The Serological Identity of Sabin's Murine type C *Mycoplasma* and *Mycoplasma pulmonis*

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SUMMARY

Serological evidence is presented that Sabin's murine type C mycoplasma belongs to the species *Mycoplasma pulmonis* and not to a separate species, *M. histotropicum*.

Between 1938 and 1941, several apparently distinct mycoplasmas were isolated from mice by Sabin (1938, 1939 *a, b*, 1941). These organisms were designated types A, B, C, D and E and assigned to three species, '*Musculomyces neurolyticus*' (type A), '*Musculomyces arthrotropicus*' (type B) and '*Musculomyces histotropicus*' (types C, D, E) (Sabin, 1941). The toxinogenic type A was evidently the same as the organism first isolated by Klieneberger from mice with rolling disease (Findlay, Klieneberger, MacCallum & Mackenzie, 1938; Klieneberger, 1940) and later classified as *Mycoplasma neurolyticum* (Edward & Freundt, 1956). However, the relationship of types B, C, D and E to the other recognized rodent mycoplasmas, *M. pulmonis* and *M. arthritis* (Edward & Freundt, 1956) could not be determined from Sabin's descriptions. In 1963, types A and C were recovered from cultures which had been lyophilized in 1943 (Tully & Ruchman, 1964): type A proved to belong to the species *M. neurolyticum*, while type C was provisionally designated, on the results of indirect fluorescent antibody tests, as *M. histotropicus* (Tully, 1965), a species distinct from the other rodent mycoplasmas. Since *Mycoplasma* is a neuter noun, with which the specific epithet must agree, the name of this organism was corrected by Edward & Freundt (1969) to *M. histotropicum*. More recently, the electrophoretic pattern of the cell proteins of type C (*M. histotropicum*, PG40, obtained from Dr D. G. ff. Edward) was found to resemble that of *M. pulmonis* strains (Razin, 1968). We show here that two lines of type C are antigenically indistinguishable from *M. pulmonis*.

A culture of type C, which had undergone 60 passages since recovery from Sabin's lyophilized material, was received from Dr J. G. Tully (National Institutes of Health, Bethesda, Md., U.S.A.) and examined at the Lister Institute in 1964. The culture was cloned by selecting a single colony at each of two successive subcultures, and tested by complement-fixation and gel-diffusion (Lemcke, 1964, 1965) and by an agar growth-inhibition technique (Clyde, 1964) against antisera to 17 serologically distinct mycoplasmas (Lemcke, 1964). It reacted only with antisera to two strains of *Mycoplasma*

pulmonis, KON from the lung of a rat and M I from the lung of a mouse (Lemcke, 1961). No reaction occurred with antisera to *M. hominis* (strain H34), *M. pneumoniae* (FH), *M. salivarium* (B3), *M. orale*, type I (823), *M. fermentans* (G2), *M. arthritis* (CAMPO, PG27), *M. neurolyticum* (KSA), *M. gallinarum* (FOWL), *M. gallisepticum* (T), *M. laidlawii* (type A), *M. agalactiae* (agalactia), *M. bovigenitalium* (PG11), *M. mycoides* var. *mycoides* (GLADYSDALE), *M. mycoides* var. *capri* (pp. goat) or two strains, NAVEI and A36, not yet accorded species rank.

More recently, antiserum was prepared against the same cloned line of type C, and further complement-fixation, gel-diffusion and growth-inhibition tests were made with this antiserum and antisera to KON and M I. The gel-diffusion antigens were mycoplasma suspensions lysed with the non-ionic detergent Triton X-100 and not the sonicated suspensions used previously (Lemcke, 1965). The complement-fixation and growth-inhibition tests confirmed the close relation of the three organisms (Table 1). In gel-diffusion tests, reactions with homologous antisera tended to be more complex than those with heterologous antisera, but the majority of the antigens was shared by all three strains (Table 1).

Table 1. *Reactions of type C and strains M I and KON of Mycoplasma pulmonis in complement-fixation (CF), growth-inhibition and gel-diffusion tests*

Complement-fixation cited as reciprocal of titre, growth-inhibition as width of inhibition zone. For gel-diffusion tests, figures in parentheses indicate number of precipitin lines shared with homologous strain. Homologous reactions in heavy type.

	Antigen	Antiserum to		
		Type C	M I	KON
Type C	CF titre	1280-2560	2560-5120	1280
	Growth inhibition (mm.)	5	5	3
	Gel diffusion (no. of lines)	6	3 (2)	4 (4)
M I	CF titre	5120	5120	2560
	Growth inhibition (mm.)	3	5-6	3
	Gel diffusion (no. of lines)	6 (5)	4	5 (5)
KON	CF titre	1280	2560	640-1280
	Growth inhibition (mm.)	3	5	5
	Gel diffusion (no. of lines)	6 (4)	3 (3)	6

Since it could be argued that this line of type C had been cloned and so might have been selected from a mixed culture, another culture of type C was examined in Glasgow by a growth-inhibition technique (Clyde, 1964), and by polyacrylamide gel electrophoresis, according to the method of Razin & Rottem (1967). This was a lyophilized culture, received from Dr Tully at the 24th passage. In tests on part of the reconstituted culture removed directly from the original ampoule, its growth was inhibited by antisera to the *Mycoplasma pulmonis* strains ASH (PG34), M50, CHENG, NEGRONI, 880 and KON, but not by antiserum to the GDL strain of *M. hyorhinis*. In polyacrylamide gel electrophoresis, the cell proteins of type C gave a similar electrophoretic pattern to those of the prototype *M. pulmonis* strain ASH (PG34).

Thus, the cultures of type C which we have examined appear to be strains of *Mycoplasma pulmonis*. Considering that cultures of type C received and examined independently in London and Glasgow were both closely related to *M. pulmonis*, it is unlikely that our results are due to accidental contamination with *M. pulmonis*,

particularly since the growth-inhibition tests on type C before subcultivation gave the same result. Moreover, since Razin (1968) obtained evidence of a similarity between the cell proteins of another culture of type C and *M. pulmonis*, it is improbable that the relationship between type C and *M. pulmonis* is confined to the cultures we examined. Manchee & Taylor-Robinson (1968) have also found a similarity between *M. histotropicum* and *M. pulmonis* in that colonies of both species adsorb rodent and guinea pig erythrocytes.

It is impossible, after nearly 30 years, to determine what relation the cultures now known as type C bear to that originally isolated by Sabin. They differ in one respect; type C originally produced arthritis in mice after intravenous inoculation, but the culture recovered from Sabin's lyophilized ampoules was not mouse-pathogenic by the intraperitoneal, intravenous or intracerebral routes (Tully & Ruchman, 1964). Nevertheless, current evidence suggests that type C belongs to the species *Mycoplasma pulmonis* and should not be classified as a separate species *M. histotropicum*. The extent to which strains differ within the species *M. pulmonis* is at present unknown, but the results of the gel-diffusion tests with type C, KON and M1, like those obtained by Fallon & Jackson (1967), suggest that serological subtypes may exist.

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Note added in proof

Since the completion of this paper, two earlier subcultures of type C, at the 4th and 7th passages from Sabin's lyophilized culture, were obtained from Dr G. J. Tully. After 4 and 2 passages, respectively, the cultures were tested by the agar growth-inhibition technique against antisera to the cloned line of type C and the 17 serologically distinct mycoplasmas cited in the text. Growth was inhibited only by antisera to type C and the *Mycoplasma pulmonis* strains KON and MI. Thus, cultures of type C passaged 8 and 9 times since recovery from Sabin's lyophilized material showed the same serological relationship to *M. pulmonis* as the later subcultures.