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PROPOSALS FOR A MODERN SEROLOGICAL DIAGNOSIS OF SYPHILIS

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PREFACE

During the last 10-15 years our theoretical and practical knowledge of the immunology of syphilis has undergone a fundamental renewal. We now have at our disposal specific serodiagnostic reactions in which the antigen is the agent of the disease itself. We are now acquiring a better knowledge of the biology of Treponema.

The result of all these newly-acquired data has been that the aura of mystery which had surrounded syphilis has been dispelled, that syphilis has been integrated into the category of infectious diseases, and that this disease has become perhaps the most complete infectious disease, one which is the richest source of information.

This understanding has also an immediate practical importance: it situates the reactions which may be used for diagnostic purposes in their right place, it unravels a very complicated Gordian knot; it makes it possible to select those reactions which deserve to survive, and increases the precision and value of the results.

There is no lack of publications discussing the serology of syphilis in its entirety. These publications include those published by the present authors in 1953 and in 1961, and the more recent one by G. Daguet (1964).

We would like, in this report, to depart from the usual scheme which begins by offering long pages of text on classical

serology. We have considered it preferable to propose the following triad: immobilization test (TIT), immunofluorescence (IF) and VDRL, and to eliminate the whole arsenal of all other possible reactions. Let us not forget that while TIT and IF have had the advantage of chronological priority, the place of reactions using cardiolipin is probably quite limited.

We shall attempt to specify the requirements and the present possibilities of the retained reactions, and we shall describe the practical arrangement which we believe we should attempt to approach. Having had the opportunity to examine a large number of bioclinical files, we shall suggest some interpretations of the titers of the different reactions and report a few special observations.

A. - THE REACTIONS

I. THE TREPONEMA IMMOBILIZATION TEST (TIT/TPI)

As we know -- and at the same time recalling the thesis of A. Touraine (1912) on Treponema (Tp) agglutination -- the TIT which has made its appearance in 1949, thanks to the remarkable work of Nelson and Mayer, was the first specific antigen-treponemic antibody reaction introduced into practice; it remains the reference reaction.

All of us know the principle of this reaction: a subject suffering from a treponemic infection soon exhibits specific antibodies in his serum which immobilize the Treponema, maintained by survival for 20 hours at 35° in anaerobiosis. However, this immobilization can take place only in the presence of a large amount of complement.

Since its development, this test has not been modified to any great extent, but in the course of years certain technical specifications and a better knowledge of the factors ensuring the precision of the reaction have in the end considerably improved the quality of the results.

We must stress the following three points:

1) The method of expression of the results which has been originally proposed was found to be very unfortunate. By expressing the percentages of Treponema immobilized, which obviously vary between 0 and 100%, and by dividing this spectrum into three zones: one -- negative -- between 0 and 20%, a second -- doubtful -- zone between 20 and 50%, and a third -- positive -- zone above 50%, a false "doubtful" zone of uncertainty has been artificially created which, in fact, does not exist, and on

the other hand, nonspecialists have been presented with the idea that a 100% reaction was strongly positive ("at the maximum reaction"), which is not at all true.

This notation has also introduced a false notion of "quantitativeness," a degree of positivity, ranging between 50 and 100%. In the classical routine qualitative reaction, the serum is brought to a final concentration of 0.1 in the tube where the reaction takes place. Now, experience has proved that the great majority of the sera of subjects suffering from treponemic infection contained by far enough antibodies to immobilize all the Treponema under these technical conditions and, in fact, a reaction of less than 100% corresponded only to a very low degree of positivity.

2) Hence, the knowledge of the degree of positivity, which is found to be more and more important, can be obtained only by means of a veritable quantitative titration (using this pleonasm to indicate that only the titer offers a definite measure). This titration is an absolute necessity in the serology of syphilis, and should be imposed on this branch of serology, as is constantly done in all other branches as well as in hematology, chemistry and all biological techniques.

In the quantitative reaction, the titer of the antibodies is expressed by the extreme dilution of the serum which is still capable of immobilizing 50% of the Treponema (this is the meaning of the very classic LD_{50}). Thus, an ordinary qualitative TIT of 50% corresponds to a titer of 10, since the serum is diluted to one-tenth its concentration for the execution of the reaction. The same qualitative TIT of 100% does not prejudge the titer, which could range between 30 and 10,000. We are aware of the importance of these titers in the interpretation of the results.

