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SUBLIMATE AND THE SERUM DIAGNOSIS OF SYPHILIS.*

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According to the studies of those who have been employing the antisheep hemolytic system of Wassermann, the serum reaction of syphilis is apt to disappear suddenly or become very weak shortly after the commencement of mercurial treatment, while the clinical aspect of the patient shows no improvement. This rapid disappearance of the reaction, although not very frequently encountered, renders the value of the Wassermann reaction quite uncertain as a prognostic measure in the treatment of syphilis.

The cause of this phenomenon has not been fully explained. As it has been observed only when the antisheep system is being used, one is justified in seeking the source of this irregularity in the fluctuation of the amount of natural antisheep amboceptor in the serum of the patient during the treatment. A serologist should expect a certain degree of irregularity due to the use of an heterohemolytic system through variable and inconstant fluctuations of the natural hemolytic amboceptors in the patient's serum. By adding immune hemolytic amboceptors, he can produce experimentally conditions which will cause a positive serum to give a negative reaction. This result is always obtained when the reaction is not very strong.

In practice it has been repeatedly demonstrated that the antihuman hemolytic system of Noguchi reveals the syphilitic antibody where the Wassermann system fails on account of the presence of natural amboceptor. Any one who has used both methods adequately must have concluded that the Noguchi system gives a more delicate and constant result with the serum of the patient under treatment. Pedersen, who utilized the antihuman system of Nogu-

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chi, in following up the effects of treatment found a perfect harmony between the intensity of the reaction and the clinical course of the disease during the treatment.

Efforts have been made by some investigators to discover whether or not the sudden disappearance or weakening of the reaction is the direct effect of the mercury upon the syphilitic antibody. Their results are contradictory, as some claim to have obtained a negative reaction with a positive syphilitic serum by mixing it with soluble mercurial preparations in test tubes, while others observed no such effects.

We have made some experiments to ascertain what effects a soluble mercurial preparation will have on the syphilitic antibody *in vitro*. As mercurial preparation, we have chosen mercuric bichloride; and for the fixation test, the system of Noguchi.

The effect of bichloride of mercury was determined upon (1) red blood corpuscles, (2) complement, (3) antihuman amboceptor (rabbit), (4) syphilitic antigen (aceton-insoluble tissue lipoids of human liver), and (5) syphilitic antibody. The results obtained are given briefly in what follows.

Hemolytic Power of Bichloride of Mercury.—In a series of tubes, each containing one cubic centimeter of a 1 per cent. suspension of washed human corpuscles, varying amounts of bichloride of mercury in suitable concentrations were added and the mixtures were incubated for two hours at 37° C. The results are as follows:

Bichloride of mercury in grams.	
0.001	No hemolysis (precipitate, discoloration)
0.0003	30 per cent. hemolysis (precipitate)
0.0001	100 per cent. hemolysis (precipitate)
0.00003	100 per cent. hemolysis
0.00001	100 per cent. hemolysis
0.000003	100 per cent. hemolysis
0.0000025	100 per cent. hemolysis
0.000002	95 per cent. hemolysis
0.0000015	90 per cent. hemolysis
0.000001	No hemolysis
0.0000007	No hemolysis

The hemolytic power of bichloride of mercury was considerably reduced by mixing it with 0.05 cubic centimeter of guinea pig complement or with two units of the amboceptor serum on paper.

Bichloride of mercury in grams.	0.05 c.c. complement (incubated 1 hour before addition of corpuscles).	2 units amboceptor (paper) (incubated 1 hour before addition of corpuscles).
0.000005	100 per cent. hemolysis	100 per cent. hemolysis
0.000003	No hemolysis	100 per cent. hemolysis
0.0000025	No hemolysis	100 per cent. hemolysis
0.000002	No hemolysis	60 per cent. hemolysis
0.0000015	No hemolysis	No hemolysis
0.000001	No hemolysis	No hemolysis

Thus, the original strength, 0.0000025, for complete hemolysis became 0.000005, and the non-hemolytic quantity, 0.000001, became 0.000003 after the bichloride had been digested with 0.05 cubic centimeter of complement. It is evident, therefore, that 0.05 cubic centimeter of complement absorbed the differences during one hour's contact with the sublimate. The absolute quantity of bichloride of mercury absorbed by this amount of serum is about 0.000002 gram. The amboceptor serum interfered in a lesser degree.

The hemolytic power of bichloride of mercury was not noticeably diminished by the antigen, which was used in doses of 0.0003 gram.

Effects of Bichloride of Mercury upon Complement and Amboceptor.—In order to detect any possible anticomplementary property of bichloride of mercury, 0.005 cubic centimeter of guinea pig complement was mixed with varying quantities of bichloride of mercury and the mixtures were incubated at 37° C. for one hour. At the end of incubation the corpuscle suspension and two units of amboceptor were introduced, and the mixtures were again incubated at 37° C.

Bichloride of mercury in grams.	Complement 0.005 + Amboceptor (two units).	Complement 0.005 + No amboceptor (control).
0.0000025	Complete hemolysis	Complete hemolysis
0.0000015	Complete hemolysis	No hemolysis
0.000001	Complete hemolysis	No hemolysis
0.0000006	Complete hemolysis	No hemolysis
0.0000003	70 per cent. hemolysis	No hemolysis
control	70 per cent. hemolysis	No hemolysis

The experiment indicates that bichloride of mercury has no anticomplementary effect in the quantities used. On the contrary, the hemolysis was more complete and proceeded more rapidly in its

presence. This is due to the injurious effect of bichloride of mercury upon the corpuscles, thus reducing the corpuscular resistance to the hemolytic action brought about by the complement and amboceptor.

