Practical Aspects of Clinical Bacteriology

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The laboratory is most often called upon to help in the diagnosis of one of the commonest clinical conditions — a pyrexia of unknown origin In Malta this may be due to various causes, but amongst the most frequent are undulant fever and typhoid fever, the former being markedly commoner. To diagnose Brucellosis two investigations can be carried out in practice: the indirect test for agglutinins, and the cultivation of the *Brucella melitensis*. Normally, it is very important, in choosing which investigation to ask for, to consider the time and period of the illness, but in undulant, which is a long-drawn out disease with specific characteristics, it is practically safe to say that by the time the patient has called in the doctor he has already had the fever for days and by then antibodies will have developed. It is, therefore, good practice to ask for an agglutination reaction as early as one suspects undulant. This can be done by the slide method, in which all one is required to send is a capillary tubeful of blood, or by sending about 5 cc. of whole blood, which will allow a titration of the agglutinins to be carried out. The first — what has erroneously become too generically called, locally, the 'blood serum reaction' — is part of the diagnostic service which the Health Dept. offers free to all practitioners. It should be easy enough to procure two or three drops of blood from the ear lobe of a patient, but, speaking from experience it seems that some doctors find difficulty in this, and we are often asked to carry out the test with about half an inch of blood in a thin capillary. This difficulty, especially in private practice, arises from the fact that the doctor has not pricked the patient's ear deeply enough. This should be done with a pin, which should be pushed right in. If the amount of blood is too little — especially if part of it has been cooked in the process of sealing the capillary, then the carrying out of the test will be quite impracticable. Incidentally, may I, in passing, mention how pleasant it is to get the accompanying note on a neat, fair-sized sheet of paper, legibly written. It is irritating to get this on a slip of paper about 3" by 1", scrawled in pencil.

The capillary tubeful method is very useful but cannot be absolutely exact. To get any exactness a titration is desirable, and for this about 5cc. of blood must be sent. The serum is separated from this and dilutions are made. The question which always arises is, 'Which dilution is diagnostic? Does a reaction at 1/80 mean the patient has actually got undulant fever, or has it to be at a higher dilution?' The answer seems to be that the higher the dilution at which a reaction is obtained the more certain it is that one is dealing with an active infection, but it seems to me, from what I have seen, that even a reaction at a low dilution is normally significant and has to be given attention. Undoubtedly, low titre reactions may mean no more than sub-clinical infection. A 1/40 or 1/80 in an afebrile goat-keeper need not cause anxiety, but there always has to be some explanation for it. May I also draw attention to two points: one is the helpfulness of observing a rising titre, an increase, that is, in the dilution at which a reaction is obtained when the tests are carried out at a fair interval — not less than a week — and secondly to the fact that a search for agglutinins is after all an indirect examination and that we can have the paradoxical picture of a patient who is very ill with undulant, but who gives a negative or a very low titre result. This is easy to understand if one realises that a patient may be very ill exactly because he is not producing those antibodies

— the agglutinins — which we are looking for for diagnostic purposes. This situation arises more commonly in the case of typhoid infection — sometimes such cases will give a persistently negative reaction throughout a first phase of the illness, will, in consequence, relapse, and will then, especially if they are about to recover, finally start giving a reaction.

The cultivation of Brucella from the blood is proof positive and incontrovertible that one is dealing with an active infection. This is relatively easy: one inoculates from 5 to 10 cc. of blood in a liquid medium such as liver broth or tryptose broth with an anticoagulant and incubates it. One point to note is the absolute necessity for guarding against the introduction of contaminants — most common of all, Staphylococci from the patient's skin or the operator's hands. One might overcome this by the additional of Penicillin to the medium but the umbrella of Penicillin should not be an excuse for bad technique. Fortunately once the Brucella has grown it is quite easy to recognise, from its morphology, colonial characters and serological reactions but unfortunately it is a slow grower and I always keep cultures for ten days, at least, before reporting a negative. This implies repeated subculturing, but I have adopted a useful technique for this. It is the use of Castaneda's method which consists in having the liquid medium in a bottle, along the side of which there is a layer of tryptose agar. One has only to slant the bottle to inoculate this agar from the liquid medium and to do so moreover with a very large inoculum. I believe we miss very few cases with this procedure. It is always advisable to take blood during the septicaemic phase, that is during a pyretic attack, and when using 50cc of broth, no less than 5 cc of blood should be taken. Do not forget that the chances of a positive result depend on the chances of there being a microorganism in the actual volume of blood you have picked up. If bacteria are scanty and you submit only a couple of millilitres you may quite easily miss getting them in that volume.

It is also practicable to look for Brucella agglutinins and to cultivate Brucella from the fluid in joint lesions. In that way I have been often able to confirm the diagnosis which had been considered on clinical grounds. Sometimes these tests are of considerable value in differential diagnosis.