3) Finally, let us point out that a recent technical modification -- namely, the addition of lysozyme to the survival medium -- makes it possible to notably increase the sensitivity of the reaction. The lysozyme (present in certain tissues, in tears, in egg white) modifies the superficial structure of the Treponema, without affecting its vitality, and permits it to effect a better fixation of the antibody molecules. This addition had been recommended by Metzger, Hardy and Neel (1961) with a view to shortening the period of incubation of the reaction, and to carrying out the reading after the 6th hour without any modification of the sensitivity. However, one of us (F.B., 1962) has shown that while this acceleration of the reaction presented almost more disadvantages than advantages, by continuing the reaction for its normal duration of 24 hours we obtained, thanks to the lysozyme, a very considerable

increase in sensitivity: sera, which were negative when tested by the classic technique, could, in this way, reveal indisputable traces of antibodies, so much so that, roughly speaking, the range of quantitative titers is multiplied by 3; this was confirmed, in particular, by Kent and De Weerdt (1963). This modification is especially useful when it confirms a weakly positive IF.

Now, let us recall the advantages and disadvantages of TIT.

Advantages:

- An absolute specificity vis-a-vis the treponemic antibodies. TIT remains THE reference reaction of treponemoses, it being understood that all species of the genus Treponema (pallidum, pertenue, carateum, cuniculi...) entail, in the infested organism, the production of nondifferentiable crossed antibodies. A TIT which is even weakly positive but which has been confirmed, must be considered as the irrefutable evidence that the subject had made contact with the Treponema.

- An excellent reproducibility under strictly defined technical conditions. We think it far-fetched to believe that the variations of the titer of the quantitative test can be interpreted only if they are quite large (x 10 for some authors); actually, a change from one value to its double (x 2) should already be retained, when the two tests have run a satisfactory course.

- An incomparably high sensitivity with respect to the cardiolipidic reactions, except in the initial stage of the treponemic infection.

Disadvantages:

- A certain delay in the appearance of the immobilizing antibody at the beginning of the disease compared with the reagins and especially with the fluorescent antibodies.

- Great technical difficulty: TIT demands sterile, limpid, nonhemolyzed sera which are nontoxic for the Treponema. Experience proves how difficult it is to satisfy even these apparently simple conditions. Let us repeat, in this connection, that contrary to a frequently heard statement, penicillin administered to a patient even an hour before the taking of the blood sample does not perturb the test at all, since the systematic addition of penicillinase to the survival medium eliminates the toxic action of penicillin toward the surviving

Tp suspension, i.e., it eliminates the falsely positive reaction. And penicillinase has no action on the antibodies.

Most importantly, however, TIT demands that the technicians exercise constant vigilance and be familiar with all the factors which could perturb the reaction. In addition, they are burdened with the difficulty of maintaining a highly virulent strain and, despite all precautions, they are faced with the risks of unexpected technical failure. These difficulties have no influence on the value of the obtained results, because the controls of this reaction make it possible to eliminate any defective test, but they rule out the systematic extension of this reaction and its use in screening.

- Finally, <u>insufficient standardization</u> of the reaction and the results from one laboratory to the other. In this connection, let us point out that the criticism which is sometimes put forth against the addition of lysozyme, namely that it increases the differences in the results, cannot be upheld, for the following reasons. It is already difficult, with the usual technique, to compare the results of different technicians; the use of this technique is necessarily aimed at the results; and, finally, it would be abnormal to discard a perfectly specific technique only because it is accused of being too sensitive.

At any event, this problem of the lack of standardization ought to be readily solved.

II. THE IMMUNOFLUORESCENCE REACTION (IFE/FTA)

Ever since its appearance five years ago, this reaction has aroused great interest. Recommended in the U.S. by Deacon in 1959, it has rapidly spread over the entire world; in our country it has been subjected to precise extensive and comparative studies.

Let us recall that the principle of this reaction is the application, to the serology of syphilis, of the scheme of Coons' indirect immunofluorescence technique which is general to all immunology: the treponemic antigen fixed on a slide is first covered with a dilution of the serum to be studied, and allowed to adsorb any of the specific antibodies which are present. These antibodies are gamma globulins which, in the second step, play the role of antigen and in turn adsorb the antibody molecules of a serum highly immunized against these gamma globulins. Thus, the Treponema are successively covered with two globulinic layers. After the anti-gamma-globulin immune serum has been labeled with fluorescein isothiocyanate, the Treponema become fluorescent and may be observed under the

microscope under very definite conditions of UV illumination; the more antibodies have been fixed by the Tp, the greater the "brightness."

