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further investigation. Another worker who has had some success with a one-day treatment is E. Ramel (*Rev. Suisse romand.*, 1940, Nos. 7 and 8). He treated 15 cases by giving them a tablet every hour for five doses and a sixth tablet was given 3 hours later. This treatment was supplemented by the induction of an attack of fever by means of an injection of Pyrifer. Miescher doubts if the Pyrifer was necessary.

IV

THE NECESSITY OF THE CLINICIAN MAKING THE BEST USE OF LABORA- TORY FACILITIES IN THE DIAGNOSIS OF VENEREAL DISEASES*

By Dr. I. N. ORPWOOD-PRICE

WHEN I was first asked to deliver a lecture on this subject, I felt a little diffident about talking, even for a short while, on what must appear to be so well-known to most people treating V.D. Further reflection, however, persuaded me that many clinicians *do not* know how to make the best use of a V.D. Laboratory. The reasons for this appear to be manifold but the chief one seems to be that the majority of practitioners have not served any apprenticeship, even of short duration, in a laboratory and many have little more than a nodding acquaintance with its work. Many years ago Colonel Harrison endeavoured to persuade the budding V.D. specialist to spend some time in the laboratory before undertaking the clinical side of this work. Unfortunately, this excellent advice was rarely followed and many deprived themselves of knowledge, the lack of which even they must occasionally regret. This lack of knowledge has been demonstrated clearly during the last few years if one reads some of the vast number of papers published about the value of the sulphonamide group of drugs. The clinical details are usually well-documented but the support or otherwise of laboratory evidence is often lacking, or is so primitive as to be valueless.

Now, as I see it, there are four main functions of a

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V.D. Laboratory and they may be summarised as follows :—

1. *To afford Evidence in support of the Clinical Diagnosis.*—It cannot be too strongly emphasised that it is no business of a pathologist as a pathologist to diagnose disease, and the onus of a diagnosis is on the clinician. Any clinician who refuses to make even a tentative diagnosis without a laboratory report is in mortal danger of sharing the fate of some who may be called administrative diagnosticians.

2. *To afford Evidence of the Effect of Treatment.*—The aid of the laboratory should never be neglected whilst the patient is in course of treatment and its services may be utilised in two directions. To assess (a) the effect of treatment on the disease itself; (b) the pathological effects (if any) of the treatment, e.g., blood dyscrasias.

3. *To afford Evidence of Cure.*—The laboratory aids to the evaluation of cure of V.D. are perhaps more neglected than anything else; particularly in gonorrhœa. A few prostatic beads stained by a simple dye such as methylene blue are often considered sufficient. Even worse, an inconveniently persistent positive blood serum test is often ignored or regarded as a false positive without any steps being taken to collect any evidence in support of this opinion. Such things are not rare in my experience, but one becomes hardened during the course of time.

4. *To afford Evidence of the Value of New Drugs in the Treatment of Disease.*—Much misdirected enthusiasm would be checked and much valuable paper would be saved if clinicians would use the laboratory facilities at their disposal and even occasionally consult the pathologist, who is usually willing to help and learn. Too often is he not consulted and his services treated somewhat like a slot machine. Too often are his efforts regarded with dark suspicion. Too often are specimens sent into the laboratory without any information and the results sent out interpreted according to the clinician's inclinations.

The role of the pathologist is to help the clinician and not to criticise him, and therefore I trust that you will treat the foregoing remarks with indulgence and allow me to give some indications of the way in which a laboratory service may be utilised to advantage. If these suggestions are followed it would not be unreasonable to hope that a lot of the present-day lip service to laboratory

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co-operation might be replaced by real co-operation. Apart from the clinician, one of the main causes of the failure of co-operation is the inaccessibility of the laboratory. I understand that some clinics are many miles from a laboratory and that rarely, if ever, do pathologist and clinician meet. This is a deplorable state of affairs and steps should be taken to remedy the position. It is unfair to the clinician and apt to make the pathologist careless. One suggestion is that, provided a clinic is not too small, a clinician pathologist should look after both aspects, but I have heard it said that there are objections to this. Perhaps members of the audience will put forth their views.

Now by far the most frequent tests done by V.D. Laboratories are serum reactions, and it is a wise precaution for the clinician to send specimens to a laboratory where tests are done at least twice a week. The reasons for this are twofold.

There will be (a) probably greater accuracy and uniformity of results; (b) the sooner a blood is tested after being taken, the better, since with complement fixation tests of any kind, the older the serum the more anti-complementary it becomes and the greater the liability to obtain doubtful or even false positive reactions. Furthermore, the percentage of false positive precipitation tests rises in an increasingly steep curve with sera more than three days old. This can easily be demonstrated with the Kahn test.

