TRIMETHYLAMINE OXIDE REDUCTION AND THE EIJKMAN REACTION IN THE GENUS *ERWINIA*

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The success that has been attained in separating species of *Shigella* by means of the trimethylamine oxide reduction test and the Eijkman reaction has prompted this investigation. Past studies with the genus *Erwinia* bearing on trimethylamine oxide reduction (Wood and Baird: J. Fisheries Research Board Can., 6, 194) and the Eijkman reaction (Stuart *et al.*: J. Bact., 43, 557) have not

TABLE 1
Trimethylamine oxide reduction and the Eijkman reaction with species of Erwinia

ERWINIA SPECIES	NUMBER OF CULTURES	TRIMETHYLAMINE OXIDE REDUCTION		EIJKMAN REACTION*		
		Positive	Negative	O	G	A
<i>E. amylovora</i>	4	0	4	4	0	0
<i>E. tracheiphila</i>	3	0	3	3	0	0
<i>E. phytophthora</i>	4	0	4	4	0	0
<i>E. solanisapra</i>	3	0	3	3	0	0
<i>E. carotovora</i>	13	3	10	13	0	0
<i>E. aroideae</i>	15	4	11	15	0	0
<i>E. carnegiana</i>	1	1	0	0	0	1
<i>E. nimipressuralis</i>	1	1	0	0	1	0
Totals.....	44	9	35	42	1	1

* O, no growth and not viable; G, growth; A, acid.

indicated the species employed. In the former study it was found that the genera *Erwinia* and *Shigella* were the only ones in the family *Enterobacteriaceae* which contained species that did not have the ability to form trimethylamine from the oxide. It was revealed that 8 out of 17 cultures of the plant pathogens were able to reduce trimethylamine oxide. Applying the Eijkman test, Stuart *et al.* (*loc. cit.*) showed that, with 24 cultures of *Erwinia*, 20 failed to survive at 45.5 C for 24 hours. Later Wood *et al.* (J. Bact., 46, 106) stated that in the genus *Shigella* a direct correlation existed between the Eijkman reaction and the ability to reduce trimethylamine oxide.

Comparative studies pertaining to the capacity to reduce trimethylamine oxide and the Eijkman reaction in the genus *Erwinia* are presented herewith. Forty-four cultures of *Erwinia* representing 8 species have been utilized. The results are given in table 1. All available cultures of *Erwinia amylovora*, *E. tracheiphila*, *E. phytophthora*, and *E. solanisapra* failed to form trimethylamine. Out of 13 isolates of *E. carotovora*, 10 were unable to reduce trimethylamine

oxide, but 3 had this capacity. With 15 cultures of *E. aroideae*, 11 were negative in their action on trimethylamine oxide, whereas 4 were able to produce trimethylamine. Single strains of *E. carnegieana* and *E. nimipressuralis* had the property of reducing trimethylamine oxide.

All of the *Erwinia* cultures, except *E. carnegieana* and *E. nimipressuralis*, failed to grow or remain viable under the conditions of the Eijkman test (glucose was substituted for lactose in the medium employed). These two Eijkman survivors, which were also trimethylamine-positive, are like *Shigella alkalescens*, *S. dispar*, and *S. sonnei* in respect to the Eijkman test and trimethylamine oxide reduction. With *E. carotovora* and *E. aroideae*, however, no correlation exists between the trimethylamine-positive strains and the ability to withstand the conditions of the Eijkman test.

No comparative studies have been made between trimethylamine-positive and trimethylamine-negative strains of the latter two species. Nevertheless, the faculty to macerate carrot tissue is maintained by negative and positive isolates of both species. The existence of strains in the soft-rot group that have the power of reducing trimethylamine oxide preclude the use of the test in distinguishing these species from members of the genus *Aerobacter*.

THE ANTIBIOTIC ACTIVITY OF VIOLACEIN, PRODIGIOSIN, AND PHTHIOL

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The water-insoluble pigments violacein, prodigiosin, and phthiol are produced, respectively, by *Chromobacterium violaceum*, *Chromobacterium prodigiosum*, and *Mycobacterium tuberculosis*. The antibiotic activity of violacein has been reported (Lichstein and Van de Sand: J. Infectious Diseases, **76**, 47) but to our knowledge there have been no similar studies with phthiol. Although whole cultures of *Chromobacterium prodigiosum* have long been recognized as possessing antibiotic activity, there has been a difference of opinion regarding the nature of the active substance. Hettche (Arch. Hyg. Bakt., **107**, 337) believes the pigment to be the active agent, but Eisler and Jacobsohn (Z. Hyg. Infektionskrankh., **117**, 76) suggest that the substance is a nonpigmented material present in broth cultures of the organism.

Violacein, extracted and purified according to the method of Strong (Science, **100**, 287), exerts a bactericidal action against *Staphylococcus aureus* in concentrations of 0.001 to 0.01 per cent when the number of bacterial cells is approximately 5,000 per ml, and a bacteriostatic action against larger inocula. The pigment exhibits no *in vitro* antiphagocytic activity against guinea pig exudative polymorphonuclear leucocytes in concentrations of 0.05 to 0.5 mg. Mice (8.5