PLURALITY OF ANTIBODIES IN SYPHILITIC SERUM AND CLINICAL PRACTICE*

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The presence of several antibodies in the serum of syphilitic patients is neither a new nor even a recent finding. Early in the 20th century many workers, including Hoffmann, Prowazek, Touraine, Kissmayer, Jeanselme, and Ebersson, had demonstrated the presence of several antibodies exerting a more or less obvious agglutinating, opsonizing, immobilizing, spirochaetolytic action on Schaudinn's *Treponema pallidum*. Such knowledge, no matter how interesting from a merely scientific standpoint, could find no practical application in the current serology, not so much because of the difficulty of translating it into practice, as because of the uncertain and inconstant relationship between serological and clinical data.

In consequence the serological reactions based on complement-fixation and flocculation were widely adopted, because the antigens were readily available and showed a better correspondence between serological and clinical data.

In the following paper the serum antibodies in syphilis are divided into two main groups :

(1) *Immune-Antibodies*.—Shown by their visible action on the *Treponemata* (agglutinins, opsonins, immobilizing antibodies, treponemicidal antibodies, etc.).

(2) *Diagnostic Antibodies*.—Requiring a diagnostic system (fixation of complement, flocculation) to demonstrate their presence.

Such a sub-division is scientific insofar as the detection of immune-antibodies in the serum may be used for diagnostic purposes, and in so far as the diagnostic antibodies exert an antitreponemal action. They are in any case true antibodies in the meaning of modern immuno-chemistry *i.e.*, globulins particularly modified through the action exerted by the partigens of the pathogenic treponema on the proteidopoietic stratum.

Immune-Antibodies.—The study of these antibodies, rather neglected during the last few years, has lately taken on a new interest owing to the investigations carried out by Nelson and Mayer (1949) who, by developing the Treponemal Immobilization Test (T.I.T.), have revived the subject of the immunology of syphilitic infection.

The extensive literature on the T.I.T. makes any report upon it superfluous. Yet, although the great sensitivity and high specificity of Nelson's test give this antibody an outstanding diagnostic value, it is doubtful whether it can be easily adopted as current serological practice, as the expense of the intricate equipment and the difficulty of carrying out this test are bound to limit examinations of sera for the immobilizing antibody. From a clinical standpoint, the Nelson test is most valuable in diagnosis, notably in dubious or controversial cases ; the value of a positive test is however lower in treated syphilis, as the immobilizing antibody test tends to remain positive in the serum long after the infection has been cured. Conversely, a negative T.I.T. is valuable in treated syphilis.

Nelson and Mayer's standardized and well-defined system of spirochaetal diagnosis is of paramount importance in that it provides a valuable means of investigation with wide possibilities in the study of immunity in syphilis.

Diagnostic Antibodies.—The investigation of these antibodies has had some important results in recent years. The studies carried out by Pangborn (1950) on cardiolipin had led to the development of a highly sensitive, highly specific antigen, and cardiolipin has proved ideal for the demonstration of antilipoid antibodies, so that its adoption in lieu of alcoholic extracts is already in progress. Here again the vast literature available makes comment unnecessary.

Other Investigations.—The present paper comprises a report of the recent observations of Italian serologists and dermatologists, which are perhaps still insufficiently known, though some of their results have already been made public (Puccinelli, 1951a, b). Three antigens were demonstrated and isolated from Reiter's cultivable *Treponema pallidum*; in syphilitic serum an antibody corresponds to each of these antigens. Consequently, the formation of these antibodies in the patient is obviously caused by similar antigens of the pathogenic *T. pallidum*, which can therefore be regarded as a serological twin of Reiter's cultivable *Treponema* (D'Alessandro and others, 1949 ; D'Alessandro and Baccaredda, 1951).

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These antigens are of three types :

- (1) Protein, destroyed by heat, or thermolabile (TL).
- (2) Polysaccharide, resistant to boiling, or thermostable (TS).
- (3) Lipoid, serologically similar to the active lipoid of the alcoholic extracts and, as shown by some recent investigations, also similar to cardiolipin (L).

The nature and chemical serological characteristics of these antigens have already been described (Puccinelli, 1951a, b). The three antigens (TL, TS, and L) correspond in the syphilitic serum to three antibodies.