On the other hand, if the sus[illegible] serum placed in contact with the Treponema does not [illegible]ic antibodies, the fluorescence conjugate will b[illegible] during the washing operation and these Treponema will be invisible in UV light: the reaction will be negative.

We must stress the importance of the titer.

Here, we touch upon a very important problem, which links up with what we have said above in connection with the TIT: sometimes it is considered sufficient to evaluate the intensity of the positivity on the basis of the intensity of the brightness; in this way one limits oneself to a qualitative reaction where the results are expressed by a variable number of crosses. We believe that this is insufficient. On the other hand, the evaluation of this brightness is rather subjective and depends both on the observer and on the quality of the equipment or reagents; on the other hand, a notation by means of crosses does not make it possible to evaluate the degree of positivity which can only be known by a quantitative reaction, which consists in examining the positive sera at successive dilutions until defining the extreme dilution which still gives a perceptible fluorescence. Finally, the quantitative reaction alone permits evaluating the quality of the reaction and of the reagents by means of sensitivity and specificity controls necessarily included in every procedure.

Advantages and disadvantages of IF:

Advantages:

The advantages are obvious:

- First of all, the sera to be examined need not be sterile (except, of course, if they must be preserved for more than 24 hours at ordinary temperature); they may be turbid or hemolyzed; they need not be tested for toxicity against surviving Tp, which is so important for the TIT. We shall see that it is apparently possible to carry out the test on dried blood.

- The speed and ease of the reaction are such that the latter compares very well, both with respect to time and cost, with the "battery" of cardiolipid reactions currently in use. A trained technician aan easily carry out about 100 reactions in 2-3 hours. This permits the use of qualitative IF as a routine test in systematic screening, followed by the obligatory

quantitative study of all the positive sera, permitting the quantitative expression of the results.

The standardization of the technique and the reagents should be easy in the near future, thus ensuring a reproducibility of the results which has been unknown so far with the other reactions. To be sure, the performance of this technique requires the examination of rather large series of tests at a fast rate, but we believe that this is a desirable and beneficial development from every point of view. Recently, a criticism has been leveled at the IF, to the effect that the antigen lacks stability. This is not so at all: while in the TIT the treponemic antigen must be prepared anew for each reaction and is never absolutely identical to the previous one, in IF a suspension of known quality may be used for several weeks, and transported without inconvenience from one laboratory to the next. On the other hand, the quality of the suspension can be readily checked prior to each use. To be sure it must be realized that the currently available commercial antigens are, in fact, generally unusable: they are too poor, too impure, too greatly altered morphologically, or too hyposensitive.

Let us recall that we have to do with a dead antigen, which eliminates the risks of contamination of the personnel.

- The sensitivity of the reaction is extremely high, and exceeds by far that which may be obtained by the other techniques: the range of positivity extends, in effect, until 1:100,000 (1 drop of serum in 5 liters of water).

- Finally, the early appearance of the fluorescent antibodies at the start of the infestation is, in fact, an indispensable diagnostic aid from the very first suspicion of the disease.

Disadvantages:

There is only one indisputable disadvantage which prevents this reaction from supplanting the TIT, but even this disadvantage has only a moderate effect in practice: the specificity at low dilutions is not an absolute one. If we allow pure serum to act on the Treponema, we frequently note a positivity due to the fixation of nontreponemic antibodies of undetermined nature. However, this nonspecificity never attains high quantitative titers.

This important question should, in effect, be evaluated with respect to the dilution. Two to three percent of the sera of subjects having no history of detectable syphilis attain a

titer of 150 and even, exceptionally, of 300; by contrast, we have never observed any nonspecificity at higher titers in more than 20,000 tests which we have carried out. Contrary to certain publications, there do not seem to exist any affections which are capable of making the cardiolipid reactions positive or of being responsible for a falsely positive IF test; in particular, malaria, leprosy, viroses, reticuloendothelial affections, acute or chronic hepatites, hyper-gamma-globulinemias have no effect whatever.

Actually, this question of specificity is a complex one, since until now the threshold of acceptance has varied according to the biologists. This is due to their personal experience with the patient and also to the reagents available to them. Hence, a given group of biologists can, for the time being, speak only on the basis of their own observations and of the comparisons which this group was able to make with the clinical data.

