For deciding when a patient is free of infection, repeated urine and stool cultures are necessary. The Vi agglutination test may also be of some possible value in this regard.

For the tracing of chronic carriers, the Vi test in South Africa has sometimes proved unreliable and its use is often abused by local health authorities. The tracing of chronic carriers depends first upon 'police' epidemiological investigations to find the suspects and secondly on bacteriological tests to prove which suspect is guilty. Whenever a case of typhoid fever occurs, every effort should be made by the local health authority to trace the responsible carrier.

**THE LABORATORY DIAGNOSIS OF TYPHOID FEVER**

**AN OUTLINE OF THE METHODS USED AT THE GOVERNMENT PATHOLOGICAL LABORATORY, CAPE TOWN**

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The bacteriological diagnosis of typhoid fever is, for all practical purposes, dependent upon the recovery of *Salmonella typhi* from specimens of blood, faeces and urine, and to a lesser degree upon the measurement in the patient's blood of the specific antibody response to the flagellar (H) and somatic (O) antigens.

The techniques used at the Government Pathological Laboratory, Cape Town, for the isolation of *S. typhi* will be outlined. We have found these methods also suitable for the isolation of the other members of the salmonella-shigella group.

The essence of the scheme is to isolate non-lactose-fermenting organisms (into which category the salmonella-shigella organisms fall) from the specimen, to separate off from these the large group of proteus organisms by utilizing the fact that they rapidly hydrolyse urea, and then submitting the remainder to tests for motility, the ability to ferment dextrose, mannite, sucrose and salicin, to produce indole, and to form sulphide. An assessment of these biochemical properties permits of a presumptive diagnosis, and confirmation is obtained by agglutination tests with specific antisera.

**ISOLATION AND IDENTIFICATION OF S. TYPHI FROM STOOL AND URINARY SPECIMENS**

**Stools.** On receipt at the laboratory each specimen of stool is plated onto selective differential media, viz. (a) McConkey's agar and (b) salmonella-shigella agar (Difco). About 1 g. of faeces is also emulsified into a tube of selenite enrichment broth which, after about 18 hours' incubation, is subcultured onto a plate of salmonella-shigella agar.

**Urine.** Equal quantities of the urine and of double-strength selenite broth are mixed and incubated for about 18 hours. Subculture is then made onto a salmonella-shigella agar plate.

**Subsequent procedures.** After 18-24 hours' incubation, all the McConkey and salmonella-shigella agar plates are examined for non-lactose-fermenting colonies with characteristics suggestive of pathogens. At least 6 suspicious colonies are then picked off and seeded into tubes of Singer's urea broth. These tubes are incubated for about 6 hours and are then examined. Tubes showing an alkaline change from the rapid hydrolysis of the urea are discarded as containing proteus organisms. From those tubes which show no signs of urease activity or of lactose fermentation, subcultures are made into the following tubes of differential media: Kligler's iron agar (Difco), semi-solid mannite medium, sucrose-lactose-salicin medium and tryptone water. After 18-24 hours' incubation these tubes are examined for changes indicative of the presence of intestinal pathogens. The presumptive diagnosis of the type of organism suggested by the reactions in these tubes is then confirmed by slide agglutination tests with the appropriate specific antisera.

If *S. typhi* is identified, pure cultures of the organism on plain agar slopes are submitted to the Phage-Typing Unit of the Institute of Pathology, Pretoria, for phage typing.

This scheme we have found to be economic and reliable.

Kligler's iron agar is a useful differential tube medium in that in a single tube of medium it is possible to confirm that the organism is a non-lactose-fermenter, and to decide whether it ferments dextrose (and whether with acid only or acid and gas) and produces hydrogen sulphide.

The semi-solid mannite medium is equally useful in that in one tube it can be decided whether the organism is motile, whether it ferments mannite and whether with acid only or acid and gas.

SLS medium, which contains sucrose, lactose and salicin, is useful in discarding organisms of the paracolon group, which may closely resemble salmonellas but can be differentiated by the fact that paracolons sooner or later always ferment one or more of these sugars, which the salmonellas never do.

Tryptone water is used to test for indole production (Kovac's reagent is used in the test).

The use of Singer's urea broth has the advantage that it not only indicates urease activity of the seeded organism but also, by virtue of the fact that it contains lactose, acts as a confirmatory test for the absence of lactose fermentation of the organism.
ISOLATION OF S. TYPHI FROM BLOOD CULTURES

As the chance of isolating S. typhi from blood cultures is virtually 100% in the first week of the disease, too much stress cannot be placed on the desirability of undertaking this procedure in all cases of clinically suspected typhoid fever, and it is regrettable that this most useful laboratory investigation is not called for more frequently. Blood specimens for culture are submitted in bile-salt broth media (5 c.c. of the blood in 100 c.c. of the broth), and on arrival at the laboratory are incubated overnight and then plated onto McConkey's agar, which, after 24 hours' incubation, is examined for characteristic growth. If this is present, tubes of Kliger's iron agar are inoculated and suspicious growths are then identified by slide agglutination tests. Absence of growth on primary plating necessitates repeated platings from the original broth on the 2nd, 4th and 8th days, before final discarding as negative. Usually, if S. typhi is present, it will be isolated on the primary plating. Occasionally only, clot cultures are performed from clotted blood specimens submitted for Widal tests.

