SEROLOGY OF SYPHILIS

By DR. INGLE WRIGHT* (London)

TECHNIQUES are available now which enable a routine laboratory to determine with considerable accuracy whether a patient has active syphilis or not. It is usually possible to determine whether low-titre antibodies are present, but of little significance, as for example following adequate treatment; or whether the results of the test suggest that the serum is giving a false positive type of reaction, which itself may be of importance. Every clinician is entitled to have a battery of modern techniques applied to patients' sera: these can prevent wrongful diagnosis of syphilis, and subsequent needless or even harmful treatment.

Wassermann and his co-workers described the use of the complement-fixation test in the diagnosis of syphilis in 1906. As is well known, the antigen at first consisted of a saline extract of syphilitic fœtal liver, a tissue abounding in *Treponema pallidum*. Soon it became apparent that extracts of non-syphilitic liver and other tissue would give equally good results. Today, alcoholic extract of beef heart forms the basis of the antigens used to detect syphilitic antibodies.

In general, such antigens have one big disadvantage: antibodies which will react with them *may* be found in the serum of patients who have never had any treponemal disease. This means that the results of a complement fixation-test (C.F.T.), or a precipitation test such as the Kahn, are not specific enough for complete reliability. Very early syphilis may be missed on the one hand, and Biological False Positives (B.F.P.) obtained on the other; some of the tests may give a positive result in an adequately-treated case. Thus the accuracy of the Kahn Test is probably only 70 per cent., while the W.R. is 80 per cent. or more.

Since 1906, the methods of the Wassermann Reaction have been modified repeatedly to give greater specificity. The standard method today should be the Whitechapel technique (Price and Wilkinson, 1952). Another antigen which may be used in the same technique is cardiolipin, which is another beef-heart extract, made more sensitive by the addition of lecithin and cholesterol. The increase in sensitivity was designed to give a positive result in early syphilis, where it is valuable, but this antigen has the disadvantage of extreme sensitivity, and may cause B.F.P. reactions

* From The Department of Pathology, The Institute of Laryngology and Otology, London.

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in pregnancy sera and sera from blood donors, or if the serum has not been refrigerated properly.

The precipitation tests are open to the same objection, and the best of these is Price's Precipitation Reaction (P.P.R.) (Price, 1950), a test which gives a good agreement between the titre of the patients' serum and the clinical condition, and seems to be of value in estimating response to treatment. It is a good deal less sensitive than the Whitechapel complement-fixation technique; that is to say, the P.P.R. will only become positive after the C.F.T., as the antibody level rises. It is subject to the prozone phenomenon, and may appear negative in the presence of very high levels of antibody in the patients' serum. The V.D.R.L. slide test (except in the exclusion of primary syphilis) appears too sensitive. It has however advantages in some forms of neurosyphilis.

Specific tests, using antigen derived from Treponemes, or whole Treponemes, are now available. Only one, the Reiter's Protein Antigen C.F.T. (R.P.C.F.T.), is suitable for routine testing. The antigen is derived from a culturable non-pathogenic treponeme known as Reiter's Treponeme. The protein fraction of its antigenic components appears to be a group antigen for the treponemata, which accounts for the detection of antibody to a different member of the group. The specificity of the R.P.C.F.T. is at least 95 per cent., and probably more.

A serum or a C.S.F. subjected to a battery of these tests will yield results which are straightforward in most cases. The table shows the characteristic types of pattern which may result: for example the results in the last column are fairly frequently seen in West Indian immigrants in this country, who have had yaws in the past, and in occasional patients with neurosyphilis, whose serum may also give a positive V.D.R.L. test.

Test	Syphilis	Biological False Positives			Treated Syphilis or Yaws			
		1	2	3	1	2	3	4*
Wassermann CFT	+	−	±	+	+	+	±	−
Cardiolipin CFT	+	+	+	+	+	+	+	−
Reiter's Protein	+	−	−	−	+	+	±	±
PPR	+	−	−	+	+	−	−	−

* Note that these results may occur in neuro-syphilis.

In 1948 Nelson described the *Treponema pallidum* immobilization test (T.P.I.). This has been regarded as having 100 per cent. specificity of results, and therefore all problem sera should be tested by this method. The test depends on the activity of a patient's serum containing antibodies to *T. pallidum* which inhibit the motility of the organism. As a suspension of the organism requires extraordinarily rigid conditions, including strict anaerobiosis, during the 16 hours of the test, the T.P.I. is cumbersome,

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consuming time and materials, and requiring very highly trained staff. This test is therefore reserved as a reference test for problem sera. It may remain positive after adequate treatment, and this seems to be true in some patients of all the treponemal antigen tests: it might perhaps be mentioned here that recent work has shown the presence of spirochætes in human lymph-nodes many years after the completion of treatment (Collart, Borel and Durel, 1964). It is considered possible that these are debased non-virulent forms of *T. pallidum*, which are nevertheless able to keep the specific antibody titre from falling to negative, perhaps because these organisms contain at least group and perhaps specific antigens. Tabetic and other neuro-syphilitic sera will give a positive T.P.I., even if the reagin tests are negative.

