Evidence that *Streptobacillus moniliformis* is an Intermediate Stage between a *Corynebacterium* and its L-form or Derived PPLO

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SUMMARY

Morphological and serological evidence is presented to demonstrate that an organism morphologically identical with *Streptobacillus moniliformis* is a stage intermediate to the L-form of a strain of *Corynebacterium cervicis*, that it is serologically closely related to a type culture of *S. moniliformis*, as well as to strains of *Mycoplasma hominis*; thus supporting the conclusion that L1, derived from *S. moniliformis*, and *M. hominis* have a similar origin, and suggesting that *S. moniliformis* is an intermediate stage between a corynebacterium resembling *C. cervicis* and an L-form.

INTRODUCTION

Streptobacillus moniliformis is commonly found in the nasopharynx and infected middle ear of rats (Strangeways, 1933; Klieneberger, 1936; Nelson, 1930, 1931, 1933); it causes rat-bite fever (Blake, 1916; Blake, Horstmann & Arnold, 1944; Brown & Nunemaker, 1942; Dolman, Kerr, Chang & Shearer, 1951; Hamburger & Knowles, 1953; Lominski, Stewart-Henderson & McKee, 1948; Scharles & Seastone, 1934) and similar conditions without a history of rat-bite (Hazard & Goodkind, 1932; Levaditi, Nicolau & Poincloux, 1925); epidemics of Haverhill fever (Parker & Hudson, 1926; Place & Sutton, 1934); a disease of mice (Freundt, 1956; Levaditi, Selbie & Schoen, 1932; van Rooyen, 1936); and calf pneumonia (Smith, 1918). It has also been isolated from brain abscesses (Oeding & Pederson, 1950) and subacute bacterial endocarditis (Blake, 1916; Hamburger & Knowles, 1953; McDermott, Leask & Berrit, 1945; Peterson, McCullough, Eisele & Goldinger, 1950; Stuart-Harris *et al.* 1935). It has generally been regarded as an unusual organism and difficult to classify, especially since it appears not only in its typical, Gram-negative, filamentous phase, with irregular swellings associated with the production of its L-form (first described by Klieneberger, 1935, 1936, as a symbiotic mycoplasma) but also in the form of short, regular, Gram-positive rods (Dubos, 1948). In its filamentous phase, and in the phenomenon of L-form production, *S. moniliformis* simulates the appearances produced in other bacteria by the influence of adverse conditions, notably the presence of penicillin. When in this state, *S. moniliformis* is impossible to separate from its L1 phase (Klieneberger, 1935, 1936). In fact, the difference between a culture of *S. moniliformis* and of L1 is simply that the latter lacks the filamentous stages (Heilman, 1941*a*; 1941*b*).

The purpose of this paper is to show that *Streptobacillus moniliformis* represents a semi-stable form intermediate between the L-stage and the Gram-positive, bacillary phase of a corynebacterium. Corynebacteria have been shown to produce

streptobacillus-like variants in cases of subacute bacterial endocarditis (Wittler *et al.* 1960) and when grown in the presence of fresh blood (Bisset, 1938); they are also known to produce variants resembling L-forms and mycoplasma (Minck, 1953; Wittler, Cary & Lindberg, 1956; Smith, Peoples & Morton, 1957; Pease & Laughton, 1962), and an antigenic relationship between *S. moniliformis* and certain mycoplasma is known to exist (Brown & Nunemaker, 1942).

The present investigation arose by the isolation of a micro-organism possessing the characters of *Streptobacillus moniliformis* from a culture of *Corynebacterium cervicis* derived from the human genital tract. This organism and its parent bacterium were compared with a standard strain of *S. moniliformis* and with cultures of *Mycoplasma hominis*.

METHODS

The strain of *Corynebacterium cervicis* (strain P; Pease & Laughton, 1962) from which the streptobacillus variant was obtained, and two strains of mycoplasma (Pease A and B) serologically identical with Edwards Type I, were isolated from vaginal swabs in this laboratory; other strains of mycoplasma were obtained by courtesy of Dr D. G. ff. Edward. The standard strain of *Streptobacillus moniliformis* was NCTC 8069.