ISOLATION OF S. TYPHI FROM CHRONIC URINARY AND FECAL CARRIERS

As we feel a little sceptical about the value of the Vi test in tracing carriers, encouragement is given to the epidemiological approach to the problem and, once a few persons are suspect on epidemiological grounds, repeated specimens of stool and urine are submitted for cultural examination. Supplies of selenite broth are supplied to the health officials undertaking the investigations, and are seeded by them with the requisite amount of faecal material. These specimens, together with the urinary specimens, are forwarded to the laboratory, where the broths are subcultured onto a salmonella-shigella agar plate, and growths with the colonial characteristics resembling those of S. typhi are passed through Singer's urea media and then subcultured only onto a Kliger's iron agar. Characteristic findings are confirmed by slide agglutination. The urinary specimens are dealt with described above.

Where specimens of stool may be long delayed in transit, they may be preserved by the addition of Sach's solution, obtainable from this laboratory on request. We have, however, seldom found this necessary with S. typhi specimens.

FORMULAE OF MEDIA

S.L.S. Medium. Peptone 10 g., sodium chloride 5 g., agar 3 g., distilled water 1,000 ml. These ingredients are steamed to dissolve, and after cooling the following are added:

Sucrose 10 g., lactose 10 g., salicin 5 g. Adjust the pH to 7-8. Add 10 ml. of Andrade's indicator and 4 ml. of a 0·4% solution of brom-cresol purple. The medium is dispensed in 5-ml. amounts in tubes and the tubes are steamed for 3 successive days for 15 minutes.

Semi-solid Mannite Medium: Proteose peptone 5 g., sodium chloride 5 g., agar 5 g., distilled water 1,000 ml. Adjust the pH to 7-8, and then add 10 g. of mannite, and 4 ml. of a 0·4% solution of brom-cresol purple. The medium is dispensed in 5-ml. amounts in tubes and the tubes autoclaved at 15 lb. pressure for 25 minutes.

Singer's Urea Medium: Difco tryptone 20 g., sodium chloride 5 g., distilled water 1,000 ml. These ingredients are steamed to dissolve and then cooled. The pH is adjusted to 7-3 and 4 ml. of cresol-red solution, 10 ml. of brom-thymol-blue solution and 10 ml. of thymol-blue solution are added. 100 ml. of 10% lactose solution and 80 ml. of 20% urea solution are then added by Seitz filtration. The medium is dispensed in 3-ml. amounts and incubated for 24 hours, and then refrigerated until required.

INTERPRETATION OF BIOCHEMICAL REACTIONS

Urine specimens are passed onto a Kligler's iron agar. Characteristic findings are confirmed by slide agglutination. The urinary specimens are then subcultured onto a salmonella-shigella agar plate, and growths with the colonial characteristics resembling those of S. typhi are passed through Singer's urea media and then subcultured only onto a Kliger's iron agar. Characteristic findings are confirmed by slide agglutination tests. The urinary specimens are dealt with described above.

Differential presumptive diagnosis according to biochemical reactions.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Motility</th>
<th>Dextrose</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Mannite</th>
<th>Indol</th>
<th>H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Other salmonella</td>
<td>+(-)</td>
<td>AG(A)</td>
<td>AG(A)</td>
<td>-</td>
<td>-</td>
<td>+(-)</td>
<td></td>
</tr>
<tr>
<td>Sh. paradysenteriae</td>
<td>(Newcastle)</td>
<td>+</td>
<td>AG</td>
<td>AG</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Sh. dysenteriae (Shiga)</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh. ambigua (Schmidt)</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh. sonnei</td>
<td>+(-)</td>
<td>AG</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh. paradysenteriae</td>
<td>(Flexner and Boyd)</td>
<td>-</td>
<td>A</td>
<td>A</td>
<td>- (-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh. alkaicolaens</td>
<td>+</td>
<td>AG</td>
<td>AG</td>
<td>+</td>
<td>+(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus group</td>
<td>+</td>
<td>AG</td>
<td>AG</td>
<td>+</td>
<td>+(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus morganii</td>
<td>+</td>
<td>A(A)</td>
<td>A</td>
<td>+</td>
<td>+(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>A(A)</td>
<td>A</td>
<td>+</td>
<td>+(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracolobactrum</td>
<td>+(-)</td>
<td>AG</td>
<td>AG(-)</td>
<td>+</td>
<td>+(-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A = acid formation only AG = acid and gas formation
**TESTS FOR (a) 'FREEDOM FROM INFECTION' OF CONVALESCENTS AND (b) DETECTION OF CARRIERS**

**Faeces**
1. Forward to laboratory in Sel. Br. Incubate up to 18 hours.
2. Subculture onto SS. Incubate 18-24 hours.
4. Discard blue or yellow tubes. Subculture green tubes.
5. Examine KI tubes. Discard tubes which do not show red slope with yellow butt and no gas and (usually) blackening.
6. Do Gram stains of smear.
7. Do slide agglutination tests with Vi, H and O typhoid antisera.