Another highly specific reference test is the Fluorescent Treponemal Antibody test (F.T.A.), described by Deacon, Falcone and Harris in 1957. This involves prepared slides on which active suspensions of *T. pallidum* are dried. A serum is tested by layering it over the treponemes and incubating it: after washing off, an anti-human-gamma-globulin serum, conjugated with a fluorescent dye, is applied to the same area. If an antigen-antibody reaction has taken place, i.e. if there were syphilitic antibodies in the patient's serum, the patient's serum globulin would fluoresce all over the treponeme, when viewed under ultra-violet light.

Yaws, it should be noted, may give serologically-indistinguishable results from syphilis; clinically there is little difficulty.

Cerebrospinal fluids may of course be tested by complement fixation tests: the serum should always be sent for testing at the same time.

The biological false positive phenomenon

Enabled by these highly specific tests to interpret the serology, we may be left with a false positive reactor. What is a false positive, and is it significant? The answer is that the false positive is a syphilis-suggesting result, in a patient who has had no treponemal disease, and it is not due to technical error in the performance of the tests. As shown above, the R.P.C.F.T. is of great value in diagnosing this reaction at the first time of testing. The Biological False Positive (B.F.P.) may be acute or chronic. An acute B.F.P. may occur during a febrile illness, sometimes quite trivial. A chronic B.F.P. is one that is repeatedly present. In the last decade or so, it has become a commonplace that such a reaction no longer causes a sigh of relief to the clinician, for syphilis can be treated, but the chronic B.F.P. reactor may very likely progress to serious disease. The type of illness is often a collagen or an auto-immune disease. The patients of this type are most commonly women.

One series of 148 B.F.P.R. was followed by Moore (1956) for periods up to 20 years. Ten cases of undoubted systemic lupus erythematosus had

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developed, and a further 45 were probably suffering from this disease. Eight cases had developed rheumatoid arthritis, and five cases were seriously ill, though no diagnosis could be made with certainty.

Catterall (1961) described a series of 54 such patients, followed up for 1 to 5 years: in addition, some who had been diagnosed as "latent" syphilis before the advent of the (*Treponema pallidum* immobilization test) T.P.I. test, and given anti-syphilitic treatment, had been followed for twenty years. (One of the characteristics of the B.F.P. response is its fluctuation, but it shows no response to anti-syphilitic treatment. Such treatment may do considerable harm, as a high proportion of these patients are, or become, hypersensitive to penicillin.) Thirty-six of the patients were women: their diagnoses were as follows: six developed systemic lupus erythematosus; one, discoid lupus erythematosus; nine had possible collagen diseases; and twelve had hæmatological abnormalities only, such as persistently raised E.S.R. Among the men, one died of periarteritis nodosa, two had discoid lupus erythematosus, three had possible collagen disease, and four had hæmatological abnormality only at the time the paper was written. Thus, only 16 patients were left by the end of the follow-up who had only a B.F.P. reaction.

It must therefore be concluded that evidence of B.F.P.R. must be carefully followed, and if the test remains persistently positive, the patient should be seen at regular if infrequent intervals by his physician.

Some chronic inflammatory condition may in fact be present when the B.F.P. is first diagnosed; Hansen's disease ("Leprosy") very often gives the reaction. Lupoid hepatitis may also do this.

Occasionally, a serum has to be reported as anti-complementary, though usually this character can be overcome in the laboratory, and a true positive or negative result may be obtained in the C.F.T. Anti-complementariness is held to be due to the presence of long-chain gamma globulins in the patient's serum, and it is therefore found in the chronic inflammatory and dysproteinæmic diseases, sometimes the same diseases as give the B.F.P. It does not affect the precipitation tests; it should be remembered that it will make a T.P.I., which is a test involving complement, well nigh impossible.

Changing levels of syphilitic antibody in the serum

Early syphilis may well be sero-negative. Cardiolipin and Reiter's protein will detect earliest appearance of antibody. If a possible chancre is accompanied by negative serology, tests should be repeated. Very high titres accompany *secondary spyhilis* and are probably associated with immunity.

Latent and all forms of tertiary syphilis should at least have positive results in all tests. Very high titres may be obtained in the cardiovascular

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cases, especially at the beginning of treatment. Gummata are always accompanied by positive tests.