TL	..	Antitreponemal antibody	TL	) Complement-fixing antibodies (RW)
TS	..	Antitreponemal antibody	TS	
L	{	Antilipoid antibody	L	
		Flocculating antilipoid antibody		

The latest investigations seem to suggest that the complement-fixing and the flocculating antilipoid antibodies are two different antibody globulins ; further research still in progress will provide an answer to this interesting question (Rein and Kostant, 1949 ; Rein, 1950 ; Puccinelli, 1952).

All diagnostic antibodies can be easily demonstrated by performing a direct complement-deviation test on the syphilitic blood in two ways :

(i) with the various partigens separately (D'Alessandro and Baccaredda, 1951),

(ii) with the different antigens (cardiolipin, normal treponemal suspension, and treponemal suspension maintained at 100° C. for 60 min.) after selecting and separately removing the different antibodies by means of specific adsorptions.

This second procedure is made considerably simpler and easier by using a suspension of Reiter's treponemata serologically free from lipoidal antigen (for technical details and interpretation of results see Puccinelli and Pezzi, 1949).

Diagnostic Antibodies in Different Periods of Syphilitic Infection.—A systematic examination of the various diagnostic antibodies and their relationship with clinical symptomatology (a procedure already performed on tens of thousands of syphilitic sera) leads to the following conclusions :

(1) *Primary Syphilis.*—During this stage the antibody TL is the first to appear (it can be found even after the 3rd or 5th day from the onset of the chancre), and is followed a few days later by the antibody TS (7th to 10th day). The last to appear are the antilipoid antibodies, the flocculating one showing up a little before the complement-fixing one. Since cardiolipin has been used in serological practice, the time interval between the appearance of antitreponemal and antilipoid antibodies has

been considerably reduced, and the positivity of the antibody L sometimes precedes that of the flocculation reactions. (Puccinelli and Oddo, 1946 ; Puccinelli and Pezzi, 1949 ; Panti and Zar, 1950 ; Rasponi, 1951 ; Riggio and Oddo, 1948 ; Fusella, 1950 ; Paoletti, 1950).

(2) *Recent Secondary Syphilis.*—At this stage when the infection flourishes all antibodies are present in the blood in large amounts (D'Alessandro and Oddo, 1946 ; Rizzi, 1951 ; D'Alessandro and Puccinelli, 1944).

(3) *Late Secondary Syphilis and Tertiary Syphilis.*—The absence of L and sometimes also of TS is not rare in late secondary syphilis (after the 2nd and 3rd year from the onset of the infection), whereas the flocculating antibodies are seldom absent, and TL is always present. The shifting from a complete range of diagnostic antibodies to a partial serological picture is perhaps the expression of the passage from the stage of treponemal septicaemia to one in which the condition becomes localized. In tertiary syphilis a serological picture similar to that of late secondary syphilis is observed.

(4) *Neurosyphilis.*—Whenever a clinical condition exists affecting the nervous system, TL is always present both in the blood and in the cerebrospinal fluid, whereas L and the flocculating antibodies are often absent. TS, on the contrary, is often present, even in subjects who have been submitted to a prolonged and efficient treatment (Meneghini, 1951, Binazzi and Marchini, 1950). This last finding, in view of the easy disappearance of TS under specific treatment, even if poor and irregular, indicates a serious clinical condition and failure of treatment.

(5) *Latent Syphilis.*—In treated syphilitic subjects TL is often found alone, or accompanied by TS, whereas the antilipoid antibodies, notably the complement-fixing ones, are often absent. This finding, rather frequent when lipoid organ antigens were used, has been less often seen since the introduction of cardiolipin. The presence of TL alone in a considerable percentage of treated syphilitic subjects has been much discussed from the standpoint of diagnosis and prognosis. Investigations have been carried out for several years on a large number of cases, and we now think that the presence of TL in the serum may be regarded as a sign that the spirochaete is still present in the organism. The presence of TL alone in latent syphilis is observed in a low proportion of efficiently-treated patients (10 per cent.), whereas in patients who either failed to follow an efficient, well-planned treatment, or were late in beginning treatment, the rate is 62 per cent. or more (Olivetti, 1951). The result of an

accurate clinical investigation on a large group of subjects whose serum appeared to contain TL alone is shown in Fig. 1. The presence of TL alone in cases entirely free of clinical symptoms (15 per cent.) might be interpreted as the "serological echo" of a residual genetic function of the antibody, which has continued even after the spirochaete has disappeared from the organism. These results also seem to suggest that these patients should be judged very conservatively and rather regarded as carriers of Schaudinn's *Treponemata*.