Certain authors perform IF's by diluting the sera to 1:200 (IF/200); others do so by diluting to 1:50; we ourselves perform IF's at a dilution of 1:150.

For us, an IF titer of 150 should be considered with reservation. It is of interest only if a preceding examination was found to be absolutely negative.

The criticism of hyposensitivity compared with the TIT in old cases of treated syphilis is not borne out in practice and should be more or less ignored: in the majority of cases the IF remains, even at this stage, more sensitive than the TIT. To be sure, in 5-10% of old treated cases of syphilis immobilization titers of 20 or 40 are attained, while the IF attains only the 150 whose debatable significance we have just discussed. It seems that this is compatible with the state of "clinical cure." At this stage, any discussion of this topic would be of interest only to theoretical immunology.

The lack of technical standardization of the IF is an important criticism leveled against this process; this lack is due

a) to the impossibility of finding any irreproachable reagents commercially available at the present time;

b) to certain uncertainties regarding the choice of the best optical equipment.

It is hoped that these problems will soon be solved, in particular thanks to the present efforts of WHO. For the time

being, as we have just said, the results obtained at different laboratories are not always comparable; the figures which we shall present are valid only for our own personal experiments.

Let us end this chapter by discussing the probable interest of the use of IF on dried blood:

The execution of the IF test on dried blood is also receiving a very special attention of the WHO, because this technique would, in particular, very much facilitate the serological surveys of large communities which are distant from well-equipped diagnostic centers. Recommended by Vaisman, Hamelin and Guthe (1963) on calibrated rings of Canson paper, this technique was employed by Lesinski in Poland on blood dried on a slide; our tests, using a slightly different method, seem to give good results. A broad survey of this topic is under way, and we can reasonably hope that it will confirm the interest of this technique.

We shall not talk in this article about any other specific reaction using Treponema pallidum, which was recommended during these last few years: reactions such as immuno-adherence, agglutination; these reactions have shown themselves to be afflicted with many difficulties, instability or insufficiency, so that they cannot at the present time be introduced into practice.

The complement fixation reaction using Tp as antigen is used abroad, particularly in the US (TPCF). We have no experience with this reaction and do not, a priori, seen any practical interest in it, beside the treponemic reactions available to us.

III. THE CARDIOLIPIDIC REACTIONS

The cardiolipidic reactions are now 50 years old, and nobody denies their usefulness and the services which they have rendered. We know that all of these reactions use the same nontreponemic tissue antigen which is now rather well defined; this antigen is the cardiolipin which reveals an antibody called reagin. The technique of these reactions is well codified and easy, perhaps even too easy.

One of the most difficult tasks of the human mind is to overcome the force of habit. If we examine "classic" serology as a function of the specific reactions, it seems obvious that the flocculation and hemolysis techniques lack the indispensable qualities of sensitivity, specificity and reproducibility; that they have only a few quantitative applications, and that they

permit the exploration of only a few years of the long medical history represented by treponemic infection.

In 1947, students taking the serology course of the Pasteur Institute studied in detail 17 main reactions and 8 variants; although the very number of these reactions is condemnatory, it still takes a special effort to admit that these reactions may be reduced to a single one.

Nevertheless, these "batteries" of habitually performed reactions are justified only by their respective deficiencies, by their frequent total contradiction of the anamnesis or clinical signs, and by the absence of a reference system.

A few years ago, the administrative provisions demanded three "classic" reactions; at the instigation of one of us, this figure was reduced to two; a hemolysis reaction on heated serum and a flocculation reaction; this has resulted in considerable economies.

Nevertheless, the time has no doubt come when the number of these nontreponemic reactions can be further reduced.

Of the flocculation reactions there are two which can be very rapidly performed on a slide and which are perfectly adapted to a series operation: these are the VDRL reaction, used in all the English-speaking countries, and the Kline reaction, which is frequently preferred in France because of the difficulty of finding a good VDRL reagent in our country. These two reactions are parallel, and it is useless to administer both of them: one can choose one or the other.

It is almost without interest to carry out, in addition, one or several hemolysis reactions. These reactions are long and delicate, they demand preliminary titrations of the antigen, of the complement and of the hemolytic system which are not always adhered to; these reactions are frequently less sensitive than the flocculation reactions and, in the rare cases where they are more sensitive, the IF would always yield an even sharper and more certain response.

