

*Discussion*

A skin test has been widely and successfully used as both a diagnostic and an epidemiological tool in South American cutaneous and mucocutaneous leishmaniasis. False positive reactions occur, especially in patients with lupus vulgaris, but false negative reactions are uncommon. On the other hand, false negative reactions are the rule in patients with active visceral leishmaniasis.<sup>c, d</sup> The leishmanin skin test has not been extensively employed in the Near Eastern and African endemic zones of *L. tropica* infection.

The high positive rates in adults confirm the impression that *L. tropica* infection was hyperendemic

in south-eastern Turkey. The immunological phenomenon of anergy is considered responsible for the false negative results in geriatric and tuberculosis patients. There were no fresh leishmanial ulcers seen during the present survey; local Turkish physicians assert that the infection rate has been extremely low during the past eight years. In 1956 a malaria eradication programme was initiated in this area, and it is highly probable that DDT sprays intended for *Anopheles* control also reduced markedly the population of *Phlebotomus papatasi*, the vector of oriental sore in Turkey.

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<sup>d</sup> Manson-Bahr, P. E. C. (1961) *E. Afr. med. J.*, **38**, 165.

## Comparative Studies of the Polymyxin-B Test with Bacteriophage Typing for the Differentiation of *Vibrio cholerae* and *Vibrio El Tor*: Preliminary Report

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The differentiation between *Vibrio cholerae* and *Vibrio El Tor* can be carried out by comparative testing of the biochemical reactions, the haemolytic reaction on plated blood-agar and blood-broth, the agglutinating property of anti-cholera serum by heating at 56°C for three hours (Gispén<sup>b</sup>), the Tanamal<sup>c</sup> tests, the agglutinating property of anti-cholera serum after treatment of the cholera vibrios with 2% chloroform (Meyer<sup>d</sup>), the action of CuSO<sub>4</sub> on vibrios (Wahba & Takla<sup>e</sup>), the chicken red blood cell agglutination test (Finkelstein & Mukerjee<sup>f</sup>), the polymyxin-B test (Gan & Tjia<sup>g</sup>), and with the aid of bacteriophage (Mukerjee<sup>h</sup>).

Outbreaks of cholera due to *Vibrio cholerae* and *V. El Tor* in several countries have emphasized the need for a simple laboratory technique that could provide rapid and reliable bacteriological identification of the type of cholera infection.

It was therefore felt that it was worth undertaking a comparative study in order to evaluate the above-mentioned laboratory tests by their sensitivity in differentiating the two types of agglutinable vibrios. The haemolytic test was performed with the Greig method and with a new haemolytic procedure, which will be published by Yen & Radojcic<sup>i</sup>. The polymyxin-B test was performed as described by Gan & Tjia,<sup>g, j</sup> while the cholera bacteriophage typing was essentially as devised by Mukerjee,<sup>h, k</sup> using the critical dilution as the working phage concentration.

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<sup>b</sup> Gispén, R. (1938) *De herkenning van vibrio cholerae en vibrio El Tor*, Amsterdam (Thesis).

<sup>c</sup> Tanamal, S. J. W. (1948) *Ned. T. Geneesk.*, **92**, 1370.

<sup>d</sup> Meyer, F. H. (1939) *Onderzoek van vibrienen uit Nederlands-Indië en de Hejaz*, Amsterdam (Thesis).

<sup>e</sup> Wahba, A. H. & Takla, V. (1962) *Bull. Wld Hlth Org.*, **26**, 306.

<sup>f</sup> Finkelstein, R. A. & Mukerjee, S. (1963) *Proc. Soc. exp. Biol. (N.Y.)*, **112**, 355.

<sup>g</sup> Gan, K. H. & Tjia, S. K. (1963) *Amer. J. Hyg.*, **77**, 184.

<sup>h</sup> Mukerjee, S. (1961) *J. Hyg. (Lond.)*, **59**, 109.

<sup>i</sup> Yen, C. H. & Radojcic, V. (to be published).

<sup>j</sup> It should be stressed that in order to obtain the most favourable results it is necessary to press the sensitivity disc slightly with a sterile applicator after placing the disc on the surface of the medium, 20 minutes after inoculation of the medium with the test bacterium.

<sup>k</sup> Mukerjee, S. (1963) *Bull. Wld Hlth Org.*, **28**, 331.

The Gispén, Meyer and Tanamal tests were carried out as described in the original papers.

In a comparison of the polymyxin-B test with Mukerjee's bacteriophage typing procedure, there were no failures with either method in a series of tests on 177 non-haemolytic *V. cholerae* strains and 163 haemolytic *V. El Tor* strains. It can therefore be concluded that the polymyxin-B test (using Difco sensitivity discs containing 50 units of polymyxin-B), as developed by Gan & Tjia,<sup>g</sup> and the bacteriophage typing (using cholera phage type IV), as described by Mukerjee,<sup>h,k</sup> show complete agreement and that, therefore, the sensitivity of both tests for distinguishing between *V. cholerae* and *V. El Tor* is equally high. Using 20 cholera and 15 *El Tor* strains in four repeated tests with the same strains, it was also shown that the polymyxin-B test gives 100% reproducible results.

Of the other differential tests, those of Tanamal, Gispén and Meyer have been used most frequently, and have been strongly recommended as a routine measure in the diagnosis of vibrios. It was therefore felt worth undertaking a comparative study of the polymyxin-B test with the above-mentioned tests.

Tests in which 27 non-haemolytic agglutinable vibrios (*V. cholerae*) and 140 haemolytic agglutinable vibrios (*V. El Tor*) were used indicated that the polymyxin-B test resulted in less failures than the Gispén test, the Meyer test and the Tanamal tests (see accompanying table). However, it should be mentioned that the deviations of the Tanamal serum-soda test and the Meyer test may still be considered to be within the limits of statistical variation.

From these studies, the conclusion may be drawn that the polymyxin-B test may be considered a

THE RESULTS OF COMPARATIVE STUDIES WITH THE GISPEN TEST, THE MEYER TEST, THE TANAMAL TESTS AND THE POLYMYXIN-B TEST

Method	<i>V. cholerae</i> : 27 strains tested (non- haemolytic)	<i>V. El Tor</i> : 140 strains tested (haemolytic)	Total : 167 strains tested
	No. (and %) of failures	No. (and %) of failures	No. (and %) of failures
Polymyxin-B test	0 (0)	0 (0)	0 (0)
Gispén test	6 (22)	0 (0)	6 (4)
Meyer test	2 (7)	0 (0)	2 (1)
Tanamal soda-sublimate test	1 (4)	5 (4)	6 (4)
Tanamal serum-soda test	0 (0)	2 (1)	2 (1)

simple and satisfactory additional test to differentiate between *V. cholerae* and *V. El Tor* in the average laboratory.

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