Media used were Difco PPLO medium, sometimes with 3.5 % (w/v) sodium chloride added, nutrient agar + 10 % (w/v) glucose and Hartley's broth + 10 % (v/v) horse serum or human plasma and 0.5 % (w/v) yeast extract.

Gel-diffusion tests were made by double diffusion precipitation in agar/phosphate gel at pH 7.4. The antigens and the antisera were prepared according to the techniques given by Pease & Laughton (1962).

RESULTS

The vaginal corynebacterium (strain P) was grown upon Difco PPLO medium containing 3.5 % sodium chloride, and a strip of filter-paper soaked in penicillin solution (300 units/ml.) was placed on the agar. After 48 hr. incubation at 37°, L-form colonies appeared between the normal bacterial growth and the zone of complete inhibition. Between the L-colonies and the bacterial forms there were some colonies of intermediate appearance. These consisted of bacteria in the centre and a thin transparent periphery of L-organisms. Subculture of the pure L-colonies and the mixed colonies upon the same medium without the penicillin usually resulted in the growth of corynebacteria only.

The pure L-colonies were not stable. If inoculated upon medium without penicillin, they grew in the form of corynebacteria, and if upon medium containing penicillin, they died out progressively in the course of one or two subcultures.

However, subculture of the pure L-colonies sometimes resulted in the growth of filaments resembling *Streptobacillus moniliformis*. This form remained antigenically unaltered when subcultured upon medium without penicillin, but was somewhat less filamentous in morphology.

The colonies of the streptobacillus form consisted of filaments (Pl. 1, fig. 1) and chains of bacillary elements (Pl. 1, fig. 2), sometimes containing swellings (Pl. 1, fig. 3). There also occurred small colonies of flat disk-like elements (Pl. 1, fig. 4) similar to the X colonies of Brown & Nunemaker (1942). These forms were usually

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Gram-positive, but mixed with some of them were Gram-negative L-forms (Pl. 1, fig. 5), which were morphologically and serologically identical with *M. hominis* (Pease & Laughton, 1962). The entire appearance was markedly similar to that of *S. moniliformis* (Pl. 1, fig. 6) except that the latter was usually Gram-negative in the filamentous phase.

The streptobacillus form grew well in Hartley's broth with serum and yeast-extract. No growth was obtained in this medium or in nutrient broth without serum. Growth was, however, obtained on nutrient agar without serum.

The results of the serological tests are shown in Table 1. The antisera were prepared against the corynebacterium and its streptobacillus variant, *Streptobacillus moniliformis* and a strain of *Mycoplasma hominis* Type 1 (Pease A) isolated in this laboratory. Antigens were made from the same organisms and also from a strain of L1, which was isolated from *S. moniliformis* with the aid of penicillin, and from *M. hominis* Types 1 (H26) and 2 (Campo) supplied by Dr Edward, and Type 1 (Pease B).

Table 1

Antisera	Antigens						
	Coryne- bacterium	Strepto- bacillus	<i>S. monili- formis</i>	L	<i>M. hominis</i> 1		
					Pease		H26
					A	B	
Corynebacterium	2	1	1	0	0	0	0
Streptobacillus	1	1	1	1	1	1	1
<i>S. moniliformis</i>	1	1	2	2	1	1	1
<i>M. hominis</i> 1 A	1 (weak)	1	0	0	1	1	1

Note: figures indicate number of precipitate lines appearing at junction.

The corynebacterium tested against its homologous antiserum showed two lines of precipitation, whereas with antiserum for the streptobacillus, only one line was formed indicating the loss of one antigen. A test in which the corynebacterium antigen was placed in the centre cup and alternating streptobacillus and *S. moniliformis* antisera in the outer six cups resulted in the formation of a hexagon (Fig. 1), showing that the corynebacterium, the streptobacillus and *S. moniliformis* have an

Fig. 1

Fig. 2

Fig. 3

Figs. 1-3. Diagrams of gel diffusion precipitin reactions. SV = Streptobacillus variant serum; SM = *S. moniliformis* serum; C = Corynebacterium serum; MH = *Mycoplasma hominis* Type 1 (Pease) serum.

antigen in common. In a similar test with three alternating antisera including the corynebacterium antiserum, a hexagon and a triangle (Fig. 2) were formed, thus showing that the streptobacillus and *S. moniliformis* both lack one antigen possessed by the corynebacterium.

