THE ROLE OF THE LABORATORY IN THE DIAGNOSIS AND CONTROL OF TYPHOID FEVER

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Typhoid fever is a serious infectious disease which is widely endemic in the rural areas of the Union of South Africa and which occasionally spreads into the urban areas. It is a preventable disease.

As the disease and its carrier state, which is responsible for its persistence in a community, can only be diagnosed with certainty by laboratory tests, an adequate public-health laboratory service must be an essential feature of any practical programme for its control. The Union Health Department provides such a service.

LABORATORY SERVICES PROVIDED

Pursuant to Government Notice No. 1073 of 22 June 1956, a pathological laboratory service is provided, free of charge, by the Union Health Department to medical practitioners, and to local authorities which have established services under the Public Health Act 1919 for the control of infectious diseases, for the performance of such laboratory tests as may be reasonably required for the diagnosis and control of certain scheduled infectious diseases. Amongst these scheduled diseases is enteric fever, i.e. typhoid and paratyphoid fevers. This service, as provided for medical practitioners, is restricted to tests necessary to confirm the presumptive clinical diagnosis in patients who are suspected to be suffering from these scheduled diseases. For local authorities, however, the service provided is of much wider scope and includes not only tests required for the diagnosis of these diseases but also those required for the treatment of patients 'for ascertaining when such patients have become free of infection, (and) for the public-health control of such diseases, including the detection of human carriers . . . and the tracing of outbreaks . . . to their sources with the object of preventing further spread'.

These services are at present provided either directly by the Union Health Department through its own laboratories at Cape Town and Durban, or indirectly on its behalf by other organizations, e.g. by the South African Institute for Medical Research, Johannesburg, and its various branch laboratories in other centres, and by the laboratory of the Cape Provincial Administration at East London.

The typhoid bacterium is a relatively hardy organism which readily survives in specimens sent through the post even though several days may elapse before they are delivered to the laboratory. There are, therefore, few areas in the Union which are so distant from the nearest laboratory that they cannot be effectively served with tests for enteric fever infections. It is unfortunate from a public-health point of view that so many medical practitioners and local health authorities fail to make full use of these free services provided by the Union Health Department. There is really no sound excuse for this and, by ignoring these services, medical practitioners and local authorities hinder public-health efforts for the control of this unnecessary disease and so render the country a disservice.

LABORATORY TESTS FOR THE DIAGNOSIS OF TYPHOID FEVER

Typhoid fever results from an alimentary infection with *Salmonella typhi* which rapidly develops into a generalized condition characterized by septicaemia. Very soon, however, the infection becomes largely localized to the mesenteric lymph nodes and the Peyer's patches of the small intestine. Both of these tissues undergo inflammatory hyperplastic changes and, towards the end of the 2nd week, necrotic changes occur in the Peyer's patches and lead to bowel ulceration. Gradually, also, antibodies are produced against the invading organism and, during the natural course of the disease, may lead to clinical recovery of the patient, which normally is expected to commence about the 4th week unless shortened by antibiotic therapy.

Because the disease commences as a septicaemia, blood cultures during the 1st week are positive in virtually 100% of cases. Thereafter the percentage of positive blood cultures diminishes so that during the 3rd week of the disease they are usually negative.

Though stool cultures may be positive during the 1st week, it is not until the 2nd week, when ulceration may begin

in the bowel, that the organisms are excreted in such numbers that they may be readily recovered by stool culture in almost all cases. This abundant intestinal excretion of *S. typhi* continues until convalescence when, in the majority of cases, it gradually ceases. The minority become chronic carriers and continue to excrete the organisms indefinitely though outwardly they may have completely recovered from the effects of the disease.

Typhoid bacteria may also be excreted in the urine during the 2nd and 3rd weeks of the disease and occasionally this urinary excretion may persist until long after convalescence has passed. These latter cases constitute the chronic urinary carriers.

