

AN EXPERIMENTAL VERIFICATION OF THE
SIGNIFICANCE OF THE DELAYED NEGA-
TIVE WASSERMANN REACTION

RALPH R. MELLON, M.D., DR. P.H.,

AND

PAULINE M. AVERY, B.S.

ROCHESTER, N. Y.

Several years ago, Olson¹ and more recently, McConnell² have called attention to a phenomenon known as the "delayed negative" Wassermann reaction. In essence, it has to do with the time at which the reaction is read. If read in about thirty minutes, it is positive; but at the end of an hour or two hours, as the case may be, it is negative. They are inclined to the opinion that such a reaction should be regarded as at least a partial one. McConnell has shown that it occurs in about 1 per cent. of his cases, which he has divided into three groups: the first group comprises known syphilitic patients, most of whom had received treatment; the second, those who probably had syphilis, and the third, those presenting no evidence of syphilis. As is to be expected, the greatest percentage of these reactions is to be found in the first group.

In this connection, however, it is worthy of note that Strickler,³ Munson and Sidlick have observed that arsenic itself may produce a positive Wassermann reaction when administered to patients suffering from diseases other than syphilis. The significance of this fact is of course obvious, as it has been common practice to use the Wassermann reaction itself as a control to treatment, and to regard the partial reactions obtained after treatment as an indication of decreased activity on the part of the disease. Personally, I have never been able to believe that this apparent correlation was very well founded, knowing as little as we do about the actual mechanism of complement fixation. The fact that arsenic administration is often followed by a disappearance of the earlier lesions of syphilis is not sufficient ground for drawing conclusions regarding a connection between the absence of a Wassermann reaction and the latency or disappearance of the disease itself.

1. Olson, G. M.: The "Delayed Negative" Wassermann Reaction, *J. Lab. & Clin. Med.* **1**:704, 1916.

2. McConnell, G.: The "Delayed Negative" Wassermann Reaction, *ibid* **5**:43, 1919.

3. Strickler, A.: A Positive Wassermann Test in Nonsyphilitic Patients After Intravenous Therapy, *J. A. M. A.* **75**:1488 (Nov. 27) 1920.

Attempts to increase the delicacy of the Wassermann reaction for diagnostic purposes are worth while, although such attempts have usually introduced the nonspecific factor. The probable freedom of the "delayed negative" phenomenon from that implication would add greatly to its value from the standpoint of increased delicacy. To my knowledge, no observations have been recorded in which the connection is particularly direct. For this reason I desire to record the following observation, which concerns complement fixation as it occurred in a patient suffering from blastomycosis and in animals experimentally infected.

In this case we were able to isolate three distinct varieties of *Oidium* from the various lesions, and we were interested to know whether the patient's serum contained bodies immune to more than one of them. The whole study will be made the subject of a special communication in the future. The antigens were prepared from thoroughly washed suspensions of the organism, which had been grown from 48 to 72 hours on agar slants. After the washing, they were killed with 0.25 per cent. formol. They were not anticomplementary or hemolytic, and their fixing power was fair. A suspension was prepared, of such turbidity as to give a quantity of 0.1 c.c. for the antigenic unit, which was one fourth of the anticomplementary dose. The patient's serum was used in 0.15 and 0.3 c.c. quantities, and was run side by side with the serum of rabbits which had received three injections of the live organisms.

The control rabbit serum and normal human serum came down promptly at the end of fifteen minutes; but at the end of thirty minutes, the reaction of both the immunized rabbit and the patient was ++++. At the end of forty-five minutes, a marked hemolysis developed, which at the end of the hour left the reaction approximately + or doubtful. At the end of twenty-four hours the reaction was unchanged, and it was perfectly obvious that there was indeed a slight inhibition. More precisely, the reaction in this case should be styled a delayed doubtful, but probably the phenomenon is essentially the same.

We wish to call attention to the fact that the rabbits used were inoculated with pure living cultures of one type of the organism, freshly isolated from the patient's lesions. Some of them succumbed to the infection, as was definitely proved at necropsy and by culture, while others, which had received smaller doses, were made very sick. This was shown by a pronounced drop in weight which they failed to regain after a period of two months. Moreover, they were scrawny and gave the impression that they might die at any time. The blood used in the test was drawn after a period of two months from animals that were definitely proved to have blastomycosis. Their serum was

inactivated at 62 C., as Kolmer⁴ has shown some time since that this temperature destroys the tendency for nonspecific reaction which occurs at times with these animals.

The fact that in the serum both of the patient and of the immune rabbits we obtained this delayed doubtful or practically negative reaction, when it was definitely known that both were suffering from the blastomycotic infection, seems significant in the interpretation of this type of reaction. It is always a step toward understanding an obscure process to put it alongside of another which is similar to it and which may be more amenable to experiment. It is obvious that, short of a spirochete demonstration, it is not possible to make a positive diagnosis of syphilis with finality in the individual case.

It is of interest also that the patient's serum showed definite agglutination to two of the three types of yeasts with which it was tried, although this occurred in a dilution of 1:1. It was completely negative in dilutions of 1:25. However, the control with normal serums was entirely negative, and the results are in accord with those originally reported by Ricketts⁵ in his study of blastomycosis.

It is likewise of importance that control titrations of the positive serums with an antigen prepared from *Endomyces albicans* were negative within the quantitative limitations of the test as employed. The latter yeast strain was isolated by Tanner⁶ from an ordinary pyogenic infection of the finger.

The tendency, then, to regard the delayed negative Wassermann reaction as essentially positive is strengthened by the appearance of the same phenomenon in a patient and in animals definitely proved to have been suffering from blastomycosis.

4. Kolmer, J. A., et al: Studies in Nonspecific Complement Fixation, J. Infect. Dis. **18**:20, 1916.

5. Ricketts, H. T.: Oidiomycosis (Blastomycosis) of the Skin and Its Fungi, J. Med. Res. **6**:377, 1901.

6. Tanner, F. W., and Feuer, B.: Cultural Studies on an Infection of the Skin by *Endomyces Albicans*, Arch. Dermat. & Syph. **1**:365, 1920.