Of the cardiolipin reactions, a rapid reaction is used in the US on total fresh blood: this is the Rapid Plasma Reagin Test (RPR). The reaction, which can be carried out on plasma without having to separate the serum, is more rapid; it is within the reach of any medical assistant, thanks to a highly developed equipment. The RPR is proposed as the type of rapid reaction for screening, at the frontiers of a country or during prospecting work in the bush. The reaction is an attractive one

but, since we are not too familiar with it, we are reluctant to take any position with respect to this reaction. Later on we shall discuss the important problem of mass examinations.

Perhaps we will be criticized for not examining here the complement-fixation reaction with cardiolipin antigen, not cardiolipin but a suspension or an extract of Reiter's spirochete which, in our opinion, is too readily classified among the Treponema. No doubt this spirochete presents an antigenic relationship with the Treponema, and the reactions using it are certainly more specific and more sensitive than the other hemolysis reactions using cardiolipin. However, on the one hand, this antigen is unstable, difficult to handle and often anticomplementary; and, on the other hand, it cannot be compared, either in sensitivity or in specificity, to the specific reactions. Hence, its use is justified only when the performance of these specific reactions is really found to be impossible.

B. - SUGGESTIONS FOR THE MODERN PRACTICE OF THE SEROLOGY OF SYPHILIS

Hence, what has just been discussed makes it necessary to sort out the methods, to apply a choice and, at the same time, to adopt a practical organization which would offer a solution of the problems of rapidity, sensitivity, specificity, reproducibility and also of the cost.

1) <u>In the general practice of clinical diagnosis, exclusion of infected persons and systematic screening</u>, the coupling of a cardiolipid-type flocculation reaction and immunofluorescence already solves almost all these problems.

As we have said above, the flocculation reaction used may, in our opinion, be the Kline or the VDRL reaction: it will yield the first approximation.

After this reaction, immunofluorescence will be carried out: all the sera positive to flocculation will be <u>quantitatively</u> examined, together with the sera of patients under surveillance whose previous titers are already available.

The sera negative to flocculation will be examined <u>qualitatively</u> by IF -- at an initial dilution of 150 to 200 -- in order to detect the beginning of syphilis (the IF precedes the reagin reactions) or a history of syphilis (IF survives the disappearance of reagin). The sera which have been found positive to the qualitative IF (and only these sera) will be subjected to a quantitative IF reaction.

If, despite everything, certain doubtful cases do remain, it will be obviously the TIT on which the decision will be based.

We think that we can state that, if all qualities of the reagents have been ensured and the techniques adhered to, no patient suffering from evolutive or latent treponemosis will elude this double examination. Two technicians working in a correctly equipped laboratory will execute in a single day all the manipulations necessary for the examination of more than 100 sera.

Thus, two of us have examined, during the second quarter of 1964, 5,700 sera originating from the Systematic Screening Centers of the Social Security Agency. Kolmer's hemolysis technique, generally considered as the best, was used parallel with the VDRL and IF method, but it has contributed almost no additional information. All the reactions which were positive in either of the two reactions were checked by TIT.

The analysis of this large number of examinations, compared with the anamnestic and clinical data, has not yet been completed: it will be published at a latter date; however, some broad outlines of the results of this analysis already become evident:

a) Reagin serology has furnished 118 doubtful or dissociated responses. The treponemic reactions have confirmed the existence of treponematosis in 101 cases and have led to the conclusion that a "falsely positive reaction" is obtained for the other 17 cases. Hence, in this series, 14.40% of falsely positive reactions were obtained for the sera suspected on the basis of the cardiolipid test.

b) The IF carried out -- for a first series -- at a dilution of 1:150 -- (deliberately chosen to be slightly too sensitive) was positive for 473 sera out of 5,700 (8.30%).

c) For these 473 sera, the IF reaction was carried out at stronger, more specific dilutions:

- 215 sera were found not to exceed 1/150; hence, 211 were considered negative and not subjected to TIT (which is less sensitive); the other 4 sera, which gave uncertain results to flocculation, were therefore examined by TIT: 2 of them were found to be positive to this test;

- 58 sera (1%) remained positive at 1/300. The TIP could be performed only for 53 of these sera, with a positive result in 28 cases. In general, this positivity of the TIT was weak,

showing the small degree of antigenic action but also the specific significance of the IF 1/300;

- 200 sera (3.5%) were positive to the IF 1/450 or more. The TIT could be carried out only for 196 cases; in 35 cases, the TIT was found to be negative (this seemed to correspond generally to an IF not exceeding 1/450); in 161 cases, the TIT was very positive, with a titer attaining 3,000.

