



Figure 1. a. Habit; b. Spores; c. Basidia; d. Peridial structure; e. Capillitial threads.

Sporocarps epigeal, 2–5 cm high and 1–3 cm broad, obconical to pyriform with a distinct base which is elongated and stalk-like (subgleba). Surface orange grey (5B2) at the centre, creamish all over when young and becoming brownish orange (5C3) to dark blond (5D4) when old. The surface spiny, spines bigger and numerous towards apex and lesser and smaller towards base; in older sporocarps spines fall off and leave depressed areas; dehiscence is by apical torn aperture. Sterile base (subgleba) chambered, occupying almost half of the length of the sporocarp, chambers up to 1 mm diam., brown, separated from the gleba by a conspicuous diaphragm, confluent with the endoperidium. Gleba white when young, brownish olivaceous when mature, composed of minute chambers which are lined with hymenium; the chambers collapse when spores mature. Basidiospores brown with a distinct olive tint, globose, 3–4 μm , asperulate, pseudomyloid and cyanophilic; spines up to 1 μm long. Basidia clavate, 7–9 \times 4–5 μm , tetrasporic, bearing 4 unequal, long, slender 5–10 μm long sterigmata. Capillitium thread-like, continuous with the inner peridium and subgleba, 2–4 μm , diam., sparingly branched, thick-walled (1–1.5 μm thick), aseptate with pitted walls. The mounds or the spines on the surface are formed by groups of pseudoparenchymatous hyphal chains, individual elements of which are globose to elliptical, 13–40 \times 9–35 μm , thick-walled (up to 1 μm thick). Clamp connections absent.

In group, in *Pinus patula* plantations, Kodaikanal and Nilgiris in Tamil Nadu, India.

Pure culture from the sporocarps has been

obtained for further *in vitro* studies of mycorrhizal synthesis.

Thanks are due to Dr V. Demoulin of Liege University, Belgium for confirming our identification of the fungus. One of us (KBP) is grateful to UGC, New Delhi for a fellowship.

1 May 1987

1. Trappe, J. M., *Bot. Rev.*, 1962, 28, 538.
2. Kornerup, A. and Wanscher, J. H., *Methuen handbook of colour*, Methuen and Co., London, 1967, p. 243.

SERODIAGNOSIS OF TYPHOID FEVER BY INDIRECT HAEMAGGLUTINATION USING CRUDE LYSATE ANTIGEN

K. ZACHARIAH and G. P. RAI

Division of Microbiology, Defence Research and Development Establishment, Gwalior 474 002, India.

ENTERIC fever is prevalent all over the world. In India most of the infections are caused by *Salmonella typhi*^{1,2}. The diagnosis is confirmed by the isolation of causative organism or by the detection of specific antibody from clinical samples. Several immunodiagnostic procedures such as widal agglutination³, haemagglutination inhibition⁴, indirect haemagglutination⁵, counterimmunoelectrophoresis⁶ etc are available for diagnosis of typhoid fever.

Culture methods are time-consuming and counterimmunoelectrophoresis is less sensitive⁷. Indirect haemagglutination is not routinely practised and moreover the antigen coated on the RBCs should be pure⁸. George and Vaughan⁹ showed that mixed antigens can also be coated on the RBCs but their coating efficiency decreases considerably.

Gupta and Rao¹⁰ used centrifuged ultrasonic lysate antigen in counterimmunoelectrophoresis for detection of typhoid. This lysate contains several antigenic components including lipopolysaccharides and protein. Tanned RBCs coat for protein and untanned RBCs coat for lipopolysaccharides. In this communication we attempted to evaluate the efficacy of *S. typhi* lysate antigen in IHA (indirect haemagglutination) by coating on to both tanned and untanned SRBC (sheep erythrocytes).

Fifty-five serum samples from suspected typhoid patients admitted at the GR Medical College, Gwalior were collected during the first and second week of fever. Serum samples were inactivated at

56°C and stored at -20°C until used. Twenty-two normal control samples from healthy individuals who were not immunized for at least six months prior to the collection were also included in the study. All sera samples were absorbed with *E. coli* and *S. paratyphi A* to remove the cross-reacting antibodies.