**Urine**
2. Subculture onto SS and proceed as for faeces.
3. Subculture at least 6 lactose fermenting colonies onto SS. Incubate 18-24 hours.
4. Discard blue or yellow tubes. Subculture green tubes.
5. Examine KI tubes. Discard tubes which do not show red slope with yellow butt and no gas and (usually) blackening.
6. Do Gram stains of smear.
7. Do slide agglutination tests with Vi, H and O typhoid antisera.

**TYPHOID FEVER : PREVENTIVE MEASURES**

R. M. LANGERMAN, M.B., CH.B., D.P.H. (CAPE TOWN), Assistant Medical Officer of Health, City of Cape Town

Typhoid fever is world-wide in distribution. Generally speaking, the disease may become endemic wherever the water supply is subject to human excremental pollution, the standard of sanitation is low, the people are less enlightened, and overcrowding is present. By this standard, many rural areas in South Africa, particularly where there are big aggregations of Bantu, and also the peri-urban areas of the cities, where there is no water-borne sanitation or adequate, safe, reticulated, water supply, may not only be considered endemic, but even potentially epidemic. As a large proportion of the labour force in the cities and bigger towns is drawn from these areas, the situation can be likened to an unexploded bomb in the back garden, surrounded in some instances by an ornamental trellis.

The reservoir of infection is constituted by the permanent carriers, the transient carriers, the ambulatory cases, and also those diagnosed cases for whom there is no adequate isolation. The problem of preventing the spread of typhoid fever is one of pure basic hygiene, consisting in the blocking of the various paths by which the causative organism might pass in the faeces or the urine of an infected individual into the mouths of others. These routes are:
1. **Direct**, in which foodstuffs are contaminated with faeces or urine by the unwashed hands of carriers or cases. This may also occur with raw fruit and vegetables; urination in the lettuce patch and the use of human excreta as manure is not unknown in market gardens in some areas.
2. **Water supplies** may be contaminated by sewage from a mixed population or excreta from carriers or cases, the effects depending upon the degree of contamination. Gross contamination of public supplies may give rise to an epidemic explosive in character.
3. **Milk supplies** may be directly or indirectly contaminated by carriers or cases, with consequent infection also of milk products, such as cream, cream cakes, ice cream, immature cheese, etc. With infection of milk from a bulk source explosive epidemics may occur.
4. **Flies** may act as vectors. After they have eaten infected excreta they may regurgitate it onto foodstuffs to which they are attracted, or they may infect the foodstuff (including milk) by contamination from the surface of their legs or bodies.

Typhoid fever may occur at any age, but is commonest in older children and young adults. The incubation period is usually 9–14 days, but may range from 1–3 weeks.

The illness commences with malaise, lassitude, headache and pyrexia. This stage of vague toxæmia (bacteraemia) lasts about a week, during which time the patient is generally non-infectious. Having regard to the prevailing conditions of hygiene or the occurrence of confirmed typhoid cases in the district the practitioner should make use of available laboratory facilities, for blood culture is the only means of establishing the diagnosis beyond doubt in this important non-infectious stage.

After isolation of the case, all contacts should be observed for a period of 3 weeks. Those employed in the handling or preparation of foodstuffs should be excluded from this occupation during the period of observation.

The Permanent Carrier

It is the permanent carrier who is the original source of every outbreak. There are usually no short cuts in detection, and a thorough investigation of all the patient’s movements and places of eating in the month before the onset of illness is frequently necessary before any clue can be obtained. More often than not the individual and his family are of a low standard of intelligence and extreme patience is required.

On detection, a carrier should be admitted to an infectious diseases hospital, where treatment should be given to bring the carrier condition to an end, if possible. Before discharge from hospital, permanent carriers are given instruction regarding their danger to others, the need to wash their hands after visiting the toilet, and if they reside in unsewered areas, the necessity for disinfecting the stercus pail after defaecation. They are forbidden to handle or prepare any foodstuffs, even in their own homes if this is possible. They may not go away on holiday, change their address, or except in emergency enter hospital without informing the local authority.

They should be visited at least once a month and the whole lecture repeated ad nauseam. Carriers often become