The rise in antibody titre does not reach significant levels until well into the 2nd week of illness. The rise is chiefly in the H and O agglutinins; the Vi agglutinins usually only appear in low titre during the 3rd week and then soon disappear except in cases in which a chronic focus of infection persists.

The early presumptive clinical diagnosis of typhoid fever during the 1st week—the most important time to make the diagnosis—is therefore best confirmed by blood culture and thereafter by stool culture. Because of its insidious onset, it may be difficult to decide clinically whether the disease is in its 1st or 2nd week. For this reason it is recommended that, when typhoid fever is first suspected, both specimens of blood and of faeces should be sent to the nearest laboratory for diagnostic tests.

The typhoid organism is not fastidious in its growth requirements, so that a simple broth may be used for blood culture. The addition of bile salts in small quantities to the medium does not interfere with the growth of enteric organisms, though it will inhibit the growth of most organisms likely to contaminate a specimen of blood during its collection. The medium of choice for the isolation of *S. typhi* from the blood is, therefore, bile broth. Because antibacterial substances may be present in the plasma, it is wise to dilute the blood specimen with such quantities of medium as to render these substances inactive. Not more than 5 ml. of blood, therefore, should be added to 100 ml. quantities of medium. Suitable culture bottles containing adequate quantities of medium for the diagnosis of typhoid fever by blood culture may be obtained by medical practitioners and local authorities in reasonable quantities to meet their requirements on request to the laboratory which serves their area.

Specimens for blood culture should be collected by venipuncture with the usual aseptic precautions and the inoculated bottles should then be forwarded to the laboratory with the minimum of delay.

For stool cultures fresh specimens of stool should be collected, in quantities of about a teaspoonful, in containers with airtight screw-cap stoppers, such as are supplied by the laboratories for this purpose. In order to avoid drying of the specimen if it is to be sent a long distance by post, it may be desirable to preserve it either in Sach's solution or in selenite broth, but usually this is quite unnecessary. Specimen bottles containing these solutions may be obtained from the laboratories on special request.

It is most important that specimens of blood and stools for cultural investigations should be collected before any antibiotic therapy is commenced.

Once collected, the specimens should be forwarded to the laboratory with a minimum of delay. Cultural tests for *S. typhi* on blood and stools are usually completed within 72 hours of the receipt of the specimens at the laboratory, but results of examinations are sometimes delayed for a week or so because of such causes as the tardy growth of the organisms. It is the usual practice of laboratories promptly to notify the positive result of any cultural test for the diagnosis of typhoid fever by telephone or telegram to the medical practitioner who forwarded the specimen. All reports are then confirmed in writing.

Widal tests have been employed extensively in the past for the diagnosis of typhoid fever but today they may be considered to be of no real value for this purpose. For the effective treatment of typhoid fever with chloramphenicol, early diagnosis is of paramount importance, since the earlier such treatment is commenced after diagnosis the quicker is the cure and the less the chance of a chronic carrier state developing. A diagnostically significant titre of H and O agglutinins in the serum of the patient is normally not to be expected before the end of the 2nd week of the disease, which is too late. Difficulties also often arise in the interpretation of the results of this test. Thus, the quantitative immunity response of individual persons varies greatly, so that it is difficult to decide what is the lowest agglutinin level that should be regarded as diagnostically significant. This position is further complicated by the fact that persons who have received previous TAB vaccine inoculations may show a non-specific anamnestic rise of the typhoid agglutinins to any febrile disturbance, so that further tests, causing more delay, may be necessary to decide whether the titre is a rising one and so of diagnostic importance. Now that blood and stool cultural tests give such excellent results during the earlier stages of the disease, Widal tests may be regarded as more of historical interest than of practical value, except in recovered cases in which it may be desirable to make a diagnosis in retrospect. The Vi agglutinins, which are more tardy in their development than the H and O, also rise too late to be of any help in the early diagnosis of the active disease.