The clinician can be of great help to the pathologist if he will only take a little care and trouble in the matter of taking bloods. Firstly, by always taking enough—5 c.c. is suggested as a minimum. Secondly, having got a requisite amount of blood in the syringe, by being careful during transference to the blood tube or screw-capped bottle. "Frothing" should be avoided, since not only is the yield of serum poor, but the red blood cells get smashed with the consequent liberation of hæmoglobin. This hæmoglobin is apt to give rise to non-specific precipitates in precipitation tests, which may confound even the elect. In order to avoid this, the blood should never be squirted through the needle. The needle should be removed and the blood pushed out of the syringe gently into a blood tube, or the plunger of the syringe should be removed and blood poured into the screw-capped bottle.

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I do not propose to discuss the use or merits of the Wassermann Reaction, the various precipitation tests for syphilis or the complement fixation test for gonorrhoea, but I would remind you of one use of the Kahn Test in particular. When the result of a serum test is required in a hurry for special reasons, the Kahn test is very useful since an answer *can* be given within an hour of the blood being collected.

The recording of serum results should be standardised and it is to be hoped that after this war, one of the minor reforms effected will be the use of the standardised notation of serum results. The least one is entitled to expect is a statement in plain English as to whether a result is positive, doubtful or negative. This may sound obvious, but not infrequently I have been confronted by a clinician holding up a pathological report and demanding to know what it means. In all humility I disclaim responsibility for these reports, but on scrutiny they often resemble an algebraical formula and require an intimate knowledge of the particular pathologist's methods before interpretation becomes possible. Such things should not be, and any pathological report put out from a V.D. Laboratory should be easily understood by any clinician from Land's End to John o'Groats.

The solution of this problem is in the hands of clinicians themselves, who should demand from the pathologist that in any report on a serum, the notation used should be in accordance with resolution 4 of the Report of the Second Laboratory Conference on the Sero-diagnosis of Syphilis under the auspices of the Health Committee of the League of Nations in 1928. This resolution is fully explained in circular 1256 issued by the Ministry of Health.

The relevant resolution (No. IV) of the Conference is to the effect that since serological tests for syphilis are primarily for the purpose of assisting clinicians in diagnosis, in observing progress under treatment, and in tests of cure; and since also patients frequently pass from the care of one clinician to that of another with the result that the serum of one patient may be tested from time to time in different laboratories, a uniform method of denoting serological results which bear approximately the same clinical interpretations would be of great value to the clinician.

Accordingly the Conference proposed rules to the following effect:—

- (1) That a negative reaction should be reported as "—" or "negative."

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- (2) That a reaction which in the hands of the serologist is given practically only by sera from cases of syphilis (and of a few well-defined pathological conditions) should be reported as " + " or " positive."
- (3) That a reaction which is neither positive as defined in (2) nor negative should be reported as " $\pm$ " or " doubtful."

In making these recommendations the Conference remarked that there was nothing in them which would prevent the serologist adding to his report any amplifying or explanatory note which might be considered desirable (*e.g.*, signs expressing the strength of the reaction).

After serum tests the most sought-for advice is the identification of the causative organisms of the various venereal diseases. I do not propose to go into the laboratory technique of identifying these organisms, but rather into what I consider the best means of conveying the material to the laboratory so that it will reach there in a sound condition for examination.

Firstly, the recognition of *S. pallida* from primary sores or mucous patches will be considered. Serum (and if possible not an admixture of blood and serum) should be drawn up into a capillary tube, 3 inches in length, which is subsequently sealed. This is achieved by the simple method of warming the closed end of the tube and then applying the open end immediately to the sore or mucous patch after scarification. The contraction of the air in the closed end on cooling will automatically draw up the serum. When the tube is not less than half-full, the open end may be sealed off by heat. The capillary tube should be transferred to a test tube and lightly packed around with cotton wool at the bottom, top and sides, leaving room for the insertion of a water-tight rubber bung. The test tube is wrapped round completely with at least two layers of lint, kept in place with rubber bands, and placed in a thermos flask containing water at 37° C. The flask is then conveyed to the laboratory as soon as possible. In this way, the serum is prevented from drying up, whilst the temperature can be maintained at slightly below body temperature for at least 24 hours, and the chances of finding active *Spirochaeta pallida* are almost as great as on immediate examination.

Many clinicians prefer the open capillary tube method, in which the fluid is allowed to flow in by capillary action and the end removed from the point of entry is subsequently sealed.

