STARCH-FERMENTING, GELATIN-LIQUEFYING CORYNEBACTERIA AND THEIR DIFFEREN- TIATION FROM *C. DIPHTHERIAE GRAVIS*

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(RECEIVED FOR PUBLICATION DECEMBER 4, 1951)

During recent years attention has been drawn to the isolation of starch-fermenting, gelatin-liquefying corynebacteria from acute cases of sore throat (Jebb, 1948; Saxholm, 1951). Fourteen strains have been isolated in this laboratory between January, 1946, and June, 1951, 12 from acute tonsillar infection, one from the throat of a symptomless carrier, and one from a lesion on the hand. In addition, a further 31 strains from other laboratories have been investigated by one of us (W. H. H. J.). With the decrease in the number of cases of *gravis* diphtheria these 14 strains formed a considerable proportion (35%) of all starch-fermenting corynebacteria isolated in this laboratory during the last five years (Table I). The strains can be distinguished from *C. diphtheriae* both biochemically and because they produce two toxins, only

TABLE I

NUMBER OF STRAINS OF *C. diphtheriae gravis* AND STARCH-FERMENTING, GELATIN-LIQUEFYING CORYNEBACTERIA ISOLATED BETWEEN JANUARY, 1946, AND JUNE, 1951

Year	Strains of <i>C. diphtheriae gravis</i>	Starch-fermenting, Gelatin-liquefying Strains
1946	15*	3
1947	5	4
1948	3	2
1949	2	1
1950	1	2
1951 (Jan.-June)	0	2
Total	26*	14

* Including seven strains isolated from two small outbreaks.

one of which is neutralizable by diphtheria antitoxin (Petrie and McClean, 1934), but as they may be confused with *C. diphtheriae gravis* their correct and rapid identification is a matter of some interest and importance.

Until recently this identification has taken some time; thus gelatin liquefaction may take up to 10 days, trehalose fermentation up to 16 days (Jebb, 1948), and nitrate broth is commonly incubated for five days before testing by the Griess-Ilosvay method (Wilson and Miles, 1946). The most rapid method in use heretofore

has been intradermal inoculation into protected and unprotected guinea-pigs (Jebb, 1948), but even this, allowing time for the preparation of test and control cultures and the development of the lesion, may take several days.

Diagnostic Methods

All strains of starch-fermenting, gelatin-liquefying corynebacteria tested by us for urea hydrolysis and nitrate reduction have split urea rapidly and have failed to reduce nitrates; similar results have been reported by Saxholm (1951). *Gravis* strains, on the other hand, are generally considered to reduce nitrates but not to hydrolyse urea; all such strains tested by us have behaved in this way. Tests for urea hydrolysis and nitrate reduction provide a rapid method for distinguishing these two organisms. By the use of a urea slope (Christensen, 1946) and a nitrate strip on a blood agar plate (Cook, 1950) an indication of the nature of the culture being tested can be obtained within 18 hours and frequently within six hours.

In view of the atypical colonial appearances sometimes shown by corynebacteria it is suggested that the following procedure be followed with all organisms which on primary isolation colonially and morphologically appear to be diphtheria bacilli. A single colony is picked from the tellurite agar or blood agar plate to a Löffler serum slope. The slope is incubated for six to eight hours and then are inoculated (1) a blood agar plate (for purity, haemolysis, and colonial appearances), (2) starch, glucose, maltose and sucrose serum-water "sugars," and serum broth, (3) a urea slope, and (4) a second blood agar plate to which a nitrate strip is then added. The reactions of the three types of *C. diphtheriae* and of the starch-fermenting, gelatin-liquefying strains are summarized in Table II. The results of urea hydrolysis and nitrate reduction will be available after overnight incubation and will form a basis for further investigations, though some of the fermentation reactions may

TABLE II
RAPID BIOCHEMICAL REACTIONS OF THE THREE TYPES OF *C. diphtheriae* AND OF STARCH-FERMENTING, GELATIN-LIQUEFYING STRAINS

Strain	Fermentation Reactions				Nitrate Reduction	Urea Hydrolysis
	Glucose	Maltose	Sucrose	Starch		
<i>C. diphtheriae mitis</i>	AC	AC	—	—	usually +	—
<i>C. diphtheriae intermedius</i>	AC	AC	—	—	+	—
<i>C. diphtheriae gravis</i>	AC	AC	—	AC	+	—
<i>Starch-fermenting, gelatin-liquefying strains</i>	AC	AC	—	AC	—	+