Neurosyphilis may however be associated with a negative Wassermann and other reagin tests. In such cases, the T.P.I. is positive, and the R.P.C.F.T. is weakly reactive; the V.D.R.L. may be positive. Thus the patient with, for example, perceptive deafness and a positive V.D.R.L. and positive R.P.C.F.T., should certainly have fresh serum tested for immobilizing antibodies (T.P.I.) before the diagnosis of neurosyphilis could be excluded. The cerebrospinal fluid should be examined in such a case, for protein, cells, colloidal-gold precipitation, and Wassermann, R.P.C.F.T. and T.P.I. Smith (1964) has described seven cases of sero-negative neurosyphilis with positive T.P.I. tests: unfortunately, these sera were not subjected to R.P.C.F.T.

Treatment will cause a fall of antibody titre; the reaction may revert to negative very quickly, if treatment is instituted early in the disease. The titres fall rapidly if primary syphilis is treated, and less rapidly if secondary syphilis is established. If treatment is not given for many years, as in late latent syphilis, or in active tertiary disease, all antibody titres may fall very slowly: the T.P.I. and Reiter's test may never become completely negative. This is of course seen in treated Yaws in just the same way.

Serology can in fact provide no substitute for history-taking and examination. Nor can it distinguish with certainty between the untreated active case, and the adequately-treated case who still has some positive tests. There can in fact be no clear-cut rule on the need for further treatment; every case requires individual clinical judgment. Every late latent and tertiary case should have a normal C.S.F. protein and cell-count before being discharged as adequately treated.

The incidence of syphilis today

Since 1958, the incidence of infectious, i.e. primary and secondary, syphilis has been on the increase in some areas of this country. London is one of these areas. In other areas, a new case is a rarity, e.g. Manchester. New cases of syphilis are much commoner in men than in women, and it is worth remembering that in London today, much of the infectious syphilis is due to the male homosexual.

All forms of tertiary syphilis are still at a fairly low level, some 2,900 in 1961 and 2,500 in 1962. These figures are from the annual report of the Chief Medical Officer of the Ministry of Health, and are compiled from the returns sent in by the venereal disease clinics. How many other cases exist and are treated, but are not seen at these clinics, can only be surmised, but a study in 1959 by the B.M.A. V.D. Group Committee suggests that a further 25 per cent. should be added to the official figures for all stages.

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There is considerable evidence that a possibly large number of patients with active syphilis are not only not notified, but *not treated, and in many cases not even suspected.*

In 1959, Sequeira (1962) analysed the records of 1,000 consecutive C.S.F. samples sent to his laboratory. Forty specimens from 39 patients gave positive reactions. Only ten were from V.D. clinic patients, and four were untreated, and thus were new patients whose disease must have eluded diagnosis until there were changes in the C.S.F. Of the 30 patients from other sources, 19 were untreated and many were unsuspected. We must not forget the patients whose C.S.F. was never sent at all, because the disease was unsuspected.

In conclusion it may be said that serology is an aid only to the clinician, who may rely, if further help be needed, on the venereologist for the decision to treat or not. Positive serology, when it suggests an active case, must always be followed by treatment: cases of tabo-paresis still occur, and some are known to have been hospital patients previously. It still happens that lack of communication within hospitals, and between the hospital and the doctor at home, results in a diagnosed case remaining untreated. When this happens, the results may be of the very gravest for the patient and his family. Much may be said in favour of routine testing of patients' sera, not because the disease is prevalent enough to warrant our suspicion in every patient seen: it is not, but if we confine ourselves to the patients whom we suspect of active syphilis, we must inevitably miss those whom we do not suspect, but nevertheless have active untreated syphilis.

REFERENCES

British Medical Association Venereologists' Group Committee in association with the British Co-operative Clinical Group (1959) *Brit. J. vener. Dis.*, **35**, 111.

Catterall, R. D. (1961) *Quart. J. Med.*, **30**, 41.

Collart, P., Borel, L. J. and Durel, P. (1964) *Brit. J. vener. Dis.*, **40**, 81.

Deacon, W. E., Falcone, V. H. and Harris, A. (1957) *Proc. Soc. exp. Biol. (N.Y.)*, **96**, 477.

Moore, J. E. (1956) *Brit. J. vener. Dis.*, **32**, 217.

——— and Lutz, W. B. (1955) *J. chron. Dis.*, **1**, 297.

Nelson, R. A., Jr. (1948) *Amer. J. Hyg.*, **48**, 120.

Price, I. N. O. (1950) *Brit. J. vener. Dis.*, **26**, 172.

——— and Wilkinson, A. E. (1952) *Brit. J. vener. Dis.*, **28**, 16.

Sequeira, P. J. L. (1962) *Brit. J. vener. Dis.*, **38**, 9.

Smith, J. L. (1964) *Amer. J. Ophthal.*, **57**, 973.

Wassermann, A., Neisser, A. and Bruck, C. (1906) *Dtsch. med. Wschr.*, **32**, 745.

Institute of Laryngology and Otology,
330/332 Gray's Inn Road,
W.C.1.

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