white mycelial growth (some portions slightly tinted pink) on the platform extending a short way down the stilt but not to the medium; there is a reddish pink color at the base and where the mycelia adhere to the glass; there is no growth in the medium. On broth the growth is loose, mycelial, shows a tint of yellow at the pellicle base, shows slight layering and extends from a sunken plant to a mycelial pellicle on the surface.

Peptone medium. On the platform is a mycelial growth with a port wine base which extends down the stilt, attempts to form a pellicle at the medium where it may show a light brown base; there is variation in the mycelia, some being coarse and as port wine colored spicules; there may be colored frosting on the platform; when the mycelia are coarser it seems the color imparted is more a port wine and when the mycelia are finer and whiter this color shows a tendency to browning; where there is more browning there tends to be more powderiness. On broth the growth is loose, mycelial and somewhat layered, apparently grown up from the sunken plant to form a downy pellicle; there is pigment of a light yellow at the base of the pellicle.

*Indiella americana*. Asparagin medium. In broth there is a bit of growth of a verrucose-like nature at the bottom of the tube. There is practically no growth on the platform but a few small balls of growth at the bottom of the tube.

Peptone medium. On broth a somewhat dense mycelial growth extends from a sunken plant  $\frac{1}{3}$  way up the medium; the surface of the growth seems as though powdered with a light colored cinnamon; at its edge are very fine rootlets of mycelia adherent to the glass. On the platform is sparse mycelial growth; this growth stains the paper a light grayish brown.

*Microsporon felineum*. Asparagin medium. The growth is a floater from which extends a fine feathery mycelial growth into the medium for a short way; the medium is slightly milky. On the platform is a mycelial growth sending a long coned tongue down one stilt into the medium and holding the broth to the base of the plant proximal to one wall of the tube; there is a caramel color at the base of the growth and where it touches the glass. The medium is slightly lighter than the control.

*Monosporon apiospermum*. Asparagin medium. On broth there

is loose, white mycelial growth from a sunken plant toward the surface; there is a suggested layering but no color; the growth surface is downy. On the platform the growth is sparse, white, fuzzy, mycelial, extending a short way down the stilt but not to the medium; there is no growth in the medium.

Peptone medium. A mycelial growth fills the broth extending from a sunken plant to form a pellicle which shows slight black in areas. On the platform is a somewhat soft compact white mycelial growth extending down stilt to medium where it forms a partial cone into the medium; a light caramel coloration shows especially where the growth approximates the stilt. The medium is slightly lighter color than the control broth.

*Trichophyton granulorum*. Asparagin medium. Growth is most extensive in broth at a white, loose mycelial pellicle from which scant, filmy, loose mycelium extends in diminishing amounts to a sunken plant. On the platform is a white, mycelial, downy growth extending only a slight way down the stilt.

Peptone medium. In broth there is a fairly thick mycelial pellicle, pink where the mycelia touch the glass, blending into a light brown at the edge of the base and a brownish green at the base centrally; from the base hangs a bit of very loose mycelia having a greenish hue becoming looser and less extensive as it extends to the sunken plant in the bottom of the tube. On the platform there is thick, soft, downy mycelium extending down the stilts to the medium where there is attempted pellicle formation; where the mycelia touch the glass is a deep brown pigment and where growth touches the medium is a light yellowish-brown pigment; the medium is a shade lighter than the control broth.

*Trichophyton gypseum asteroides*. Asparagin medium. In broth mycelial growth fills the bottom  $\frac{1}{3}$  extending from the sunken plant like a powder puff; between sunken plant and upper surface of growth is an irregular band of yellowish-green pigment; on surface of plant and in medium above is a slight pink hue. There is sparse growth on the platform.