TESTS TO ASCERTAIN WHETHER THE PATIENT IS FREE OF INFECTION

All cases of typhoid fever excrete typhoid bacteria in their stools and many cases also excrete them in the urine. This excretion usually only continues until convalescence but, in a few cases, it may persist indefinitely despite the patient's apparent full clinical recovery from the disease and despite adequate chloramphenicol therapy. Such patients become 'healthy' carriers. The fact that the subject has made a full clinical recovery from the disease is thus no evidence whatever that he is not a carrier and still excreting the pathogens. Such carriers are a definite danger to others because they are capable of transmitting the disease to them. This carrier state can only be diagnosed by laboratory means. It is therefore most essential in the public-health interest that no patient who has clinically recovered from typhoid fever should be regarded as free of infection and fit for discharge from medical surveillance until it has been proved, by adequate bacteriological tests, that he is no longer excreting the organisms in his stools or urine.

As all typhoid patients continue to excrete the organisms until well into the convalescent stage, it may be regarded as

a waste of effort to submit specimens for testing for freedom from infection until 3-4 weeks have elapsed since the commencement of chloramphenicol treatment. Thereafter, at least 3 specimens each of stool and urine should be sent to the laboratory at intervals of not less than 4 days for cultural testing. Some authorities also think it wise at the end of this period to send a sample of whole blood or serum for a Vi test to confirm that no focus of infection is persisting. If all these specimens of stool and urine prove negative and the Vi agglutinin titre is less than 1 in 10, the subject may be regarded as free of infection and fit for discharge from medical surveillance. If, however, any one of these tests prove positive, he should be regarded as a potential chronic carrier. In such a case a further series of not less than 6 specimens each of stool and of urine should be sent for testing at similar intervals and the Vi test should again be repeated at the end of this series. If all these specimens prove to be negative and the Vi agglutinin titre below 1 in 10, the subject may now be regarded as free of infection, but if any of these specimens for cultural test prove positive and, possibly, if the Vi agglutinin titre remains unduly high, the subject should be regarded as a carrier and a potential danger to others. He should therefore be kept under surveillance by the local health authority and should not be allowed to carry on any work associated with the handling of food, and all necessary hygienic instructions should be given to him to minimize the possibility of his infecting others. The pathologist in charge of the laboratory should be consulted on what further tests should be carried out, and when, so as to determine whether there is any change in the carrier state of the subject and whether he is responding to any treatment that may have been prescribed. It is unfortunate that in these cases further treatment with antibiotics is seldom of any value and that the problem of how best to cure the chronic carrier has still found no really satisfactory solution. Nevertheless, by keeping known carriers under proper medical surveillance a great deal may be done by health authorities in preventing them from spreading the disease to others.

Experience at Cape Town shows that, if the diagnosis of typhoid fever is made in the early stages of the disease and adequate chloramphenicol treatment is immediately instituted, the chronic carrier rate is kept low—in the neighbourhood of 2% for faecal carriers and less than 1% for urinary carriers.

LABORATORY TESTS FOR THE DISCOVERY OF CHRONIC CARRIERS

The only natural reservoir for typhoid bacteria is the human being, i.e. the patient suffering from or recovering from the disease and the 'healthy' chronic carrier. Both patients and chronic carriers excrete typhoid organisms in their faeces or urine and the spread of disease from them to others is usually by excremental contamination of water or foodstuffs, including milk.