Effects of Bichloride of Mercury upon the Syphilitic Antigen and Antibody.—To determine the effect of bichloride of mercury upon the antigen, it was mixed with the latter and incubated for one hour at 37° C. before introducing the syphilitic serum and complement. After the incubation, the syphilitic serum and complement were added and incubated, as usual. Amboceptor and corpuscles were added and the whole was again incubated.

To determine the effect of bichloride of mercury upon the syphilitic antibody, it was mixed with the latter and incubated for one hour at 37° C., then, at the end of incubation, the complement and antigen were introduced, the remaining procedures being the same as usual.

The amount of complement employed was 0.05 cubic centimeter; that of amboceptor, two units; that of antigen, 0.0003 gram of aceton-insoluble lipoids; that of syphilitic serum, 0.02 cubic centimeter; and that of corpuscles, 0.1 cubic centimeter of a 10 per cent. suspension. The total volume of fluid in each tube equalled one cubic centimeter.

The foregoing experiments show that bichloride of mercury in the highest non-hemolytic quantity (0.0000025 gram) failed to remove, to a noticeable degree, the syphilitic antibody, and the reaction in two strongly positive sera remained unchanged. In two weaker sera there was a little more hemolysis than in the control tubes which contained no bichloride of mercury. This slight hemolysis may be explained, (1) by the removal of some of the antibodies by the bichloride, (2) by the injury inflicted by the bichloride upon the red blood corpuscles, rendering these more readily hemolyzable by the complement and amboceptor introduced afterward, or (3) by the combined action of (1) and (2). Of these possibilities, the second is the more probable, this assumption being supported by the fact that, in the controls in which no bichloride of mercury was present, and in which no hemolysis occurred at first, there was a trace of hemolysis after these had stood for six

TABLE I.

Experimental arrangement.	Effect of $HgCl_2$ upon syphilitic antigen.						Effect of $HgCl_2$ upon syphilitic antibody.			
	Antigen (0.0003 gram) + $HgCl_2$; incubation.						Syphilitic serum (0.02 c.c.) + $HgCl_2$; incubation.			
	Then, complement (0.05 c.c.) and syphilitic serum (0.02 c.c.); incubation.						Then, complement (0.05 c.c.) and antigen (0.0003 gram); incubation.			
	Then, corpuscular suspension and amboceptor; incubation.						Then, corpuscular suspension and amboceptor; incubation.			
Varieties of serum.	Secondary lues. Serum No. 1.		Secondary lues. Serum No. 2.		Tertiary lues. Serum No. 3.	Latent lues. Serum No. 4.	Serum No. 1.	Serum No. 2.	Serum No. 3.	Serum No
$HgCl_2$(gram) 0.0000025	No H.	Absolute inhibition.	No H.	Absolute inhibition.	Slight H.	Complete H.	No H.	No H.	Slight H.	Complete I .
0.000002	No H.		No H.		Trace H.	Almost complete H.	No H.	No H.	Trace H.	Complete H.
0.0000015	No H.		No H.		No H.	Much H.	No H.	No H.	No H.	Much H.
0.000001	No H.		No H.		Trace H in 20 hours.	Moderate H.	No H.	No H.	Trace H in 20 hours.	Moderate H.
0.0000006	No H.		No H.		No H.	No H.	No H.	No H.	No H.	No H.
0.0000003	No H.		No H.		No H.	Trace H in 6 hours.	No H.	No H.	No H.	Trace H in 6 hours.
control	No H.		No H.		No H.	No H.	No H.	No H.	No H.	No H.

H = hemolysis.

hours or longer, showing that the complement was not completely fixed. The same is true also in the experiments relating to the effect of sublimate upon the antigen. When working without proper controls (such as determine the effects of non-hemolytic doses of bichloride of mercury upon the complement-amboceptor hemolysis), one is apt to consider the above experiments as constituting evidence of the removal of the syphilitic antibody by the sublimate. It may be added here that the strongly positive sera (No. 1 and No. 2) contained only about two units of syphilitic antibody in 0.02 cubic centimeter. In test tube experiments it seems to us that the only way to determine the slightest possible effect of sublimate upon syphilitic antibody is to test the action of the highest non-hemolytic quantity of bichloride of mercury upon the smallest detectable number of antibody units. This was done in our present determination.

Whether the same results are obtainable with the antisheep hemolytic system is another problem. If the resistance of sheep corpuscles to the hemolytic effect of bichloride of mercury is greater, thus permitting the use of a larger amount of the mercurial salt, a more distinct effect might be secured. But the work of Satta and Donati shows it to be about the same. One might imagine that the hemolytic effect of the mercurial salt can be prevented by adding certain indifferent proteins to the mixture, but, unfortunately, according to our recent studies, the indifferent proteins can themselves saturate the fixing affinity of the mixture of syphilitic serum and antigen and render the positive reaction negative.

The test tube experiments do not, however, exclude the possibility that bichloride of mercury acts in the body upon the syphilitic antibody. In the body the sublimate circulates constantly and may remove the syphilitic antibody little by little. Besides, the rich protein content of the body fluids, especially the blood serum, protects the red blood corpuscles from the injurious effect of bichloride of mercury to a far greater degree than the conditions *in vitro* permit. The question is not adequately answered by means of test tube experiments. It is well to recall here that the administration of the powerful spirochæticide, dioxy-diamido-arsenobenzol,

of Ehrlich and Hata can lead to the disappearance of the syphilis reaction in a comparatively short period, yet this preparation contains no mercury. The disappearance of the reaction under the mercurial treatment may, therefore, be due, not to an influence on the antibody, but to a destruction of the spirochæte.