FIG. 1.—Syphilitic patients presenting TL antibody only in blood.

(6) *Congenital Syphilis*.—These patients often show a prevalence of antilipoid antibodies, notably the complement-fixing one, even when the anti-treponemal antibodies are absent (Puccinelli and Vivarelli, 1950). Prolonged observation of many cases has however shown that such a condition may be present without specific clinical signs. The new born child and the congenital syphilitic with evident and active clinical symptoms, present a serological picture of diagnostic antibodies which match those of patients with acquired syphilis. The positivity of the antilipoid antibodies when the antitreponemal ones are absent is observed:

(a) in children clinically negative but born from syphilitic mothers who have had some treatment during pregnancy; such a serology, which practically affects only the antibody L, becomes spontaneously negative in a few weeks' time without treatment, and is interpreted as a passive passage or transfer of L from the mother to the child by way of the placenta;

(b) in subjects treated more or less extensively during their childhood or youth, who show a persistence of L without clinical symptoms, or, at the worst, with some cicatricial elements (stigmata).

These serological findings, which constitute the so-called resistant or irreducible reactions, may be modified by such aspecific interventions as may

alter the normal proteidopoiesis (fever, fasting, intercurrent diseases, proteidoplasmodopathies, etc.); such findings suggest a proteidopoietic "mutation", in an antibody direction, brought about by the action exerted on the reticulo-endothelial system of the patient during its development, and continuing even after recovery from the infections has taken place.

Diagnostic Antibodies under Treatment.—During specific treatment of syphilis with clinical manifestations the first antibody to disappear is always TS; the rapidity of its disappearance suggests that its production may be dependent upon the vitality of the *Treponema*. The speed of the other antibodies disappearance is linked with the period of contagion and the gravity of the symptomatology.

In cases of recently acquired syphilis with active symptoms, L is usually the first antibody to disappear.* On the other hand, in cases of old syphilis, or where there is little clinical symptomatology or none, TL is always the first to disappear, and case L may survive unaltered for a long period.

Quantitative Analysis.—This confirms the findings described above. Their behaviour during the treatment of syphilis is illustrated in Figs 2 and 3.

FIG. 2.—Behaviour of diagnostic antibodies in blood during and after treatment of recent syphilis with clinical symptoms.

FIG. 3.—Behaviour of diagnostic antibodies during and after treatment of late, latent, and congenital syphilis without clinical symptoms.

* An exception to this rule may be found in very early syphilis (chancre) in which TL is sometimes the first to disappear.

False Positive Reactions.—The presence of several antibodies in syphilitic serum is of considerable practical importance in defining the specificity of the reactions. The serological or biologic false positives (B.F.P.) depend upon the presence in the blood of such pathological or physiological globulins as may react with the antigens used. If this event rarely occurs with a single "antibody-type globulin" the presence of several non-specific globulins in a single subject should become so exceptional as to lose any practical significance. The presence of several diagnostic antibodies in the serum under examination is therefore important in showing the specificity of such reactions. The clinical and serological study of a large number of cases has shown that serological false positives are observed in a comparatively large number of cases when lipoid antigens are used (presence of "L-type" globulins during the alterations of the proteidopoesis); but they are exceptional with the protein and polysaccharide antigens; the presence of "TL-type" and "TS-type" globulins in the absence of syphilitic infection requires careful search for another kind of spirochaetosis in the patient (group reactions).

Biochemical Data

A biochemical investigation of the different diagnostic antibodies (TL, TS, L, and flocculating) has made it possible to show that these antibody globulins differ from each other and are partly characterized by distinctive chemical, physical, and biological features. This applies particularly to TL, TS, and L, whereas ample data are still required to differentiate between complement-deviating and flocculating antilipoid antibodies.

Electrophoretic investigation has made it possible to establish in the meantime that they are four antibody globulins, all with similar electric surface charges, and all intermediate between the slower beta-globulins and the fast gamma-globulins. This intermediate electrophoretic position confirms their antibody nature.