As patients suffering from acute typhoid present the typical clinical picture of the disease, the diagnosis of which may be confirmed with certainty by appropriate laboratory tests, and as by such tests it may also be determined with reasonable certainty when they become free of infection and so incapable of spreading the disease to others, the spread of disease from such patients may be readily controlled by simple public-health measures. Far more difficult, however, is the control of the spread of the disease from the unknown healthy carrier. These carriers present no clinical signs and may not even have a history of typhoid fever, since they may possibly have acquired the carrier state from a subclinical infection. They may, therefore, long remain unsuspected in a community and perhaps give rise to repeated epidemics. Until these carriers are detected they remain a potential and unknown source of danger. The hygienic measures that are necessary to completely obviate the danger of acquiring a typhoid infection are difficult to practise effectively in rural areas, particularly amongst primitive non-European communities. Moreover, typhoid infection when present in one community may readily cross to another, and vaccination confers only a relative and not very lasting degree of immunity. For these reasons, one of the most important factors in the attempted control of typhoid fever in South Africa should be well directed efforts towards the tracing of carriers. To attempt, even in a small community, to test and, possibly, periodically retest everybody to ascertain whether any carriers exist in it is obviously quite an impracticable procedure. When, however, a case of typhoid fever, which may herald an epidemic, occurs in a community, besides tightening up local hygienic measures, effectively controlling the patient, and perhaps initiating a vaccination campaign, every effort should be made by the local health authorities to trace the source of the infection back to the original carrier. This is obviously often a very difficult task involving much hard and persistent effort, which must be intelligently directed. Unfortunately there are no reliable short cuts and, until the unknown carrier is detected, there is always the danger that he may start fresh epidemics.

To help trace chronic carriers, the Vi agglutination test has been extensively used in South Africa. Theoretically these tests depend upon the assumption that the active focus of infection which may persist in the subject and render him a carrier is associated with a persistent rise of Vi agglutinins in his blood. These agglutinins may be demonstrated by a relatively simple inexpensive serological test. The Vi agglutination test should therefore be a very valuable screening test for tracing potential carriers. Our practical experience, however, is that this test is a disappointing one. Firstly there are various technical difficulties. The antigen, which is the essential reagent used in the test, is often unreliable as evidenced by the fact that antigens obtained from different and presumably reliable sources, when tested in parallel against unknown sera, often give widely differing results despite the fact that they have supposedly been standardized. There is also the difficulty in deciding what is a diagnostically significant titre. Generally a titre of 1 in 10 with a standardized antigen is regarded as suggestive of the carrier state, but there is evidence that proved carriers may on occasion show a lower titre than this—even none at all. A further difficulty is the fact that so many persons who show a titre of Vi agglutinins of 1 in 10 or more are obviously not carriers in that there is no epidemiological evidence to implicate them with any typhoid cases and, more important, in that despite extensive bacteriological investigations there is no evidence to indicate that they ever excreted typhoid bacteria. Finally, and most serious, is the frequent misapplication in South Africa of this test by local health authorities. Some of them require all food handlers to be periodically subjected to Vi tests, and those who are found to give positive tests are then immediately, and often without any further investigation, taken off their jobs. So

ridiculous has been the position that I have even known persons who were employed as 'food handlers' in handling hermetically sealed metal cans of food in a store to be dismissed from their jobs because they gave Vi-positive tests. A Vi-positive test by no manner of means proves that a person is a carrier of typhoid fever. Even provided that the positive result is a reliable one, it at best merely suggests that the person may be a carrier; and the only way to prove that he is in fact a carrier is by bacteriological cultural tests to prove that he is actually excreting the organisms. For a person to be dismissed from his employment as a food handler merely because he unfortunately gives a Vi-positive test appears to be both unwarranted and unfair. If a Vi-positive test is to be regarded as suggestive evidence that a food handler may be a typhoid carrier, the proper procedure is to suspend him temporarily from the handling of food and then to investigate the position by appropriate bacteriological tests to ascertain whether he is actually excreting typhoid bacteria.

I personally think that the Vi test, as at present generally used in South Africa, is sometimes so unreliable in its results and so often misused in practice that it should be dropped from routine usage until the technique for its performance has been suitably improved, particularly in respect to the provision of reliable antigens, and its value in detecting carriers more effectively assessed by a properly carried out research programme.

There would thus appear to be only one reliable way for tracing the carrier who has initiated a typhoid epidemic, and that is the hard way. The first step is 'police' epidemiological investigations by health officials to trace all possible suspects, and the second step is to find which one of these is guilty by the performance of appropriate bacteriological tests on properly collected urinary and faecal specimens.