The remaining ones of these 5,700 sera were negative to all tests.

On several occasions the positivity of the IF or the TIT -- revealing the indisputable existence of a treponemosis -- was associated with a negative reagin serology.

To sum up: Cardiolipidic serology brought to light 1% of treponemosis, the TIT 3.33%, the IF 4-4.5%, according to the accepted threshold.

Let us point out, finally, that the time devoted to the complete execution of the VDRL/IF coupling -- on these 5,700 sera in 3 months -- has not exceeded the normal work schedule of a single technician.

2) By contrast, for the supervision of a proven syphilitic patient and the control of the efficacy of treatment it is necessary to combine quantitative IF and quantitative TIT. These two reactions back each other up and complement one another. Their dissociation makes control indispensable, and in this way prevents any gross material error.

The therapist will frequently be aided in the choice of the dosage and rate of the injections by the knowledge of the antibody titers and their variation.

On the biological level, we must consider that the condition of a syphilitic patient is really followed up when one knows, with precision, the titers of his specific antibodies, thus, grosso modo and in the name of what is taught by infectious pathology in general, the intensity and development of his treponemosis. One often reads publications reporting some interesting or clinical case, without any indication of the real humoral state.

The rate of the serological examinations varies a great deal, depending on the case in question. We believe that the majority of clinicians would agree that any suspected contamination should be examined by IF once every week for three months,

and that a recent treated infection should be checked once every three or four months, at least by means of IF, until a negative or stable result is obtained. On the contrary, an old treated and stabilized affection, regardless of the level of its antibodies, would not justify more than one safety checkup once every year or every two years. Despite the high cost of the quantitative reactions, the patient's financial status and society's budget rapidly benefits from a suitable frequency of examinations. Serologists are very often asked to repeat, at very brief intervals, qualitative TIT's -- which, inescapably, are 100% positive -- for subjects who, obviously, would require only rare but precise examinations. Nor should we neglect the often disastrous psychological result, for these subjects, of these heart-breaking "100%" positive results. In effect, as all of us know, the psychological element is frequently of major importance in our specialty, and frequently dictates the physician's attitude.

C. - THE PROBLEM OF MASSIVE AND URGENT SCREENINGS

We shall discuss this problem without knowing how to solve it: this problem arises under two circumstances:

a) Prospecting Work

In a rather large number of countries, it is necessary to screen and treat persons suffering from yaws or syphilis without having the financial and technical means for carrying out any "total mass treatment." Hence, it is necessary to concentrate the whole population, to take blood and carry out a serological examination, select the patients to be treated, and let everyone go before the end of the day after having waited a few hours.

b) Temporary Immigration of Personnel

Here, the problem is similar: a more or less sizable group of persons presenting themselves at a port of entry or at a land frontier. If this group contains contagious individuals, they will transmit the infection; if the group contains patients, they will, perhaps, be taken into charge by the receiving community.

Actually, we believe that the problem can be broken up into two parts:

- In prospecting work in the bush, one must proceed rapidly and if there are errors on the side of excess (in these countries where allergy to penicillin is negligible), these errors are rather profitable ones. It is necessary to choose the most rapid reaction. In certain African prospecting

operations, we have seen 240 "Rapid Plasma Reagin tests" carried out in a single day; despite certain remarks which we may make with regard to this reaction, this figure is worthy of consideration.

- As far as immigration is concerned, one has time. If an error is committed, this error risks preventing the subject from earning his living, and this is a serious matter; while an error in the opposite direction would approve the entry of a contagious person, and this, too, is quite serious. Until we have a better understanding of the RPR test, we consider it wise to adhere to our scheme, because sera travel more rapidly than the individuals, and the administrative formalities of immigration are longer than a well executed serological test.

Three solutions, which we consider to be prudent ones, present themselves:

- Carry out both the VDRL and the IF test on the spot;

- Decant the serum in an efficient manner and send it to a competent serological center;

- Take a blood sample on Canson paper and send it to a highly specialized laboratory which will obtain from it the material required for the IF. This latter method is still under study, but it undoubtedly represents the solution of the future.