Overnight growth of *S. typhi* on nutrient agar was scraped off and suspended in sterile saline and sonicated at 20 kilocycles for 10 min in cold. This lysate was centrifuged at 2500 g for 15 min to sediment unbroken cells. Supernatant was collected and adjusted to 2 mg protein per ml. Heparinized SRBC was collected in Alsevier solution. After washing, tanning was carried out according to Fulthroe *et al*¹¹, and IHA was done according to the method of Clark and Cassals¹² adapted to microtitre system.

Out of the 55 sera collected, 41 were culture-positive and 34 were widal-positive. From table 1 it is clear that all the widal-positive samples (a+c) were also positive by IHA using tanned SRBC (tSRBC) whereas only 24 samples were positive by untanned SRBC in IHA. Seven out of the 15 widal-negative (culture-positive) samples (b) were positive by tSRBC in IHA. Forty-nine samples (a+b+c) were confirmed as positive either by culture or by widal or by both. Out of 49 confirmed cases, 41 (83.67%) were positive by IHA using tSRBC whereas untanned SRBC showed only 26 (53.06%). IHA by tSRBC showed 14.29% more positivity than the widal test (34 cases) alone whereas untanned SRBC showed 16.32% less than the conventional widal test. Two of the culture-negative and widal-negative samples (d) were also

positive when tested by IHA using tSRBC. This implies that IHA using tSRBC is more sensitive than untanned SRBC as also conventional widal test.

This study further shows that in IHA admixture of lipopolysaccharides, protein and other cellular components in the antigen preparation do not matter much as regards the sensitivity of the test. By dispensing with cumbersome procedures of purified antigen preparation the crude lysate antigen used in this test is a promise for routine laboratory tests. Moreover, this is less time-consuming than widal test and/or culture methods, and needs less quantity of antigen and antibody.

The authors are grateful to Dr P. K. Ramachandran, Director, for his keen interest and encouragement, and to Brig K. M. Rao, Head, Microbiology Division for able guidance and suggestions.

8 June 1987

Table 1 Comparison of tanned and untanned SRBC in IHA

Type of samples	Number of samples	Indirect haemagglutination	
		Tanned SRBC	Untanned SRBC
(a) Culture +ve Widal +ve	26	26	17
(b) Culture +ve Widal -ve	15	7	2
(c) Culture -ve Widal +ve	8	8	7
(d) Culture -ve Widal -ve	6	2	1
Total	55	43	27

1. Agrawal, S. C., *Bull. WHO*, 1963, **29**, 113.
2. Basu, S., Dewan, M. L. and Suri, J. C., *Bull. WHO*, 1975, **52**, 331.
3. Widal, G. F. I. and Sicard, A., *Bull. Soc. Med. Paris (3rd series)*, 1896, **13**, 681.
4. Buxton, A. and Davis, J. M., *Immunology*, 1963, **6**, 530.
5. Morello, J. A., Digenio, T. A. and Baber, E. E., *J. Bacteriol.*, 1964, **88**, 1277.
6. Gupta, A. K. and Rao, K. M., *J. Immunol. Methods*, 1979, **30**, 349.
7. Sivadasan, K., Betty, K. and Jacob John, T., *The Lancet*, 1984, **1**, 134.
8. Herbert, W. J., In: *Handbook of experimental immunology*, (ed.) D. M. Weir, Blackwell Scientific Publications, Oxford, London, 1979, p. 20.
9. George, M. and Vaughan, J. H., *J. Immunol.*, 1962, **88**, 191.
10. Gupta, A. K. and Rao, K. M., *J. Immunol. Methods*, 1981, **40**, 373.
11. Fulthroe, A. J., Roitt, I. M., Doniach, D. and Couchman, K., *J. Clin. Pathol.*, 1961, **14**, 654.
12. Clark, D. H. and Cassals, J., *Am. J. Trop. Med. Hyg.*, 1958, **7**, 561.