The use of the thermos flask to maintain an even

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temperature is definitely indicated when extremes of atmospheric temperatures are encountered. To those who dislike the idea of using a thermos flask, I would suggest a thick covering of lightly packed wool as a compromise. This forms a reasonably efficient air jacket which will maintain a fairly constant temperature.

Specimens sent as wet swabs or dried smears are useless since the *Spirochaeta pallida* disintegrate rapidly in any drying process and are then impossible to identify by any method.

The next organism on the list is the gonococcus, one of the most wily of bacteria and the despair of many a bacteriologist. For successful isolation it requires a specialised medium made within a very limited range of pH, and I believe that if the organism is not transferred to the medium at the right stage of its life cycle, little or no growth will be obtained. Perhaps I had better explain what I mean by the right stage of its life cycle. This is the stage when the majority of the gonococci in the inoculum consist of well-formed, clear-cut cocci, as opposed to swollen disintegrating forms so often seen in smears made from body discharges. Furthermore, if too much pus is transferred to the medium with the inoculum, the growth is retarded if not prevented and, moreover, the organism cannot compete with other organisms which grow so much more readily. To obtain a growth of the gonococcus, all these difficulties have to be surmounted, and with the best medium in the world (except in the early acute stages of the disease) a successful culture is at present apt to be in the nature of a hit or miss experiment. In fact, a negative culture means little. With regard to the type of medium to be used, I have had little or no success with liquid media. A solid medium is essential to success, and of these the best in my experience are still hydrocele or egg albumen agar. The organism grows best at 37° C. and the culture is examined after 48 hours. To those who have no incubator, a vacuum flask can be employed if the culture tubes are closed with a rubber cork instead of the usual cotton-wool plugs. Even in a small flask of one-pint capacity containing two culture tubes, the temperature drops only 10° in 48 hours, and this does not prevent to any marked degree a successful growth. If a larger flask be used the drop in temperature is less. In performing the actual

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inoculation, attention to detail is necessary if success is to be achieved. The medium should be at 37° C. before inoculation is attempted and any inoculum from the urethra should be obtained from the interior after thorough cleansing of the meatus with sterile water. In like manner, an attempt to clean the cervix should be made before an inoculum is taken. Sodium bicarbonate or antiseptics should not be used in this cleansing process. The inoculum on the platinum wire should be transferred rapidly to the medium tube and rubbed firmly all over the surface of the medium. Too often have I seen the material slopped on to the surface of medium and, after a perfunctory rub or two, the tube plugged without any attempt at flaming either the top of the tube or the plug. Such efforts may bring success occasionally in spite of all, but when what may be termed the technical difficulties of growth are so marked, the pathologist is entitled to expect that the clinician will support him to the full and take real care and trouble with his inoculations. A laboratory report can be expected in approximately 48 hours from the time of inoculation. When vesiculo-prostatic cultures are made, even more attention to the details of inoculation is required. Not only the meatus, but also the whole urethra, should be cleaned by washing with sterile water. Massage of the prostate and vesicles should then be performed and the material obtained expelled straight on to medium contained in a 5-inch diameter Petri dish. The inoculum should then be thoroughly rubbed all over the surface of the medium as quickly as possible and the cover of the dish replaced. Incubation is then carried on for 4 days, at the end of which a report can be given.

My experience of attempts of the cultivation of gonococci from urinary threads has not been a happy one, and I think this should not be undertaken except in cases of exceptional importance. The best technique, I believe, is to fish the thread out of the urine with a platinum loop and remove as much urine as possible by leaving one end of the thread in contact with sterile blotting paper, whilst the rest of the thread is suspended in the air. The thread is then inoculated on to the medium in the usual way. Inoculation of centrifuged deposits of urine are useless in my opinion, since the only results I have ever obtained are either no growth at all, a complete

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sheet of coliform bacilli covering the surface of the medium or a mixed growth of organisms.

It may seem to some that all the work involved in taking cultures does not compensate one for the amount of information received. I do not support this view, but one thing is quite definite, and that is that if these inoculations are made with little or no attention to detail the results obtained by the pathologist will be a waste of time and material.

The identification of the *Trichomonas vaginalis* is usually a very easy task requiring only a glass slide coverslip and microscope. Therefore a laboratory is not often asked for this investigation. Should, however, it be desired to send a specimen of pus to the laboratory for this purpose, the pus should be mixed with five times its own volume of normal saline (one teaspoonful of salt to the pint) in a rubber-corked tube. The active parasite can be recognised at least 20 hours after despatch except in the coldest weather when the thermos flask (temp. 20° C.) should be used. In my experience the parasite does not tolerate very well a temperature of 37° C. for any length of time and is apt to die out. Owing to difficulties of fixation, even when using the osmic acid method, I have found smears of little use in identifying with certainty the parasite.