D. - ATTEMPT AT AN INTERPRETATION OF THE RESULTS

All through this report we have stressed the importance of the titers. On the basis of the biological and clinical comparisons which we were in a position to make, it is no doubt useful to specify the conclusions which we believe may be drawn from these titers, doing so with full awareness of the danger inherent in such interpretations, and stating again that we are presenting merely a general outline, which can be revised and subjected to criticism on the basis of special cases.

[Translator's note: Page 803 of text is missing.]

a) The proof of inflammation: cellular reaction and proteinorachia. Here, we have to do with nonspecific signs, which nevertheless are very instructive and which should disappear by treatment;

b) The presence of antibodies: they are generally on a par with blood serology and since, at any rate, the treatment is carried out "to the currently possible maximum," a positive

IF or TIT in the LCR is only of limited pragmatic importance, whether the antibodies originate from the blood by filtration or whether -- according to some opinion -- they had been elaborated on the spot.

In our opinion, the old "criterion of cure" based on the negativity of serological reactions in the LCR should no longer be taken into consideration.

Let us note that the IF is totally negative in the normal LCR's; hence, an IF of 1/50 is of value, but this positivity will be re-encountered in the serum.

E. - SOME CONCRETE EXAMPLES

It is not, perhaps, without interest to take from our files a few concrete cases which show the comparative developments -- such as they have appeared in our own experience -- of some cases which, in our opinion, call for attention.

1. Suspicion of contamination:

This observation is exceptional: we have recently been able to follow up a physician clinically and serologically during the entire incubation period; this subject had had no history of venereal disease and was highly suspected to have become contaminated on a specific date.

The following table shows that the IF has preceded the first sign of the chancre:

(1) *Jour*	*T.I.T.*	*I.F.*	(2) *Réagine*	(3) *Clinique*
0				Contamination
3	0	0	0	0
10	0	0	0	0
17	0	300	0	0
22	0	450	0	Petite papule non érodée.
23				Après grattage, fond noi- (4) positif. Traitement.
31	0	450	0	0
60	0	300	0	0
80	0	50	0	0

1 -- Day; 2 -- Reagin; 3 -- Clinical data; 4 -- Small non-eroded papula. After scraping, positive dark-field test. Treatment.

2. Favorable evolution of a secondary treated syphilis:

M. L...

Here, we have to do with a typical roseola, immediately treated with two injections of bismuth, then with 15 million units of bipenicillin (tt = titer).

Date	T.I.T.	I.F.	Reagin
10-10-62	100 % tt 3 000	12 000	+++
1-11-62	100 % tt 1 200	4 000	+++
12-1-63	100 % tt 150	1 350	±
25-6-63	98 % tt 40	900	0
15-10-63	80 % tt 25	450	0
10-6-64	40 % tt 8	300	0

(A series of bismuth injections in January and July 1963.)

It should be noted that if the first four TIT's had been only qualitative, they would not have reflected the favorable evolution of this case.

3. Syphilis with classic dissociated serology, stable TIT and IF:

Roseola discovered and treated in 1957. Patient seen again in 1960, followed up regularly since then; he received four series of 10-20 units of extencillin, 600,000 units.

Mrs. B. G...

Date	T.I.T.	I.F.	Reagin
2-5-60	100 % tt 600		——+
4-10-60	—— 300	450	—+—
5-4-61	—— 280		++—
16-12-61	—— 300	450	——+
8-12-62	—— 1 200 ×	1 350 ×	——++
12-6-63	—— 800 ×	1 350 ×	——+
22-1-64	—— 600 ×	1 350 ×	——+
29-5-64	—— 590 ×	1 350 ×	±——

The stability of the titers is remarkable. The increase of the TIT* and IF* after 1962 is due to the systematic use of lysozyme in the first case and to the improvement of the IF technique in the second case; in 1961, the latter technique was still in the development stage.

4. Latent syphilis with hypersensitive cardiolipidic serology:

Serological syphilis discovered in 1946, regularly treated until 1955, not treated since, showing no clinical signs.

Date	T.I.T.	I.F.	Reagin
11-4-61	75 %	—	++
19-10-61	80 %	300	++
18-5-62	65 %	450	+ +++ +++
14-5-63	92 %	900	++ ++ +++
20-4-64	70 %	900	++

5. Syphilitic meningo-encephalitis:

Subject showing circulatory, neurological and psychological disturbances (for the past two years) whose syphilitic origin had not been recognized. Treatment begun after diagnosis.

