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## CONCERNING LANDAU'S COLOR TEST FOR SERODIAGNOSIS OF SYPHILIS \*

JOHN A. KOLMER, M.D.

PHILADELPHIA

Recently Landau<sup>1</sup> has described an iodine color reaction in syphilis for which he claims a high degree of specificity as compared with the Wassermann reaction. On account of the simplicity of the test and the claims made it has attracted considerable attention, so that I have thought it advisable briefly to record my observations, although they have not by any means substantiated Landau's claims and show the test to be without practical value.

Landau at first used iodized petrolatum for the test, prepared by mixing 5 drops of tincture of iodine in 10 c.c. of paraffin oil. To 0.2 c.c. of the serum was added 2.5 c.c. of this reagent and the test tube set aside in a dark place for from five to fifteen hours. Syphilitic serum, it is claimed, decolorizes the mixture, while the color persists a reddish yellow with normal serum.

With this method Landau examined the serums of 90 persons regarded as syphilitics; of these 49 reacted positively with the Wassermann reaction and 55 with the iodized petrolatum test. Of 32 controls regarded as nonsyphilitic, all reacted negatively with the Wassermann reaction while 1 (a case of *ulcus cruris*) reacted positively with the iodine test.

Later<sup>2</sup> the technic was modified and a 1 per cent. solution of iodine in tetrachloride of carbon employed. To 0.2 c.c. of the fresh, clear serum is added 0.01 c.c. of the iodine reagent; they are well mixed until all color disappears from the reagent and then set aside in a dark place for four hours at room temperature. With the serum of a syphilitic the fluid is a clear transparent yellow; with a nonsyphilitic serum the fluid becomes a whitish gray and is opaque.

Because of the frequent difficulty of reading the results, the addition of starch paste to the mixtures has been advocated as a means for detecting unbound iodine in the serums, as indicated by the development of a blue color. Negative tests, therefore, are supposed to be indicated by a blue color and positive tests by no change of color on the addition of starch. I have also used this technic in the examination of a number of serums, but have not found it of any aid in interpreting the reactions, so that the iodine reaction was found far inferior to the Wassermann reaction in the

practical diagnosis of syphilis, although it may possess considerable theoretical value and interest.

Landau has advanced no adequate explanation of these reactions, but was apparently influenced in his researches by the chemical physiologic reactions of Meier and Porges, Fornet and Schereschewsky, Herman and Perutz, Klausner, Ascoli, Schurman and others who have advocated various floccule, precipitin and color reactions in the diagnosis of syphilis, on the basis that the Wassermann reaction itself is a colloidal chemical reaction with lipoidal substances.

While Landau has not laid down more definite directions, I have conducted his tests in this investigation as follows:

### TECHNIC

(a) Clear corpuscle-free serums were employed. Opalescent serums, as those obtained by bleeding shortly after a meal, are not adapted for the method, especially with the iodine tetrachloride of carbon technic. Practically all serums were used in a fresh active condition and also after heating or inactivation at 56 C. for one-half hour. Serums deeply stained with hemoglobin were found unsatisfactory and were discarded.

While Landau did not report on the results of the examination of cerebrospinal fluids, I have examined a number with both reagents, and with these the results were even more inconsistent than with serums.

(b) With the iodized petrolatum method the exact technic of Landau was observed; with the iodine tetrachloride of carbon method I have modified his technic to this extent, that five times the quantities of serum and reagent were used in order to observe the reaction in a larger volume. With the quantities he advised, namely, 0.2 c.c. serum and 0.01 c.c. reagent, the bulk is quite small even in the small test tubes ordinarily in use in a laboratory; whereas with 1 c.c. of serum and 0.05 c.c. reagent the readings are much easier and more definite. With the iodized petrolatum technic, readings were made at the end of fifteen to eighteen hours; with the iodine tetrachloride of carbon technic, at the end of four hours and again after twenty-four hours at room temperature.

The starch test for unbound iodine was applied by the addition of 0.5 c.c. of a 1 per cent. solution of starch in distilled water. This amount of starch gives a distinct blue color in the sediment when added at once to a mixture of serum and iodine reagent. In the tests I have added the starch after the mixtures of serum and iodine tetrachloride of carbon reagent have stood four hours at room temperature, and in some tests at the end of twenty-four hours. With few exceptions no change of color was perceptible, regardless of whether the serum was from a normal or luetic person. Thus, of 58 serums tested in this manner, 20 yielded positive Wassermann reactions; 38 yielded positive iodine reactions. After four hours the addition of starch to the 58 serums showed a faint blue reaction characterized by a few blue granules, not easily seen in but six tubes, and four of these occurred in clear serums or positive reactions according to Landau.

