Spirocheticidal Activity of Plasma and Serum Fractions.* (18990)

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The treponemal immobilization test by Nelson and Mayer(1) provides a convenient *in vitro* measure of at least one type of circulating antibody against pathogenic *T. pallidum*. The substances measured in the serums of syphilitic patients and animals are apparently the same as the spirocheticidal substances reported by earlier investigators(1,2). While the relationship of these antibodies to acquired immunity in syphilis is by no means clear(3), it is hoped that an understanding of the nature of these spirocheticidal substances will aid in the interpretations of the host reaction to infection. It is established that these "immobilizing antibodies" are associated with the protein fraction of serum; "that they are not reagin;" and that they are relatively heat stable. The present report is concerned with fractionation of syphilitic human serums and plasmas using Cohn's Method 10(4). It will be shown that most of the immobilizing activity is associated with Cohn's fractions III-1,2 which represents the high molecular weight isoagglutinins and a small amount of prothrombin protein, and fibrinogen when plasma was employed, and fraction I + III-3 which represents the high molecular weight antihemophilic globulin and cold insoluble globulin and small amount of plasminogen.

Experimental. Since Method 10 of Cohn was developed for the fractionation of normal human plasmas, minor adjustment in the first step of the procedure was required in order to make it adaptable to syphilitic human serums and plasmas. The aim of such adjustment was to maintain the pH and the ionic strength at the levels specified by the Cohn Method. It was necessary to commence fractionation with an adjusted "Reagent A." The original Reagent A contains 250 ml of 95% ethanol and 2.5 ml of 0.8 Γ/2 pH 4.0 sodium acetate buffer per liter. Four parts of this reagent when added to one part of Cohn's A.C.D. anticoagulant containing plasma(4) results in a mixture having the desired pH of 5.8-5.9. It was found that for most syphilitic specimens a less acid reagent was required. Thus a series of modified reagents with a lower acetic acid content was prepared and kept in stock. From these reagents a suitable one (furnishing the desired pH of 5.8-5.9) was selected for each syphilitic plasma and serum by "titration." In this procedure 4 ml of the modified Reagent A, cooled to −5°C, was added to 1 ml of plasma in a tube cooled to 0°C. It was necessary to add the reagent in small portions while the tube was rotated and kept at −5°C. 1 ml of the well mixed suspension was diluted with 4 ml of 0.02 M sodium chloride and the pH determined with the aid of a Cambridge pH meter. The other details concerning fractionation were exactly as those given by Cohn(4). The immobilization tests were performed in the manner of Magnuson and Thompson(2). Quantitative tests were controlled with a single pool of positive serum, and titers are expressed as that dilution of serum which immobilized 50% of the organisms after 16 hours incubation at 35°C.

In Table I are recorded the spirocheticidal activities of a series of plasma fractions I + III-1,2 and I + III-3 from patients of early latent syphilis. The results show that in most instances the recovered total activity was over 50%. In one instance (patient No. 239), however, recovery was very low, 15.3%,

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TABLE I. Activity of Plasma Fractions III-1,2 and I+III-3.

Patient No.	Plasma	Fraction III-1,2	Fraction I+III-3	Total activity recovered in %	Positive control
24,881	1:460	1:54	1:100	33.6	1:420
239	1:580	1:31	1:58	15.3	1:495
242	1:80	1:56	1:82	172.5	1:660
247A	1:76	1:16.5	1:23.5	52.5	1:280
247B	1:300	1:84	1:83	55.6	1:500
247C	1:510	1:86	1:220	60.1	1:500
251A	1:235	1:58.5	1:100	68.3	1:210
251B	1:990	1:125	1:420	55.1	1:210

Titers converted to dilutions which immobilized 50% of the spirochetes.

TABLE II. Comparison of Activities of Serums and Plasmas.

Patient No.	Serum	Plasma	Positive control
241	1:45	1:82	1:380
242	1:183	1:80	1:660
244	1:345	1:335	1:280
247A	1:76	1:80	1:280
247B	1:330	1:290	1:500
25601A	1:1100	1:500	1:320
25597B	1:16	1:14.5	1:258
25599C	1:320	1:540	1:320
25600D	1:123	1:66.5	1:320
23815E	1:740	1:250	1:320
251B	1:1280	1:910	1:210

Titers converted to dilutions which immobilize 50% of the spirochetes.

in another instance (patient No. 242) it was too high, 172.5%.