AC = acid and clot; + = positive reaction; — = no fermentation or negative reaction.

occasionally take rather longer to develop. If the organism appears to be a *mitis* strain of *C. diphtheriae* subcutaneous virulence tests can then be performed in the usual way, while with *intermedius* and *gravis* strains isolated from clinical cases no further action will probably be necessary. Starch-fermenting strains which do not hydrolyse urea and yet colonially and morphologically do not appear to be typical *gravis* strains should be tested for virulence by subcutaneous guinea-pig inoculation. Starch-fermenting strains which hydrolyse urea should always be further investigated

by intradermal inoculation into protected and unprotected guinea-pigs and possibly by testing for gelatin liquefaction and trehalose fermentation.

Many starch-fermenting, gelatin-liquefying strains produce two toxins, one of which is neutralizable by diphtheria antitoxin and the other resembles that of *C. ovis*. It is, therefore, important that animal inoculations with these strains be performed by the intradermal route, as the results of the classical subcutaneous virulence test may be misleading. If the subcutaneous route is used the unprotected guinea-pig may die within 24 to 48 hours with the post-mortem appearances of diphtheria infection, while 500 units of diphtheria antitoxin may protect the other guinea-pig indefinitely (Mair, 1928). If death should supervene, the post-mortem appearances are not characteristic of diphtheria but resemble those of infection with *C. ovis* (Barratt, 1933 ; Petrie and McClean, 1934). On intradermal injection, however, starch-fermenting, gelatin-liquefying strains produce a characteristic ulcerating lesion which is not neutralized by 1,000 units of diphtheria antitoxin.

Discussion

The pathogenicity and epidemiological significance of starch-fermenting, gelatin-liquefying strains are still in doubt, but their more frequent recognition in recent years is of interest. It is possible that many of them are regarded as atypical *gravis* strains and that their incidence is greater than is generally realized. Morphologically and colonially they may resemble *C. diphtheriae gravis*, and the fermentation reactions of both organisms in the usual diagnostic "sugars" are identical. Virulence tests are frequently omitted with *gravis* strains, but when they are done the subcutaneous method is usually chosen ; as has already been stated starch-fermenting, gelatin-liquefying organisms may also be expected to kill an unprotected guinea-pig on subcutaneous inoculation with the production of post-mortem changes characteristic of diphtheria. It is suggested that the routine use of tests for urea hydrolysis and nitrate reduction with all starch-fermenting corynebacteria may help to identify these strains and so reveal their true incidence.

Most of the starch-fermenting, gelatin-liquefying cultures examined by us have been isolated during the summer months from sporadic cases or carriers among children or adults living in rural areas. This pattern of distribution may perhaps be related to the consumption of raw milk, and we have had evidence of the association of these organisms with milk on three separate occasions. In one infected family the organism was cultivated from the milk bottle which had contained raw milk supplied to the household. On another occasion the organism was isolated from the milk of a cow suffering from mastitis. Recently we have also isolated a strain from an indolent ulcer on the hand of a milker.

In general, infection with these organisms clinically resembles tonsillitis rather than diphtheria, though some membrane formation in the throat may be seen. The disease is usually mild, but the severity varies from one patient to another ; this may be an expression of the amount of diphtheria toxin produced by the particular strain and the state of immunity of the individual. Epidemics of this disease are unusual and most of the 45 strains investigated by us were isolated from single cases. As many of these strains elaborate a toxin which is neutralizable in part at least by diphtheria antitoxin (Petrie and McClean, 1934), it would appear to be wise to regard patients infected with these organisms as suffering from true diphtheria.

Summary

Starch-fermenting, gelatin-liquefying corynebacteria have formed 35.0% of all starch-fermenting corynebacteria isolated at this laboratory during the past five years.

These strains can be rapidly distinguished from *C. diphtheriae gravis* by their ability to hydrolyse urea and their failure to reduce nitrates. The inclusion of tests for urea hydrolysis and nitrate reduction in the routine examination of all suspected diphtheria organisms is suggested. Starch-fermenting corynebacteria which hydrolyse urea and fail to reduce nitrates should be examined for trehalose fermentation and gelatin liquefaction and inoculated intradermally into protected and unprotected guinea-pigs. It is important to use the intradermal route, as the results of subcutaneous inoculation may be misleading.

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