

A non-starch-fermenting variant of *Corynebacterium ulcerans*

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SYNOPSIS Recently a non-starch-fermenting strain of *C. ulcerans* was isolated and a strain which did not hydrolyse urea when first isolated has also been discovered. For full identification of *C. ulcerans* both intradermal and subcutaneous tests in guinea-pigs are required, and it is recommended that these tests should be carried out with all organisms which culturally and morphologically resemble diphtheria bacilli.

Corynebacterium ulcerans was originally described by Gilbert and Stewart in 1927. Later investigations by Barratt (1933), Jebb (1948), Saxholm (1951), and Cook and Jebb (1952) have provided further tests to distinguish *C. ulcerans* from other pathogenic corynebacteria.

Wilson and Miles (1964) state that *C. ulcerans* produces acid in glucose, maltose, starch, dextrin, and trehalose (slowly), that it liquefies gelatin, hydrolyses urea, and fails to reduce nitrate. Most strains form two toxins, one immunologically identical with diphtheria toxin and one related to the toxin of *C. ovis*. Intradermal injection into guinea-pigs produces a purulent necrotic lesion that is not prevented by diphtheria antitoxin.

Recently, one of us (T.D.M.M.) isolated a non-starch-fermenting variant of *C. ulcerans* from the throat of a schoolboy with a sore throat, of which this organism was considered to be the cause. The boy had been immunized against diphtheria. Apart from its failure to ferment starch this strain produced the typical reactions of *C. ulcerans*. On intradermal inoculation it produced ulcerating lesions within two days in two guinea-pigs which had received 20 units and 1,000 units respectively of diphtheria antitoxin. On subcutaneous inoculation, a guinea-pig protected by 500 units of diphtheria antitoxin, remained alive and apparently well for the 15 days it was kept under observation. An unprotected guinea-pig died in less than 48 hours. At necropsy there was a gelatinous oedema at the site of inoculation, free fluid in the peritoneal cavity, and the suprarenals were enlarged and haemorrhagic.

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DISCUSSION

From a clinical standpoint, the most important thing to do with any organism which culturally and morphologically resembles *C. diphtheriae* is to determine whether or not it produces diphtheria toxin. This can be done both with *C. diphtheriae* and *C. ulcerans* by subcutaneous inoculation into protected and unprotected guinea-pigs or by the use of an Elek plate (Elek, 1948; Elek, 1949; Jameson, 1965). Like *C. diphtheriae*, not all strains of *C. ulcerans* produce a toxin which can be neutralized by diphtheria antitoxin (Petrie and McClean, 1934).

For full identification of strains of *C. ulcerans*, however, further tests are required. Cook and Jebb (1952) recommended that all organisms which, on primary isolation, colonially and morphologically appeared to be diphtheria bacilli should be tested for urea hydrolysis, nitrate reduction, haemolysis, and fermentation of glucose, maltose, sucrose, and starch. The use of a blood agar plate, to which a nitrate strip is added after inoculation (Cook, 1950), provides a rapid method of testing for nitrate reduction. Cook and Jebb also recommended that starch-fermenting strains which hydrolyse urea should always be further tested by intradermal inoculation into protected and unprotected guinea-pigs and possibly also by testing for gelatin liquefaction and trehalose fermentation.

The results that will be found on intradermal and subcutaneous inoculation of guinea-pigs with virulent and avirulent strains of *C. diphtheriae* and with strains of *C. ulcerans* which produce diphtheria

TABLE I

Organism	Subcutaneous Inoculation		Intradermal Inoculation	
	No Diphtheria Antitoxin	500 Units of Diphtheria Antitoxin	20 Units of Diphtheria Antitoxin	1,000 Units of Diphtheria Antitoxin
<i>C. diphtheriae</i> (virulent)	Death in 1-4 days with enlarged and haemorrhagic suprarenals	Remains alive	Localized erythematous lesion followed by necrosis	No lesion
<i>C. diphtherias</i> (virulent)	Remains alive	Remains alive	No lesion	No lesion
<i>C. ulcerans</i> (which produces diphtheria toxin)	Death in 1-4 days with enlarged and haemorrhagic suprarenals	Remains alive ¹	Large ulcerating lesion	Large ulcerating lesion
<i>C. ulcerans</i> (which does not produce diphtheria toxin)	Remains alive ¹	Remains alive ¹	Large ulcerating lesion	Large ulcerating lesion

¹Occasionally these animals die; if they do the post-mortem appearances resemble those of infection with *C. ovis* (Barratt, 1933; Petrie and McClean, 1934). The suprarenals are not affected.

toxin and those which do not are set out in Table I. Since a non-starch-fermenting strain of *C. ulcerans* has now been isolated and Shore (1964) has isolated a strain which did not hydrolyse urea when it was first isolated, it seems advisable to carry out guinea-pig inoculations by both intradermal and the subcutaneous routes with all strains of organisms which culturally and morphologically resemble diphtheria bacilli.

Of the other methods of distinguishing between *C. diphtheriae* and *C. ulcerans*, gelatin liquefaction and trehalose fermentation may both take a week or more, and failure to reduce nitrate is not a wholly reliable differential test, since occasional strains of *C. diphtheriae* also fail to reduce nitrate (Jebb, 1948).

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