The situation with regard to typhoid diagnosis is very similar only here we have to add culture of micro-organisms from the faeces to the agglutinin reaction and blood culture. It is very important in these cases to recall which test is most likely to be useful at a given period of the illness. The percentage of cases showing positive blood cultures declines steadily after the first week. The bacteraemic stage in typhoid is an early stage, so blood culture is most likely to be useful at that time. The frequency of *Salm. typhi* in the faeces rises from the first week to the third and then falls somewhat slowly. The likelihood, therefore, of getting a positive result in faeces culture becomes greater as that of getting a positive blood culture decreases. As for agglutinins to quote Topley and Wilson, "In the first week about 20% of the cases give a positive agglutination test. The curve then rises sharply, crossing the blood-culture curve just before the end of the second week, and still rising attains a value of 90% or more by the fourth week; after which it remains at a high level for several weeks". What I have said about the paradoxical negative in severe cases with regard to undulant fever applies even more in the case of typhoid fever — cases where one only gets a positive in a relapse. The agglutination reaction is interfered with by antibodies which may have followed T.A.B. vaccination. A mere 1/40 or 1/80 in a vaccinated patient means very little. One odd circumstance is that many practitioners do not realise that many young

people do receive T.A.B. vaccination.

To overcome the difficulty arising from agglutinins due to T.A.B. one may carry out tests for Vi antibodies, but in practice, I believe that it is more useful either to carry out a blood culture, or even more to perform two agglutination tests at a week's interval and to look for a rising titre.

One cause of "Pyrexia of unknown origin" which still persists amongst us is murine typhus. It started as an epidemic in 1943 but a few cases still crop up. It was the laboratory which definitely established the existence of that infection in 1943, and it can still be of help through carrying out the Weil-Felix reaction — the search for antibodies which will agglutinate a suspension of the 0 variant of Proteus X 19. We learnt, however, in 1943 that a low titre reaction (1/40 or 1/80 or even higher) could and does occur in quite healthy inhabitants of our island and the necessity for noting a rise in titre is even more important in the case of typhus than it is in undulant and typhoid.

An illness in which the laboratory can play a vitally important part is diphtheria. Between 1938 and 1953 I had to do with the bacteriological control of practically all cases of diphtheria in Malta, and that meant examining something in the region of fifteen thousand swabs. Unlike what happens elsewhere I examined these myself and not, except for very brief intervals, through the help of technicians, so that I feel I can claim a modicum of experience in the matter. The golden rule in the diagnosis of diphtheria seems to be that there is no golden rule. Neither the nature of the membrane nor the presence or absence of high temperature nor any other feature seem to be able to replace the examination of a swab — properly taken. In this connection it should be pointed out that the Medical and Health Department runs a free diagnostic service for this purpose. Some practitioners do not make use of it since they fear that if they send in a swab it means they have to report the case even as a suspected case. Obviously there is not much to be said about that — one has to report the case, but on the other hand if one does not suspect, but wants to exclude the possibility of diphtheria I feel one need not necessarily report the case.

May I emphasise, having said all that, the extreme importance of not letting treatment wait on bacteriological confirmation in dephtheria. No physician is justified in doing that. One other point: more and more I have come to realise the frequency of diphtheria away from the throat and larynx. Do not forget it as a possible cause in chronic inflammation of the nares and in chronic ear suppurations. Diphtheria in the ear may arise as a late complication of pharyngeal diphtheria or appear on its own. I have met several cases in which the *Corynebacterium diphtheriae* was causative and in which specific treatment cured the condition.

With reference to tuberculosis I will mention just one point. Sometimes the patient may be incapable of supplying a suitable sample of sputum, because he is in the habit of swallowing it. In such cases it is advisable to pass a gastric tube and examine the gastric contents — preferably a sample obtained about an hour after the patient has wakened in the morning. This should be examined not later than an hour after it has been taken, otherwise the tubercle bacilli which resist the gastric acids for a time will eventually be killed and cannot be cultivated or used for pathogenicity tests. In this connection I wish to draw attention to an excellent series of articles which appeared in the B.M.J. between October 1953 and July 1954. In connection with the gastric examination, I would remind you that it involves inoculation of guinea pigs, which are far from abundant and will involve waiting for some six weeks — an anxious period for the patient. So do not resort to it needlessly. If the patient can spit out his sputum you should always prefer

that. You should also bear in mind that what we are looking for in these cases is swallowed sputum so it is absolutely useless to examine gastric contents if the patient does not suffer from a cough at all.

One disease, cases of which I have come across within the last year — not in abundance, but disturbing enough in their frequency — is leprosy. The traditional method of diagnosis here consists in examination of scrapings from the nasal mucosa, but I believe a greater number of positives is obtained by examining a smear of the fluid which can be obtained from the skin by making a tiny slit with a sharp bistoury into the nodule or into inflamed skin. This is practically painless, can be done by the ordinary physician and need not alarm the patient. As a precaution the smear, which can be done with the bistoury itself, must always be made on a new slide, to avoid such false positives as can arise from the presence of scratches, etc.