(c) All serums and cerebrospinal fluids were tested for the Wassermann reaction with three different antigens,

\* From the Laboratories of the Philadelphia Polyclinic and College for Graduates in Medicine.

1. Landau, W.: Untersuchungen über eine Reaktion luetischer Sera mit einem Jodal Reagens, *Wien. klin. Wchschr.*, 1913, xxvi, 1702.

2. Misc. Abst., *The Journal A. M. A.*, Oct. 10, 1914, p. 1317.

namely, an alcoholic extract of human heart, reenforced with cholesterin, an alcoholic extract of syphilitic liver and an extract of acetone-insoluble lipoids of human heart. I have tabulated the results according to the Wassermann reaction, for the histories and diagnoses of the patients tested were at times incomplete and uncertain and in none of the cases regarded clinically as syphilitic was the Wassermann reaction negative.

## RESULTS

(a) *With the Iodized Petrolatum Reagent.*—Twenty-five serums and five cerebrospinal fluids were tested with this reagent. As will be noted in the table, the addition of serum to this reagent practically always resulted in the mixture becoming lighter in color, irrespective of whether the serum was of a luetic or normal person. In some instances the shades and degrees of decolorization were different, but not consistent in regard to the nature of the serum derived from a syphilitic or nonsyphilitic person. Normal as well as syphilitic serums were found to decolorize the

As already stated, the results are expressed according to whether the Wassermann reaction was positive or negative. All of the serums reacting positively in the Wassermann reaction were from persons in the secondary, tertiary or latent stages of syphilis; three were of congenital syphilis; none was in the primary stage. A few were not diagnosed by the attending physician.

The iodine reactions were conducted with fresh active serums and again after heating the serums at 56 C. for thirty minutes. Readings were made after four hours, and again at the end of twenty-four hours, the tubes remaining at room temperature.

The results observed are summarized as follows:

## 1. WASSERMANN POSITIVE SERUMS

1. Of seventy-four serums yielding positive Wassermann reactions fifty-three, or 71.6 per cent., tested in a fresh active condition, reacted positively in the

RESULTS OF LANDAU'S IODIZED PETROLATUM TEST WITH SERUMS AND CEREBROSPINAL FLUIDS REACTING POSITIVELY AND NEGATIVELY IN THE WASSERMANN REACTION

No.	Diagnosis	Wassermann Reaction			Landau's Reaction	
		Cholesterinized Alc. Ext. Human Heart	Alc. Ext. Syph. Liver	Acetone Insol. Lipoids	Active Serum	Inactivated Serum
1	Secondary syphilis.....	++++	++++	++++	Partial decolorization...	Partial decolorization.
2	Secondary syphilis.....	++++	++++	++++	Partial decolorization...	Partial decolorization.
3	Secondary syphilis.....	++++	++++	++++	Almost colorless.....	Almost colorless.
4	Secondary syphilis.....	++++	++++	++++	Partial decolorization...	Partial decolorization.
5	Tertiary syphilis.....	++++	++++	++++	Partial decolorization...	Partial decolorization.
6	Secondary syphilis.....	++++	++++	++++	Partial decolorization...	Partial decolorization.
7	Acute arthritis.....	—	—	—	Partial decolorization...	Partial decolorization.
8	Acute gonorrhea.....	—	—	—	Almost colorless.....	Almost colorless.
9	Secondary syphilis (salvarsan)....	++	—	—	Partial decolorization...	Almost colorless.
10	Acute gonorrhea.....	—	—	—	Almost colorless.....	Partial decolorization.
11	Acute gonorrhea.....	—	—	—	Almost colorless.....	Partial decolorization.
12	Pneumonia.....	—	—	—	Partial decolorization...	Almost colorless.
13	Acute endocarditis.....	—	—	—	Almost colorless.....	Colorless.
14	Tertiary syphilis.....	++++	++++	++++	Partial decolorization...	Partial decolorization.
15	Secondary syphilis (salvarsan)....	+++	+	++	Partial decolorization...	Almost colorless.
16	Infant feeding case.....	—	—	—	Partial decolorization...	Colorless.
17	Sarcoma.....	—	—	—	Partial decolorization...	Partial decolorization.
18	Chronic gonorrhea.....	—	—	—	Partial decolorization...	Partial decolorization.
19	Paresis.....	—	—	—	Almost colorless.....	Colorless.
20	Tabes dorsalis.....	++++	+++	+++	Partial decolorization...	Partial decolorization.
21	Normal rabbit serum.....	+++	++	++	Partial decolorization...	Partial decolorization.
22	Normal rabbit serum.....	+++	++	++	Partial decolorization...	Partial decolorization.
23	Normal dog serum.....	++	+	+	Partial decolorization...	Partial decolorization.
24	Normal dog serum.....	+	±	±	Partial decolorization...	Partial decolorization.
25	Normal dog serum.....	—	—	—	Partial decolorization...	Partial decolorization.
26	Cerebrospinal fluid.....	++++	++++	++++	Practically no change..	
27	Cerebrospinal fluid.....	+++	++	++	Practically no change..	
28	Cerebrospinal fluid.....	+++	+	+	Practically no change..	
29	Cerebrospinal fluid.....	—	—	—	Slightly decolorized....	
30	Cerebrospinal fluid.....	—	—	—	No change.....	

