

60°C, and 65°C respectively the diminution in complement fixation was closely paralleled by the decrease in clotting activity.

Conclusions. These results give further

evidence of the correlation between clotting and complement activity and indicate that antigens possessing thromboplastic activity may give nonspecific complement fixation.

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A Note on the Relationship Between the Intermedius and Minimus Types of *C. diphtheriae*.*

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Frobisher¹ and Eller and Frobisher² described bacteriologically and epidemiologically an outbreak of diphtheria in Baltimore and called attention to 2 hitherto undescribed types of diphtheria bacilli. One of these is a virulent saccharose-fermenting variety. The other, with which the present communication deals, is characterized by the following special properties: (a) the production, on the cystine-tellurite agar, used routinely in these laboratories and previously described,³ of very small, often hardly visible colonies that are always flat, but which may be either rough or smooth; (b) failure to ferment dextrose promptly (*i.e.*, within 48 hours at 37°C), *when first isolated*. In the article referred to,¹ attention was also called to the fact that other small-colony strains of diphtheria bacilli were encountered during the epidemic described but that the term *minimus* type was reserved for the minute-colony strains which failed to ferment dextrose as do strains ordinarily encountered in Baltimore and tested under the same conditions.

It is well known that small-colony types of *C. diphtheriae* are not uncommon. Such forms have been described by Corbett and Phillips,⁴ Morton,⁵ and Mittag.⁶ However,

failure to ferment dextrose by such forms has not been described previously and it seemed desirable to designate so distinctive a variety by a special name, following the example of Anderson *et al.*⁷ Since publishing this description several workers, in personal communications, have suggested that the *minimus* type is in reality identical with the *intermedius* type described by Anderson *et al.* It is the purpose of this note further to clarify the status of the *minimus* type among other small-colony types.

First, as to colony form, the original description of the *intermedius*-type colony states that the colonies are small as are those of the *mitis* type. In other respects the organisms of the *intermedius* type are closely similar to the *mitis* type except that in broth cultures the *intermedius* type produces a granular sediment, rather than a smooth turbidity like the typical *mitis* strains. In this respect the *intermedius* strains are also like the *minimus* strains. No pictures of the *intermedius* colonies were shown in the original article,⁷ but colored plates were presented which illustrate the *gravis* and *mitis* colonies. In a monograph by Dudley⁸ a similar colorplate shows *intermedius* colonies and these are not at all like colonies of the *minimus* type.

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¹ Frobisher, M., Jr., Adams, M. L., and Kulins, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 330.

² Eller, C. Howe, and Frobisher, M., Jr., *Am. J. Hyg.*, 1945, **42**, 179.

³ Frobisher, M., Jr., *J. Inf. Dis.*, 1937, **60**, 99.

⁴ Corbett, L., and Phillips, G. C., *J. Path. Bact.*, 1897, **4**, 193.

⁵ Morton, H. E., *Am. J. Path.*, 1932, **8**, 605.

⁶ Mittag, G., *Zentr. Bakt. Parasitenk.*, 1937, I Orig., **138**, 426.

⁷ Anderson, J. S., Happold, F. C., McLeod, J. W., and Thompson, J. G., *J. Path. and Biol.*, 1931, **34**, 667.

⁸ Dudley, S. F., May, P. M., and O'Flynn, J. A., *Active Immunization Against Diphtheria*, His Majesty's Stationery Office, 1934.

Second, no mention was made by Anderson *et al.*⁷ of unusual fermentative properties of the *intermedius* type as originally described. It was stated by those workers that the fermentation reactions of the *intermedius* type were normal, *i.e.*, *mitis*-like. This is definitely unlike the *minimus* type. It would appear then, that *intermedius* and *minimus* strains are 2 distinct varieties of small-colony types of diphtheria bacilli.

In order to clarify still further the difference between the *intermedius* and *minimus* types several strains of each were cultivated side by side on the same Petri plates and in tubes of dextrose broth all made from the same lot of medium and inoculated at the same time. It was our good fortune to be able to make such a comparison due to the kindness of Dr. J. W. McLeod, of the University of Leeds, in sending us some time ago (1936), a collection of *gravis*, *mitis*, and *intermedius* strains. The growth on the slants as originally received from England was suspended at that time in a few drops of defibrinated rabbit's blood and immediately desiccated. They remained undisturbed for 10 years. This technic and its value have recently been described by Frobisher, Pai and Hakim.⁹ In February 1946, the 10 *intermedius* strains from Leeds were taken from the vacuum jar, transferred to blood-dextrose broth to revive them and, when growth had developed (24 hours at 37°), each of 10 plates of cystine-tellurite agar was streaked on one-half of its surface with one of the 10 cultures. The other half of each plate was inoculated in exactly the same way with one of 5 *minimus* strains which had been desiccated in exactly the same manner as the English strains, but for only a few months. Simultaneously, tubes of heart-infusion broth containing 1% dextrose were respectively inoculated with one drop of a broth culture of each of the 15 cultures being compared.

After the usual period of incubation (48 hours at 37°) the growth on the plates was examined. On 4 of the plates there was no difference between the *minimus* cultures and

those labeled *intermedius*. On the other 6 plates the *intermedius* colonies were larger, more raised, darker, smoother and more glistening, *i.e.*, clearly more *mitis*-like than the *minimus* colonies. A differentiation between the 2 types of growth could easily be made with the unaided eye. However, both would certainly be classed as dwarf- or small-colony variants.

The point of particular interest was that, on examining the dextrose broth the *minimus* cultures and the 4 Leeds cultures the colonies of which were of the *minimus* type, were found to have remained alkaline. No fermentation occurred during 7 days of incubation. The 6 cultures from Leeds the colonies of which were more *mitis*-like, had produced marked acidity in 48 hours. It was clear that of the 10 Leeds strains, all were definitely of the small-colony variety, but 6 were of the type known as *intermedius*, whereas the other 4 were of the type we have designated *minimus*.

The question of growth in the broth tubes requires some discussion. The *minimus* strains, whether from Leeds or Baltimore, do not grow as luxuriantly on any media so far tested, as do the *mitis*, *gravis*, *indeterminate*, or *intermedius* strains. This is doubtless a reflection of their small-colony formation on solid media. However, they grow sufficiently in broth so that one would reasonably expect dextrose to be fermented were the organisms capable of doing so. After several subcultures on artificial media they acidify dextrose broth and then grow more vigorously, but they still grow as sparsely on media not containing dextrose. Their failure to acidify dextrose broth immediately after isolation is in contrast with other strains; a distinctive feature, whatever its explanation.

Summary. A comparative study was made of *intermedius*-type strains of *C. diphtheriae* obtained from Dr. J. W. McLeod, Leeds, England and *minimus*-type strains described by Frobisher in Baltimore. The *minimus* type was found to be a distinct colony variant and was differentiated from strains described as *intermedius* by Anderson, *et al.* Other small-colony variants have also been found differing from both the *intermedius* and *minimus* types.

⁹ Frobisher, M., Jr., Pai, S. E., and Hakim, S., in preparation.