Other people appear to have been more successful than I have, but as the result of my experiences in working in association with Dr. Mascall at the Whitechapel Clinic, and of much work done at the Hospital for Women, Soho Square, there seems to be little doubt in my mind that for diagnostic purposes wet specimens are much to be preferred to dry smears.

Perhaps after blood tests the interpretation of smears from discharges from various parts of the body is the request most frequently made. Few people appear to have been taught how to make a smear and many specimens which I receive look as though the rats have been at them. There is a proper method of making a smear and if a little attention is paid to the details there is very little loss of time, and the pathologist is enabled to give a much more accurate report. The slide should be warmed to body heat—test on the back of the hand—and a drop of sterile water or normal saline placed on it. An approximately equal amount of the discharge should be taken by

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means of a platinum loop and well mixed on the slide with the water or saline, and then spread out evenly in a thin film. The film should be dried *slowly*, and finally fixed by passing through a hot flame three times. Slow drying is the secret of success since distortion of the organised elements such as the various cells is less apt to occur. This point is so frequently ignored or forgotten that I make no apology for referring to it. If a film is made in this way it is possible to report the average number of leucocytes present in an average field under a $1/12$ magnification. Whilst I admit this to be a rough estimation, at least it gives the clinician a mental picture of what has been seen under the microscope and a standard can be established by which one can work. For example, taking a $1/12$ field as the standard measurement, I regard 5 leucocytes present as normal, 10 as doubtful and 15 as pathological. The presence of epithelial cells is not so important as a rule, and I usually report them as a few, or some, or many present. Gonococci should not be reported as present unless the organisms are Gram-negative, intracellular diplococci and come from the genito-urinary tract. Extracellular Gram-negative diplococci may be present without the intracellular variety, and in the absence of coliform bacilli may almost certainly be regarded as the genuine article; but if coliform bacilli are present as well, this fact should be reported and great care exercised before committing oneself to a definite opinion.

Cerebro-spinal fluids are often sent to the laboratory for Wassermann, Kahn, cell-counts, protein estimations and Lange reactions. For these examinations the fluid must be free from blood, otherwise a reliable opinion is impossible. It is advisable to take about 2 c.cm. into each of two tubes. The second tube is almost invariably free from blood, even if the first is contaminated. Accurate cell counts are impossible if the fluid is not examined within 5 hours of its being taken, since the cells in the cerebro-spinal fluid disintegrate very rapidly even if the fluid is kept in the cold. Protein estimations are so approximate that it seems doubtful whether these examinations are worth while. The most information can be obtained from Wassermann, Kahn and Lange reactions.

Cultures from soft sores are often made with the idea

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of isolating the causative organism, Ducrey's bacillus. This organism belongs to the hæmoglobinophyllic group. It requires a blood agar medium for growth, and it is advisable to attend to all those details of inoculation that one associates with the gonococcus. Until a year ago there was little demand for this investigation, but since the collapse of France, in spite of the ubiquitous sulphonamides, it seems likely that the demand for vaccines made from Ducrey's bacillus will increase. (Dosage should start at about 50 millions of the organism and be increased.)

Amongst other specimens received by a V.D. laboratory are blood films and these are often taken in such a care-free, happy-go-lucky manner, that whilst it may flatter the ability of the pathologist to give an accurate account of what he ought to see, it is apt to make his eyes grow dim rather more quickly than he deserves. Blood films should be carefully made in the manner learnt as a student if useful reports are to be expected. Reliable differential counts or the presence of malarial parasites cannot be detected in a dried-up mass of blood on a glass slide.

If a clinician requires a full blood count, hæmoglobin or platelet estimation, he should not attempt to take the specimens but should call in the pathologist to see to it himself. Since these estimations are relatively mathematically accurate, the taking of the requisite dilutions requires sound training and constant practice such as few clinicians have the opportunity of obtaining.

In the foregoing remarks I have attempted to give in brief detail the liaison work required from the clinician and the pathologist in the transmission of specimens from the patient to the laboratory.

In conclusion I venture to make the suggestion that under the auspices of the Society a committee be set up to consider a standard series of tests required to be completed before, apart from the clinical evidence, a patient is to be considered free from disease.

The results of their deliberations could be circulated and opinions invited from the services and from the civilian clinics, and then a scheme of tests evolved which should prove a help to all.