The relationship between the streptobacillus and *Mycoplasma hominis* (Type 1) was shown in a test in which *M. hominis* (H26, Edward) antigen was placed in the centre cup and alternating streptobacillus and *M. hominis* antisera in the outer six cups. A hexagon (Fig. 3) was formed, showing that the streptobacillus and *M. hominis* have an antigen in common. *M. hominis* Type 2 (Campo) antigen did not react with any of the antisera.

DISCUSSION

The foregoing observations show that a corynebacterium of vaginal origin is capable of giving rise to a filamentous variant resembling *Streptobacillus moniliformis*, both morphologically and serologically, which further dissociates to give an L-form resembling *Mycoplasma hominis* both morphologically and serologically. The process of variation entails the loss of an antigenic component by the original corynebacterium, which presumably represents some portion of the cell wall material. This simultaneous production of a filamentous variant and loss of a surface antigen is exactly analogous with the process of S-R variation, as shown in *Corynebacterium diphtheriae* by Bisset (1938) and leads to the rather surprising suggestion that *Streptobacillus moniliformis*, of which the corynebacterium-like rod phase is, in fact, well known (Smith, 1918; Heilman, 1941*a*; Oeding & Pederson, 1950; Parker & Hudson, 1926) and is illustrated by Dienes (in Dubos, 1948), is both a stage intermediate to the L-form and in its morphology and antigenic structure simulates a filamentous R variant of one or more corynebacteria. It is by no means certain that all strains described as *S. moniliformis* do, in fact, have a single origin. The serological relationship of these variants, and of classical *S. moniliformis* and L1 to *Mycoplasma hominis* serves to reinforce the view of Pease & Laughton (1962) that mycoplasma are, in fact, L-forms of corynebacteria, in many cases.

These observations were foreshadowed, in a notable manner, by Hadley, Delves & Klimek (1931) who recognized the progression S-R-G (gonidial) in several types of bacteria, and remarked upon the resemblance of the G-form, now called the L-form, to the organism of bovine pleuropneumonia. Hadley's studies were, however, curiously neglected, and when the same resemblance between the L-colonies of *Streptobacillus moniliformis* and the pleuropneumonia organism was again noticed by Klieneberger (1935), the earlier observations were forgotten by subsequent workers. Klieneberger at first believed that the L-form was a symbiote of the bacillus, but was later convinced of its bacterial origin. Rather curiously, it appears to have been generally accepted that recognition of the bacterial origin of L1 had the effect of disproving the inferences drawn from its very strong resemblance to the pleuropneumonia organisms, or mycoplasma, and this has delayed recognition of what now appears to be the truth: that the L1 of *S. moniliformis* is synonymous with *Mycoplasma hominis*, and that both have their origin in strains of corynebacterium.

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EXPLANATION OF PLATE

PLATE 1

Figs. 1-5. *Streptobacillus*-like variant of *Corynebacterium cervicis*, showing various appearances previously described as characteristic of *Streptobacillus moniliformis*. 48 hr. growth on Difco PPLO medium, at 37°.

Fig. 1. Filaments. ×3000.

Fig. 2. Chains of bacillary elements. ×2000.

Fig. 3. Streptobacilli showing swellings. ×3000.

Fig. 4. Streptobacilli showing a collection of flat disk-like elements, appearing as circular masses. ×3000.

Fig. 5. Gram-positive bacillary forms mixed with Gram-negative L-forms. ×2000.

Fig. 6. *Streptobacillus moniliformis* showing filamentous and swollen forms. ×2000. 48 hr. growth on Difco PPLO medium, at 37°.

Fig. 1

Fig. 2

Fig. 3

Fig. 4