Peptone medium. In broth there is a partial pellicle attached to glass on whose surface is white, fuzzy mycelium and at whose base and where attached to the glass a deep brown grey pigment; a tuft of mycelial growth extends from the partial pellicle into the broth where it floats like a fringe; in the medium which is deep amber colored are sparse bits of growth. There is slight growth on the platform which shows brownish to red tinted where it touches the glass; the growth does not extend down the stilts and there is none

in the medium which is faintly darker than the control peptone broth.

*Trichophyton gypseum lacticolor*. Asparagin medium. On broth is a powdery, low mycelial pellicle with a suggested pink tint; from the pellicle extends loose mycelium decreasing in amount to the sunken plant below; a portion of this mycelium has a faint brown tint. There is a soft downy mycelial growth on the platform extending down the stilts almost to the medium.

Peptone medium. On broth is a bowl-shaped pellicle whose upper surface is light tan, powdery with low mycelium and whose under surface is a brown; a loose mycelium extends from the under surface to the sunken plant at the bottom of the tube; the broth is amber color. On the platform is a low mycelial growth extending down the stilts to the medium where a tongue and cone are formed to the medium; on the horizontal portion is a pink powder; the medium is a shade darker than the control.

*Trichophyton niveum*. Asparagin medium. On broth a loose, fluffy growth extends from almost the surface to a yellow tinted sunken plant below. A white mycelium covers the horizontal portion of the platform and extends a short way down the stilt.

Peptone medium. On broth is a thick white mycelial pellicle; where the mycelia touch the glass is a pink tint; at the base and where the medium touches the plant is a caramel color; the pellicle sends a tongue into the medium; this tongue is wet by the medium on one side and mycelial on the other; a small amount of mycelial growth is attached to the sunken plant below. On the platform is a profuse soft white mycelial growth extending down the stilt to the medium where a cone and attempted pellicle is present. There are a few balls of fluffy mycelial growth in the medium which is a shade lighter than the control; where the mycelia touch the glass above is a brown pigment.

*Trichophyton purpureum*. Asparagin medium. On broth there is a loose, mycelial growth extending upward from the sunken plant below but not reaching the surface, its upper surface is slightly shaggy. The platform shows sparse growth.

Peptone medium. The growth on broth comes from the sunken plant, is loose, hair-like mycelial and continuous with itself; this growth gradually reaches the surface where it forms a white pellicle with a yellow base. On platform is a white soft mycelial growth extending down the stilts to the medium where it grows across as a pellicle; where the growth touches the glass and at its

base is a port wine color; there is no growth in medium; the medium is a shade darker than the control.

*Trichophyton sulfureum*. Asparagin medium. On broth there is a small amount of cottony growth sediment. On the platform the growth is sparse.

Peptone medium. On broth is a powdery white pellicle whose base is a light amber and from which hangs a loose friable mycelium extending to the sunken plant below; a light amber tint is present especially to areas of the growth. On the horizontal portion of the platform is a soft, sparse white growth, brown where the mycelium touches the glass; the broth is faintly darker than the control.

This method of growth is important whether or not there is a soluble pigment. If so, the platform can be removed and the pigment recovered. If the pigments are not water soluble the broth will be the color of the control broth which is kept for comparison. The morphology of the growths is mentioned here but is covered elsewhere in detail.<sup>2</sup>

In the instance of non-water soluble pigment the growth can be stripped from the blotting paper and the colored blotting paper used for comparison and testing of the pigment with reference to change in color to reaction, etc. The pinks which indicate an increase in alkalinity, since they brown on adding HCl, are changed in color by autoclaving but left less harmed by 10% formalin.

It will be noted that some broths with growths are lighter than the controls. Without further evidence the significance of this observation is not apparent.

This method of bringing out color is considered valuable from the point of view of contrast, as a method of preserving the color for later comparison, as a method of recovering pigment for testing, as a means of determining relative color production on different media, in determining reaction change in media with growth where the color varies with pH and as a method of organismal differentiation. Another possible application is where a colored ingredient is a part of the culture medium. If this is selectively utilized by the organism one should expect the color intensity of the medium due to this substance to decrease.