M. A. C...

Date	T.I.T.	I.F.	Reagin
5-6-61	Sérum 100 % tt 2 500	36 000	+++
	L.C.R. 100 % tt 3 000	24 000	+++
31-7-61	L.C.R. 100 % tt 600	8 000	++
30-10-61	L.C.R. 100 % tt 700	4 000	++
20-2-62	L.C.R. 100 % tt 800	1 350	+++

Note, in this observation, the considerable titers of the antibodies in the LCR in June 1961, analogous to those of the serum. The titrations were obviously very carefully checked, and we believe that we can formally exclude an error or a contamination of the LCR by traces of blood. Such figures pose the problem of the origin of the antibodies in the LCR; is it not possible for them to be formed, in the case of neuraxic lesion, by the plasmocytes present in the latter, rather than originating from the serum by filtration through the meninges?

6. Congenital syphilis:

We owe this very special observation to H. Payenneville, who will publish it elsewhere.

A woman was delivering a child on 8 December 1963, and on this occasion it was discovered that she suffered from a secondary generalized florid syphilis, with a chancre of one labium minus in the process of cicatrization. Dark-field examination positive, immediate treatment.

The father had presented an eruption a short time before: his serology was positive.

The child has no suspicious clinical symptom, and was left under supervision for four months for theoretical reasons which our colleague Dr. Payenneville will discuss in due time.

The following table shows the evolution of the IF titers:

Date	Age	I.F.	
9-12-63	1 jour ... (1) ..	36 000	
11-1-64	1 mois ... (2) ..	1 350	
10-2-64	2 mois	2 700	(3)
8-3-64	3 mois ... (2) ..	2 700	
13-4-64	4 mois ... (2) ..	36 000	Traitement
30-6-64	6 mois ... (2) ..	36 000	
21-9-64	9 mois	4 000	

1 -- Day; 2 -- Month(s); 3 -- Treatment.

It may be considered that this child was born with a supply of maternal antibodies, most of which were eliminated in three months. At that time, he was able to produce his own antibodies, and the titer rose again to a high level.

F. - CONCLUSIONS

The few technical considerations which we have indicated cannot be summed up; nevertheless, there is a notion whose importance we feel it necessary to stress again, namely the importance of the quantitative reactions. If these reactions must be followed by different laboratories, it is obvious that it is necessary to create appropriate conditions for reproducibility.

On a more general level, we believe that the time has come to replace, <u>for diagnostic purposes</u>, the battery of classic reactions with a simple arrangement which yields better data:

- First approximation by the VDRL or Kline test;

- Quantitative immunofluorescence on sera which were found to be positive to flocculation, and simple qualitative IF on sera which were found to be negative to flocculation;

- Immobilization test -- as is currently done -- for the rare doubtful sera.

For the <u>surveillance of treated patients</u>, the quantitative tests for these three classes of reactions are no less imperative, since the only important point is to follow the titer.

However, this serological scheme, while it is applicable on a limited scale already at the present time, cannot be immediately generalized.

In our opinion, it represents an objective to be attained as soon as possible, because it alone is capable of solving the theoretical, practical and social problems with which syphilographers are faced. However, this application requires a -- perhaps utopian -- set of marked modifications to be brought about in the present system:

- First, an agreement on the part of clinicians and biologists regarding the principles of the immunology of syphilis;

- Then, an agreement on the part of technicians regarding the technical methods and the methods of expression of the results;

- Furthermore, as a corollary to the above, the placing of reagents of perfect and checked quality at the technicians' disposal. In particular, IF cannot be correctly performed with the conjugates and antigens which are currently available commercially;

- Finally, a regrouping of the serological centers. In France, the smallest population center having a few thousand inhabitants has its own serological laboratory in which a few serological examinations are carried out per week, without the possibility of checking the quality of the reagents. This decentralization has been of some service, and at times makes it possible to save time; however, it seems incompatible with a more highly developed serology.

The present possibilities of packaging, transportation and transmission of results make us believe that only the concentration of the examinations in specialized laboratories will permit the solution of the problems of quality, standardization, rapidity and economy which we have discussed above.

Such a regrouping ought not to be decided on the basis of a decree which, it is to be feared, may contain some defects; we believe that it will become imperative sooner or later, in response to the advance of our knowledge and techniques.

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