reagent to an equal degree. Cerebrospinal fluids, on the other hand, produced slight or no change in the color of the mixture, regardless of whether they were from a luetic or nonluetic person.

(b) *With Iodin Tetrachlorid of Carbon Reagent.*—With this reagent I examined 162 serums and fifteen cerebrospinal fluids. The former were tested in a fresh active condition and again after heating at 56 C. for half an hour. Readings were made after standing four and again twenty-four hours at room temperature.

In examining most of the serums listed I used the technic adopted, which requires five times the quantities of serum and reagent given by Landau. Even under these circumstances it was usually a difficult matter to interpret and read the results. In most instances a portion of unused serum of each specimen was employed as a control to determine the presence of faint degrees of cloudiness or to read a serum very faintly opalescent in its natural or unused state.

iodin test as read at the end of four hours; at the end of twenty-four hours a few serums became cloudy and thereby reduced the percentage of positive iodine reactions to about 68 per cent.

2. The iodine reactions with heated serums and those more than twenty-four hours old yielded results quite similar to those tested in a fresh active condition, in that 72 per cent. were positive.

3. In general the iodine reaction was negative in about 28 per cent. of serums yielding positive Wassermann reactions and regarded as from luetic persons.

## 2. WASSERMANN NEGATIVE SERUMS

The error of the iodine reaction is even greater with serums of normal persons and those suffering with diseases other than syphilis, in that a relatively high proportion, at least 70 per cent., react positively.

1. Of sixty-one serums reacting negatively in the Wassermann reaction and tested with the iodine reagent in a fresh, active condition, but eighteen, or

29.5 per cent., reacted negatively as read at the end of four hours.

2. Of even greater importance, therefore, is the observation that about 70 per cent. of nonluetic and Wassermann negative serums yield falsely positive iodine reactions.

3. With heated or inactivated serums the iodine reaction at the end of four hours yielded results quite similar to those observed with fresh serum, in that 26 per cent. were negative, and thereby in agreement with the Wassermann reaction.

With cerebrospinal fluids the results were entirely unsatisfactory. Practically all fluids reacted alike; that is, the color persisted in the reagent collected in the bottoms of the test tubes and the fluids remained clear irrespective of whether they were from luetic or nonluetic persons. Of fifteen fluids tested, six gave strongly positive Wassermann reactions and nine reacted negatively, but as stated, all reacted in a similar manner when tested with iodine in tetrachloride of carbon reagent.

#### SUMMARY

1. Of eleven serums of persons and four of normal rabbits and dogs reacting positively, and of ten reacting negatively in the Wassermann reaction, the tests with Landau's iodized petrolatum were entirely unsatisfactory. Both normal and luetic serums alike were found to produce partial decolorization of the reagent, and complete decolorization was likewise observed with both normal and luetic serums. The results with active and inactivated serum were practically similar.

2. Cerebrospinal fluids of both syphilitic and non-syphilitic persons tested with iodized petrolatum produced similar results, in that both alike caused little or no change of the reagent.

