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DISTINCTION BETWEEN SALMONELLA AND ARIZONA
BY LEIFSON'S SODIUM MALONATE MEDIUM

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A sodium malonate medium was used by Leifson (1953) for separating Escherichia coli from Aerobacter aerogenes. Later Schaub (1948) found that her subgroup C of Group II paracolon bacilli (similar to organisms described by Peluffo, Edwards, and Bruner (1942), Edwards, Cherry, and Bruner (1943) and Hinshaw and McNeil (1945)) could be distinguished from Salmonella species by a sodium malonate test, slow lactose fermentation, and delayed liquefaction of gelatin. Using Leifson's sodium malonate medium as one of the routine biochemical tests on members of the Enterobacteriaceae, phenylalanine was added for the detection of Proteus and Providencia strains (Shaw and Clarke, 1955) and our results agreed with those of Schaub. The presence of phenylalanine did not affect the reading of the malonate test.

Methods

Strains. The cultures of Salmonella and Arizona examined were those maintained at the National Collection of Type Cultures.

Medium and malonate test. Leifson's (1933) sodium malonate and Henriksen's (1950) phenylalanine tests were combined by adding phenylalanine to Leifson's medium. 0.1 per cent Yeastrel was added to increase growth. The medium was as follows: $(\text{NH}_4)_2\text{SO}_4$, 2.0 g; K_2HPO_4 , 0.6 g; KH_2PO_4 , 0.4 g; NaCl , 2.0 g; sodium malonate, 3.0 g; DL-phenylalanine, 2.0 g; Yeastrel, 1.0 g; 0.5(w/v) ethanolic bromthymol blue, 5 ml; distilled water, 1000 ml. It was sterilised at 10 lbs/sq.in. for 10 min. Cultures were incubated overnight at 37°C; organisms using malonate produced an alkaline reaction.

Results

Table 1 gives the results of the test on Salmonella spp. and Arizona strains, and shows that most of the Arizona strains were malonate-positive, i.e., produced alkali in the malonate medium. The five Arizona strains that failed to change the pH in the malonate medium were biochemically typical strains in that they were late or non-lactose fermenters and failed to produce acid in dulcitol.

Table 1. Utilization of Sodium Malonate by Salmonella and Arizona strains.

	Number tested	Number positive	Per cent	Number negative	Per cent
Salmonella	228	11	4.8	217	95.2
Arizona	67	62	92.5	5	7.5

Salmonella spp. were mostly malonate-negative. The 11 strains that caused a pH change in the malonate medium were Salmonella dar-es-salaam (NCTC 2206 and NCTC 5773), S. canastel (NCTC 6948), S. neasden (NCTC 7835), S. marylebone (NCTC 9605), S. worcester (NCTC 8273), S. nachshonim (NCTC 8280), S. rowbarton (NCTC 9606), S. bleadon (NCTC 9604), S. taunton (NCTC 9600) and S. uphill (NCTC 9581). All these strains except S. marylebone and S. taunton were described by Kauffmann (1954) as liquefying gelatin, but when examined by us some of them failed to do so within 30 days at 22°C. All were non-lactose fermenters and produced acid and gas from dulcitol. Five of the strains belong to serological groups D or G, and the remainder are recently described strains from tortoises (Boycott, Taylor, and Douglas, 1953; Douglas and Taylor, 1954).

Discussion

Salmonella and Arizona strains have many biochemical characters in common, and differ mainly in the ability of Arizona strains to liquefy gelatin when incubation is prolonged for a month or more. However, a few Salmonella serotypes are known to liquefy gelatin, and strains of some

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of these serotypes were shown to produce an alkaline reaction in Leifson's malonate medium.

The majority of Arizona strains were malonate-positive, but the few that were negative seem to form, with the malonate-positive salmonellas, intermediate links between the Arizona and Salmonella groups. With the exception of Salmonella marylebone and S. taunton all the malonate-positive Salmonella serotypes were originally described as gelatin-positive, and consequently they differed in their biochemical characters from the Arizona group only in being dulcitol-positive.

Serologically there is much overlapping of antigens between the Arizona and Salmonella groups (Edwards, West, and Bruner, 1947) and in several instances the antigens are identical. Thus, with so many biochemical and serological characters in common, it would seem logical to regard the Arizona group as a species of the genus Salmonella. The malonate test would help to distinguish this species.

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