

It should be emphasized, however, that the titer of the hamster serum following infection is usually not so high as that of the ferret and it is therefore advisable to use not more than 100 MLD of virus in performing the neutralization-test.

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A Selective Medium for Isolation of *Hemophilus influenzae*.

EMANUEL B. SCHOENBACH AND LEAH R. SEIDMAN. (Introduced by J. F. Enders.)

From the Department of Bacteriology and Immunology, Harvard Medical School, and School of Public Health, Boston, Mass.

The isolation of Gram negative pathogenic microorganisms from the nasopharynx is often difficult because of overgrowth by Gram positive cocci. This difficulty is increased when the organism requires an enriched medium which favors the growth of normal flora. Fleming¹ inoculated plates with penicillin and noted that Gram positive cocci could be inhibited and *H. influenzae* obtained in relatively pure culture.

The determination of the incidence of *H. influenzae* in the nasopharynx, because of its possible relation to the severity of influenza epidemics, is of considerable importance. A more convenient method of isolation than those available at present would be of obvious value. Accordingly we investigated the effect on this organism of the bactericidal agent, tyrothricin, isolated by Dubos.²

The tyrothricin was prepared according to the method of Dubos.^{2, 3, 4} Following saline precipitation, it was kept in the form of a stock alcoholic solution (8 mg/100 cc). The tyrothricin was diluted in 5% glucose in distilled water. It was incorporated into the medium by placing 1.5 cc of the dilution to be studied in a Petri plate and adding 15 cc of Fildes' peptic blood digest agar at 45°C. The plates were allowed to dry at room temperature and 0.1 cc of inoculum spread over the surfaces. Chocolate agar plates were similarly made containing tyrothricin. A heavy inoculum of *Staphylococcus aureus*, mixed with varying dilutions of *H. influenzae* was used.

¹ Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

² Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1.

³ Dubos, R. J., and Hotchkiss, R., *J. Biol. Chem.*, 1940, **136**, 803.

⁴ Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.

It is clear from the data included in Table I that *H. influenzae* is resistant to the action of tyrothricin, and that by incorporation of this substance in the medium a selective killing of this strain of staphylococcus was obtained. It will be noted that in the higher dilutions of tyrothricin, due to the obscuring effect of the staphylococcus only a few colonies of *H. influenzae* were evident. The number of colonies of influenza bacilli was not reduced even in a 1:100 dilution of the stock alcoholic tyrothricin solution, although at this concentration the colonies were somewhat smaller and showed less iridescence. The bacilli from the medium containing tyrothricin exhibited typical morphology, reacted with capsular swelling in type-specific rabbit serum, and were still viable on transfer after 6 days.

Attempts to use the tyrothricin in the starch casein hydrolysate medium of Mueller and Hinton⁵ for selective isolation of meningococci and gonococci were unsuccessful. Tyrothricin inhibited the growth of these Gram negative cocci and staphylococci in the same dilutions. Further purification and the use of recrystallized gramicidin also failed to give differential inhibition of the staphylococci. Dubos⁶ has noted that gramicidin kills the meningococcus and the gonococcus. This bactericidal action is apparently an exception to

TABLE I
Effect of Tyrothricin on Mixed Cultures of Staphylococcus and *H. influenzae*

Inoculum*	Final dilutions of tyrothricin in plates of peptic digest agar				
	1:110	1:1100	1:11,000	1:110,000	1:1,100,000
Staphylococcus 10 ⁻²	0	0	+	+++	++++
<i>H. influenzae</i> 10 ⁻⁶	72 col.†	Colonies not well separated	43 col.	76 col.	75 col.
Staph., 10 ⁻² } Flu., 10 ⁻⁶ }		78 col. <i>H.</i> <i>influenzae</i> . No staph.	Moderate growth of staph. Scattered col. <i>H. influ-</i> <i>enzae</i>	Heavy staph. No <i>H. influ-</i> <i>enzae</i> visible	Heavy staph. No <i>H. influ-</i> <i>enzae</i> visible
Staph., 10 ⁻² } Flu., 10 ⁻⁴ }	<i>H. influenzae</i> only†	<i>H. influenzae</i> only	Mixed <i>H.</i> <i>influenzae</i> and staph.	Heavy staph. Occasional col. <i>H. influ-</i> <i>enzae</i>	Heavy staph. Occasional col. <i>H. influ-</i> <i>enzae</i>

*By plate count without tyrothricin 0.1 cc of 10⁻² dilution of staphylococcus culture contained 196,000 organisms.

0.1 cc of 10⁻⁶ dilution of *H. influenzae* culture contained 68 organisms.

†At this concentration of tyrothricin, the colonies of mucoid *H. influenzae* were reduced in size, and showed markedly less iridescence.

⁵ Mueller, J. H., and Hinton, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 330.

⁶ Dubos, R. J., *The Harvey Lectures*, 1939-1940, p. 223.

the restriction of the activity of gramicidin to Gram positive organisms.

The media containing tyrothricin were then tested for the cultivation of material obtained from apparently healthy subjects. The region of the pharynx was swabbed with a sterile cotton-tipped applicator which was then placed in about 1 cc infusion broth. After incubation for 1 hour a drop of the broth was placed on each of four media: Fildes' peptic digest agar, Fildes' peptic digest agar containing tyrothricin (10^{-3}), chocolate agar and chocolate agar containing tyrothricin (10^{-3}). The difference between the media containing tyrothricin and the ordinary media was strikingly apparent. On chocolate media the change in color about streptococci and staphylococci was intense and few individual colonies were apparent, while the media containing the tyrothricin revealed no discoloration and isolated colonies were easily discerned. The peptic digest medium containing tyrothricin was also more satisfactory than its control. Gram positive organisms were conspicuously absent.

Isolation of *H. influenzae* was greatly facilitated and a somewhat larger number of isolations were made on the media containing tyrothricin than on the others. Although the ease of isolation cannot be illustrated in a table, the results of the number of *H. influenzae* isolated in each case are of interest (Table II).

No attempt was made to pick colonies that might resemble *H. hemolyticus* although in a number of cases this organism was isolated.

Conclusion. Through the use of a medium containing tyrothricin in a concentration inhibitory to Gram positive cocci, isolation of *H. influenzae* from the nasopharynx may be facilitated. This medium is being tested in epidemiological surveys now in progress.

TABLE II.
Isolation of *H. influenzae* on Media with and without Tyrothricin.

	No. cultures examined	<i>H. influenzae</i> isolated
Tyrothricin in Fildes' medium	130	23
Fildes' medium alone	86	10
Chocolate and tyrothricin	86	6
Chocolate alone	86	0

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