Urinary carriers are readily proved by cultural tests on samples of urine which need not be catheter specimens. The collection of suitable faecal specimens is more difficult. The evidence is that in the faecal carrier the focus of persistent infection is usually in the gall-bladder or biliary passages, and that the excretion of the bacteria may be intermittent and their numbers in the faeces so low that they may readily be missed on routine cultural examinations. It has, therefore, been suggested that the best way to detect 'faecal' carriers is to culture samples of duodenal fluid obtained by the passage of a duodenal tube. This minor operative procedure, however, is very cumbersome and not suited for routine usage in the tracing of carriers. My personal experience is that very good results may be obtained by giving the subject a small dose of calomel followed by magnesium sulphate and then culturing a specimen from the third stool obtained by this artifically induced diarrhoea. Theoretically this procedure allows the collection of a specimen of stool hurriedly evacuated from the small intestines, into which the gall-bladder has been stimulated to empty itself.

Such stool specimens should be collected into containers of selenite broth as supplied by the laboratory for this purpose and then promptly forwarded to the laboratory for cultural studies. If the test proves negative and there is still strong epidemiological evidence to indicate that the subject is a carrier, the test may be repeated and, if necessary, duodenal intubation considered.

OTHER LABORATORY INVESTIGATIONS

Phage typing. In South Africa phage typing of *S. typhi* is carried out on behalf of the Union Health Department by a research unit, under Professor Pijper and Dr. Crocker, which is attached to the Institute of Pathology of the University of Pretoria. Pure cultures of typhoid organisms that have been isolated from patients or carriers should be sent to this institute for phage typing, for such tests may offer very valuable epidemiological information whether cases of typhoid fever originate from one or more sources, and they may also help greatly to determine whether a particular carrier is the source of a particular epidemic. It is the practice of the Government Pathological Laboratories at Cape Town and Durban to send a culture of every typhoid bacterium isolated to this institute and as a result much useful epidemiological information has been obtained. This is a practice which is therefore recommended to all pathological laboratories in the Union of South Africa.

As the vehicle for the spread of typhoid fever is often water or foodstuffs, including milk, which have been accidentally contaminated by excrement from a patient or carrier, it is a wise procedure on the part of local health authorities to ask for bacteriological tests on properly collected samples of water or foodstuffs suspected on epidemiological grounds to be involved in the spread of typhoid fever during an epidemic, in order to ascertain whether there is any bacteriological evidence of faecal contamination of such water or foodstuffs. It must, however, be stressed that the finding of faecal pollution in the water or foodstuffs is not the final solution of the problem of the source. The final solution lies in tracing the carrier who polluted the water or foodstuffs, and until he is found the possibility of future epidemics must hang like the sword of Damocles over the community.

Team Work

It is obvious that the control of typhoid fever in the community must depend upon good team work and therefore there should always be the closest cooperation possible between the medical practitioners who discover the cases, the local health authority responsible for the public-health control of the disease, and the pathologist responsible for providing the necessary laboratory services. If these various persons all play their appropriate roles and act in concert, it should be possible, in many parts of South Africa, to score a decisive victory over typhoid fever.

SUMMARY

To control typhoid fever effectively in South Africa, full use should be made by medical practitioners and local health authorities of the free laboratory services provided by the Union Health Department. Laboratory tests allow:

(*a*) of the confirmation of the presumptive clinical diagnosis of typhoid fever,

(*b*) of a decision being made on when a patient is free of infection and therefore safe for discharge from medical surveillance, and

(*c*) of the discovering or proving of chronic carriers, who are ultimately responsible for all epidemics.

For the early diagnosis of the disease, blood and stool cultures are recommended and the Widal test is to be regarded as a laboratory test chiefly of historical interest.

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.

Phage tests are of great epidemiological value and all cultures isolated from patients and carriers should be sent by laboratories to the Institute of Pathology, Pretoria, for phage typing.

To control typhoid fever, the closest possible cooperation is desirable between medical practitioners, local health authorities and public-health pathological laboratories.

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