We are frequently called upon to examine cerebro-spinal fluid in cases of meningitis and meningeal irritation. The observation to be made here is that even with purulent fluids diagnosis may be difficult. It often happens in *Neis. meningitidis* infection that the bacteria are extremely few, and it is rarely that the case is met with which provides slides full of polymorphonuclear cells and full of bacteria, the sort of case which could be called a "demonstrator's delight". Much more frequently bacteria are scanty and slides have to be looked at long and closely before microorganisms are seen which one can be convinced are really Neisseria. A common infecting agent, with purulent fluids, is *Strept. pneumoniae*. Perhaps even commoner are cases in which one finds a mixture of bacteria, quite frequently not easily identifiable but including gram positive rods, negative rods and staphylococci. These cases are associated with a perforated membrana tympani and are the result of a passage of bacteria from the outer ear.

As far as urethral discharge in the male is concerned I think a point of interest is to realise that not all cases of purulent urethritis are in fact due to an infection with *Neis. gonorrhoeae*. Non-specific urethritis is becoming more and more frequent as a diagnosis — a urethritis due to staphylococci, to *Bact. coli* and various other microorganisms. Normally when I get two slides sent to me with a smear, I stain one with Archibald's stain; a mixture of thionin and methylene blue which gives one a general idea what bacteria one is dealing with and then carry out a Gram. It is surprising how often cases which at first glance might appear to be cases of gonorrhoea turn out to be due to mixed non-specific infections.

In connection with discharges in the female, I wish to draw your attention very earnestly to the frequency with which leucorrhoea is due to the presence of a protozoal parasite the *Trichomonas vaginalis*. This, too, is a diagnosis which venereologists are making with increasing frequency. It seems to be transmitted generally by sexual intercourse. It is quite easy to diagnose provided one performs the examination as it should be done, one examines, that is, a little of the fluid discharge immediately after it is taken from the patient, because Trichomonas is easily identifiable when seen moving, but difficult to stain and to identify once it is motionless against a background of strands of fibrin, etc. Some actually advise having a microscope in the room where patients are examined. The fluid is taken up with a Pasteur pipette and if too dense, it is diluted with a little saline solution. I feel quite convinced that Trichomonas infestation accounts for a large proportion a cases of leucorrhoea; some attribute to it well over 40% of cases primarily complaining of vaginal discharge. It is certainly very common in Malta.

A word with regard to examination of faeces in general and specially with reference to the search for *Entamoeba histolytica*. My teacher in semeiotics used to

insist very strongly that the first examination consists in just looking at the sample —an undignified, and repulsive but necessary part of a doctor's work. Again and again I get samples sent which purport to come from dysenteric patients which look quite normal. Of course, one may get the encysted form in these cases, but one should at least make sure that there have been diarrhoeic periods, and moreover it is much easier and somehow more convincing to find the vegetative amoeba. If the patient is not in a diarrhoeic phase one has to wait for it and if there is not and never has been such a phase then one would be looking for what has probably never been there. Then there is the method of submission: amoebae, as is very well known, die out fairly soon and unless one sees an amoeba moving it is very difficult to be certain of its nature. The best way to get a sample is to get the patient into hospital and examine stools soon after passage. Very often this is just the thing which the private practitioner and the patient do not want to do. There is resort to the device of keeping the sample warm in a thermos flask. This would solve the difficulty, if the patient can carry out instructions accurately. The habit has come in of putting warm water in the flask holding the tubeful of faeces. The trouble here is that most often patients (or sometimes nurses, or even doctors) put in water at well over 50°C. which, of course kills the amoebae which have been saved from dying of cold to be killed by over-heating. This is due to the fact that thermometers, apart from the clinical variety, are uncommon in homes so that patients and nurses rely on their idea of an adequate temperature, which does not happen to match that of the amoeba's. It might be better to use no water at all — although it should be simple enough to get suitable thermometers — at least in hospital wards and to teach staff to use them properly. I wish also to point out the possibility that, especially severe diarrhoeas may be due to bacillary dysentery — Sh. shigae, Sh. sonnei, Sh. flexneri, Schmitz's bacillus are met with, the Sh. shigae being especially violent.

In conclusion I wish to make a plea for the proper use of the laboratory. I have already mentioned en passant certain points which must be noted with regard to the submission of samples. To overlook these is to be doing things in such a way that neither the doctor nor the patient will be getting the benefits otherwise attainable. It is worse than useless to submit samples haphazardly in the hope that the laboratory people might find something in it somehow. Nobody can find anything which is not in the material submitted. Then there is the question of examining material as part of a routine. Apart from the fact that this leads to blunting and atrophy of the clinical sense, there is the plain truth that the staff available in most laboratories is limited and if they are made to use up all their time (I beg you to note that I do not say 'waste their time') on inessentials then they will have much less time and energy available for really useful investigations. Doubtless things ought to be different; staffs should be large enough to do more work but, there is a huge lag between things as they are and as they should be. I am afraid we have to carry on under the conditions which prevail. I always feel that the laboratory should be used as one would use a razor blade provided he only has one. One would keep it for shaving where a sharp edge is really necessary and not blunt it by sharpening pencils. With these provisos the laboratory is always ready and eager to be of use, it is pleased when it serves its purpose and ready to correct and improve its shortcomings, presuming that at the other end there are understanding clinicians gifted with a sense of science and an appreciation of things as they are in actual fact.