Observations on the Weissensee G Strain of *Corynebacterium diphtheriae*

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SUMMARY

From a Park-Williams No. 8 strain (Weissensee G) of *Corynebacterium diphtheriae*, recently reported by Rajadhyaksha & Rao (1965) to be toxinogenic but *non*-lysogenic, two diphtherial strains have been isolated: 1, a typical phage-resistant, slow-growing, toxinogenic PW8 strain; 2, a fast-growing non-toxinogenic strain that is sensitive to diphtherial phages.

INTRODUCTION

The lysogenic conversion of sensitive, non-toxinogenic diphtheria bacilli to lysogeny and toxinogeny by the temperate bacteriophage β was first demonstrated by Freeman (1951). Since that time, there has accumulated a considerable body of evidence which suggests that the capacity of a given strain to produce the diphtheria toxin protein depends upon the phage genome (Groman, 1955; Groman & Eaton, 1955; Barksdale, 1959). However, expression of the toxinogenic character also depends upon certain peculiarities in host cell metabolism and the mere presence of the phage genome does not suffice (Miller, Pappenheimer & Doolittle, 1966). It still remains uncertain whether or not the β -phage DNA codes directly for the toxin protein.

Recently, Rajadhyaksha & Rao (1965), working with a variant of the classic pw8 strain (Weissensee G) reported, in this *Journal*, experiments which they interpreted as indicating that toxinogenicity depends upon an episomal cytoplasmic host factor, T⁺. They suggested that the role of phage is merely to facilitate entry, by transduction, of the T⁺ factor into sensitive T⁻ cells. According to the Indian workers, it is possible to remove the phage genome from lysogenic bacteria by treatment with low concentrations of acriflavine, without altering their capacity to produce toxin. They report that their Weissensee G strain is non-lysogenic and sensitive to phage despite its capacity to synthesize toxin in high yield.

Because of the important implications of these unexpected findings and because recent studies with our stock pw8 strain (Miller *et al.* 1966) are not in agreement with Rajadhyaksha & Rao, we decided to compare the Weissensee G strain with our own pw8 strain.

RESULTS AND CONCLUSIONS

A filter paper strip containing the dried Weissensee G organisms was kindly sent us by Dr S. S. Rao of the Haffkine Institute, Bombay. It was placed aseptically in a flask containing Casamino acid medium (Miller *et al.* 1966) and incubated overnight at 35° on a rotary shaker. The following day, the culture was streaked on chocolate

agar plates. After 48 hr of incubation, most of the colonies that grew out were tiny and resembled closely colonies of our own PW8 strain. There were, however, a few large colonies. After several subcultures and replatings, two distinct strains of diphtheria bacilli were separated from the Weissensee G specimen. The first gave small colonies on chocolate agar, grew slowly in liquid medium, did not require tryptophan for growth, was phage resistant and produced high yields of toxin. The second gave large colonies, grew rapidly on liquid medium containing tryptophan, was sensitive to phage β and its mutants and was non-toxinogenic. Table 1 summarizes the properties of the two strains separated from the Weissensee G culture and compares them with three stock strains from our own laboratory.

Table 1. *Properties of certain strains of Corynebacterium diphtheriae*

Property	Strains				
	PW8 (Weissensee G)		PW8*	P60 (β)*	c7 (β)*
	Small 160 min.	Large 60 min.	Small 160 min.	Large 60 min.	Large 60 min.
Colony size (chocolate agar)					
Generation time (35°)	160 min.	60 min.	160 min.	60 min.	60 min.
Tryptophan requirement	—	+	—	+	+
Sensitivity to phages B and β	—	+	—	—	+
Toxin production (Lf/ml.)	≥ 80	0	80–130	15–20	0
Yield of β -phage particles/10 ⁸ u.v.-irradiated organisms	1–2	0	ca. 5	ca. 10 ⁹	0
Q ₀ † (mm. ³ /mg./hr): Succinate	Not done	86	15–20	15–20	85–100
Glucose	Not done	58	15–20	15–20	40–60
Ethanol	Not done	104	35–40	35–40	100–150

* Strains marked with an asterisk are stock strains from this laboratory. The terminology is that of Miller *et al.* (1966). Strain P60(β) was formerly called PW8 (P) (Barksdale, 1959; Pappenheimer *et al.* 1962; Rajadhyaksha & Rao, 1965). Miller *et al.* have shown that the phage P is indistinguishable from β in host range, serological and other properties.

† See Pappenheimer *et al.* (1962).

The possibility, suggested by Rajadhyaksha & Rao (1965), that toxin production by diphtheria bacilli depends upon host cytoplasmic particles which are not controlled by the phage genome, cannot be rigorously excluded, as was indeed pointed out several years ago by Jacob & Wollman (1961). Nevertheless, evidence obtained in many laboratories makes such a possibility appear extremely unlikely. In any case, the results reported by the Indian workers cannot be considered as evidence since all of their observations can be readily explained by the presence of phage-sensitive fast-growing bacteria in their PW8 culture. On rich media such as are used to obtain phage plaques, the fast-growing sensitive strain would often predominate, thus accounting for the apparent phage sensitivity of the Weissensee G strain. Since PW8 strains do not absorb phage (Miller *et al.* 1966), only the fast-growing strain would be affected by treatment with β -phage and would be converted to a lysogenic strain similar to c7(β) which is fast-growing and gives low yields of toxin in comparison with PW8. Since the PW8 strains do not release phage particles except after induction with ultraviolet (u.v.) radiation (and then *less* than one β -particle/10⁷ irradiated bacteria), a mixed culture of a few sensitive bacteria together with PW8 bacteria might be maintained through many transfers without conversion of the sensitive strain to lysogeny.

The findings reported by Rajadhyaksha & Rao (1965) are not in agreement with those of Saragea & Maximesco (1964), who included the Weissensee G strain in their survey of many diphtherial strains. In their hands, this pw8 variant was resistant to phages and yielded a few phage particles after u.v.-irradiation. Our own results with the small colonies isolated from the strain received from the Haffkine Institute agree with those of Saragea & Maximesco.

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