In Table II are compared the spirocheticidal activities of a series of serums and plasmas of patients having early latent syphilis. In order to maintain the same ionic strength and pH, the same quantity of anticoagulant was added to serums as was contained in the plasmas. It may be seen in Table II that there is no appreciable difference between the serum and plasma derived from a given patient. It is evident that clotting elements do not remove any of the spirocheticidal activity.

Since it was found that serum contains as much of the spirocheticidal activity as plasma, in the following experiments fractionation was applied to a series of serums (Table III). The total activities of serum fractions I + III-1,2 and I + III-3 range from 26.2% to 72.3% with an average of 51.6%. The results shown in Table III, being more consistent than those of Table I, would indicate that serum is a more suitable starting material for the concentration and isolation of the spirocheticidal substances than plasma.

In order to maintain the same ionic strength and pH in both, the same volume of anticoagulant was added to the serums as there was present in the plasmas.

Summary. There is no appreciable difference between the spirocheticidal activity of the serum and the plasma from patients with early syphilis. Serums and plasmas were fractionated into eight precipitates by the application of Cohn's Method 10. In the majority of the experiments, more than 50% of the spirocheticidal substances were present in fraction III-1,2 associated with high molecular weight isoagglutinins and small amount of prothrombin protein (and fibrinogen when

TABLE III. Activity of Serum Fractions III-1,2 and I+III-3.

Patient No.	Serum	Fraction III-1,2	Fraction I+III-3	Total activity recovered in %	Positive control
235	1:118	1:56	1:14.5	59.8	1:270
237	1:154	1:102	1:11	72.3	1:270
239	1:502	1:56	1:75	26.2	1:540
241	1:50	1:18	1:11	58	1:380
242	1:185	1:57	1:20	41.6	1:660

Titers converted to dilutions which immobilized 50% of the spirochetes.

plasma was employed) and fraction I + III-3 associated with the high molecular weight antihemophilic globulin and cold insoluble globulin and a small amount of plasminogen.

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Effect of Cortisone and of Aureomycin on Baby Pigs Fed a Vitamin B_{12}-Deficient Diet. (18991)

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Using baby pigs as experimental animals, a vit. B_{12} deficiency has been produced on a "synthetic milk" diet by Neumann *et al.*(1). According to Johnson and Neumann(2), administration of crystalline vit. B_{12} to this diet will completely alleviate the deficiency symptoms. Stokstad and Jukes(3) reported in some experiments a "sparing effect" of aureomycin upon the vit. B_{12} requirement of chicks. Lichtman, Ginsberg, and Watson(4) administered aureomycin to pernicious anemia patients and reported a hematological improvement. Cravioto-Munoz *et al.*(5) state that aureomycin can replace vit. B_{12} in the purified diet of the rat. No work has appeared on the relation of cortisone to nutritional vit. B_{12} deficiency.

The present study was conducted to determine the effect of aureomycin and of cortisone when administered to baby pigs fed a vit. B_{12}-deficient ration.

Experimental. Six Chester White pigs (Exp. I) and 5 Poland China pigs (Exp. II) were removed from their dams at 48 hours of age and individually fed *ad libitum*. The alpha-protein "synthetic milk" ration fed was essentially the same as reported in the vit. B_{12} studies of Neumann *et al.*(6). In addition, 2% of sulfathalidine was added per liter of milk. The method of feeding and care of the animals was similar to that reported previously by Johnson *et al.*(7). In Exp. I two groups were compared. Group I received the basal ration plus intramuscular injections of crystalline vit. B_{12} at a level of 0.8 μg/kg body wt/day. Group II received the basal ration plus 100 mg of aureomycin per kg of dry matter consumed. After 49 days on this diet they were given 30 μg of vit. B_{12} by intramuscular injection for 3 successive days and remained on the experiment 14 days after therapy. The 5 pigs in Exp. II received the basal ration for 39 days. At that time they showed signs of vit. B_{12} deficiency, including a very low reticulocyte count. One pig was continued on the basal ration as a control, while the other pigs were divided into 2 groups. Group IV received 20 mg of cortisone daily by intramuscular injection, and Group V received in their diet 100 mg of aureomycin per kg of dry matter consumed.

Blood samples were taken daily during the treatment period, and reticulocyte counts were determined. The thyroids and tongues of the pigs in Groups IV and V, Exp. II, were removed at autopsy for histological examination.

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