The molecular weights of the various diagnostic antibodies are all different :

TL low (approximately 35,000 or less) ; TS between 75,000 and 150,000 ; antilipoid antibodies both considerably higher—approximately 990,000 for the flocculating ones, and even more for L.

The heat-resistance also differs widely :

TL highly heat-resistant, being still serologically active after 20 minutes at a temperature of 72° C., 10 minutes at 74° C., or 2 minutes at 78° C.

L serological activity lost after 15 minutes at 64° C., or 20 minutes at 64° C.

The two others occupy intermediate positions between TL and L.

The precipitation reactions with electrolytes (ammonium sulphate, trichloroacetic acid) and dialysis with distilled water or different concentrations of sodium chloride solutions, show a uniform precipitation for the four antibody globulins.

TL proves much more resistant to treatment of serum with alcohol or phenol and to ageing than the other antibodies which show a marked lability in such conditions.

These data show that certain differences exist between the different diagnostic antibodies according to the characters of the respective globulins, and that such differences are more evident in the body of the colloidal micella than in its electric surface condition (Puccinelli, 1951a).

Biological Data

The data so far collected suggest that L should be regarded as a large globulin of complex structure not far modified from normal globulin of which it is a derivative. Its nearly physiological constitution allows it to seep, in spite of its volume, through a physiological membrane, such as the placenta, and to be found in the serum comparatively easily whenever the normal proteidopoietic rate is altered, and even in the blood of normal animals or normal subjects. The complexity of its colloidal structure is also confirmed by the frequency with which it follows the laws of adsorption according to Freundlich (Puccinelli, 1951b). The possibility of the persistence of its formation, even after clinical recovery, is suggested by infections in young patients whose proteidopoietic systems are still in the developmental stage. The fact that in such patients its formation persists fairly frequently indicates a "mutation" of proteidopoesis. This phenomenon, only noted in connexion with L, suggests that we are dealing with a proteidopoietic action very similar to normal.

TL is much smaller and shows a greater degree of modification from the normal than L. In spite of its smaller volume, it does not seep passively through the placenta, because it is too "heterogenized" in comparison with normal globulin ; in quantitative reactions it usually follows the chemical laws of definite proportions ; its presence in the serum is not detected—or only exceptionally detected—through any alteration, even a serious one, in proteidopoesis ; and its presence when syphilitic infection is absent must be regarded evidence of a group reaction to some other kind of spirochaetosis.

Summary

The clinical and serological investigations which we have been carrying out for more than 8 years, lead to the belief that the presence of TL in the serum indicates a persistence of the *Treponema* in the organism. Whether this finding is the proof of an actual lesion can only be ascertained by a thorough clinical examination. In any case, the finding should always be interpreted as indicating the need for accurate and painstaking supervision of the patient.

The presence in the serum of L alone, without the anti-treponemal antibodies, is a finding that should always be considered very conservatively, as it constantly tends to disprove the existence of syphilis in the active stage; it may either indicate an aspecific proteidopoietic alteration, or may represent, in recently-treated congenital syphilis, a mutation of the normal proteidopoeisis in a particular section.

While we wait for the investigations now in progress to differentiate the complement-fixing antilipoid antibodies from the flocculating ones, and to prove their respective clinical significance, the great importance of the persistence of TS in the serum of treated syphilitics must be emphasized.

A systematic serological analysis of the different diagnostic antibodies in syphilitics proves their practical usefulness in diagnosis, prognosis, and therapy. Our everyday experience indicates that in the serology of syphilis, as in the immuno-chemistry of so many other infectious diseases, the protein antigens are the most active in the production of antibodies, and this not only in connexion with their "antigenic power", but also in view of the specificity of their reactions. The extensive statistical data supplied by Italian serologists lead to the belief that TL antibody (antiprotein) is the

diagnostic antibody that more than any other constitutes an immuno-chemical mark indicating the presence of Schaudinn's pathogenic *Treponema* in the organism.

Nevertheless, a thorough serological investigation of the syphilitic should always include a careful examination of the full range of the diagnostic antibodies (and, if possible, of some of the immune-antibodies) in the serum; as only a comprehensive and well-balanced understanding of these elements can provide a clear and definite diagnostic, prognostic, and therapeutical judgment of syphilitic infection.

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