3. With the iodine in tetrachloride of carbon reagent the results with fresh active serums after standing four hours at room temperature, as directed by Landau, were as follows:

(a) Of seventy-four serums giving a positive Wassermann reaction, 53, or 71.6 per cent., gave a positive iodine reaction.

(b) Of sixty-one serums giving a negative Wassermann reaction, 18, or but 29.5 per cent., gave a negative iodine reaction. As based on the Wassermann reaction, the iodine test yielded about 70 per cent. false positive reactions with Wassermann negative serums.

4. The error of the iodine test, therefore, is not only in the low percentage of correct positive results, but is especially evident in the high percentage of false positive reactions with nonluetic serums.

5. With cerebrospinal fluids the iodine reagent produced no visible changes, irrespective of whether the fluids were from normal or luetic persons.

**Free Public Laundries.**—Buffalo claims to be the first city in the United States to establish free public baths and free public laundries. They are considered among Buffalo's best investments. The first free municipal bath house and laundry was opened January 2, 1897, in a tenement and cheap lodging house district. The second was opened in a similar neighborhood January 2, 1901. Both were popular from the start. According to the Buffalo *Sanitary Bulletin*, in 1915 the number of bathers was 151,111 men, 14,154 women and 53,461 children, a total of 218,714. The laundry was used by 7,698 men and 178 women, a total of 7,876. Two other municipal baths and laundries are to be established in the Black Rock and South Side districts.

## THE WASSERMANN REACTION AS A CLINICAL TEST, WITH SPECIAL REFERENCE TO ITS BEAR- ING ON MATRIMONY

WALTER J. HEIMANN, M.D.

NEW YORK

### I

In concluding a recent article, Dr. E. L. Keyes, Jr.,<sup>1</sup> states that, "a negative Wassermann is not sufficient evidence of the cure or absence of syphilis," and that "a positive Wassermann, unsupported by clinical evidence, is not sufficient evidence of syphilis." He further remarks that "a positive Wassermann does not prohibit matrimony." With the first two statements it is impossible to disagree, although, by further study it may be shown that they are not axiomatic, and that they may be clarified in such a manner as to bring order out of apparent chaos. With the third statement cited above it is impossible to agree. The object of this article is to indicate that with the very facts from which these conclusions were drawn, other deductions might have been reached.

To dogmatize to the extent of asserting that the positive Wassermann, *per se*, indicates syphilis, is admittedly not wise, for diseases other than syphilis may give the reaction. These are yaws, lepra, tuberculosis, cancer, malaria and scarlet fever. If they are capable of causing the reaction, it goes without saying that others may do so too. We know of none, it is true, and hence, any discussion of such a possibility, however admissible, comes within the realm of medieval scholasticism. Of the diseases enumerated, yaws is at least closely related to syphilis, and the others frequently occur in syphilitics. In fact, from what we know of epithelioma of the tongue, and reasoning by analogy, it is conceivable that the Wassermann reaction is positive mostly in such carcinomas as originate in syphilitic cicatrices. Let us admit, however, that the reaction may be positive in patients suffering with the enumerated ailments, and in whom syphilis may be certainly excluded, if such a thing is possible, and what then? The Wassermann test in practice is a diagnostic and perhaps therapeutic aid to the clinician. Were there no other means to recognize non-syphilitic conditions able to produce this phenomenon, the test would indeed be worthless. All of them, fortunately, possess characteristic clinical, bacteriologic or histologic features which place their diagnosis beyond peradventure. Such being the case, it inevitably follows that a positive Wassermann in a disease obviously neither yaws, tuberculosis, lepra, carcinoma, malaria nor scarlatina, indicates the presence of syphilis.

There are other facts, however, which are more disconcerting because of their elusiveness, namely, the influence on the Wassermann reaction of various chemicals introduced into the body from without or engendered within the body by metabolic activity. Alcohol is known to render a positive Wassermann negative. Acidosis (Keyes) may excite its transitory appearance in individuals in whom it should be absent. In view of these phenomena and without theorizing about them, it is only rational for us as practitioners to study and classify them, lest they lead to misinter-

1. Keyes, E. L., Jr.: Some Clinical Features of the Wassermann Reaction, *THE JOURNAL A. M. A.*, March 6, 1915, p. 804.