THE PRESENCE OF PLASMA INHIBITORS DURING THE CRISIS PHENOMENON IN EXPERIMENTAL RELAPSING FEVER (BORRELIA NOVYI)*

BY ORNELLA CALABI, S.D.

(*From the Department of Microbiology, Harvard School of Public Health, Boston*)

Immunity in relapsing fever has been under investigation since 1906 (1, 2), but the mechanism of this immunity is poorly understood. This is because of the instability of *Borreliae*, the scanty information on the genealogy of the strains used, the wide range of experimental animals studied, the lack of quantitative data on the infective doses, and the crudity of the qualitative serological tests (3–5).

The present study was undertaken in an effort to elucidate the mechanism of the crisis phenomenon. Blood was studied in an attempt to determine the nature of the factor(s) which might be responsible for resolution of the primary infection. This necessitated the development of an appropriate method for the production of assayable infections, so that the result of treatment with serum, plasma, or their derivatives, obtained at the time of crisis, might be determined reliably.

Material and Methods

Maintenance of the Strain.—The strain of *Borrelia novyi*[1] used was maintained in 21-day-old rats (Hisaw strain) weighing an average of 45 gm. These served for the experimental studies as well as for maintenance of the strain. For this latter purpose several drops of blood were taken by cardiac puncture from infected donor rats under light ether anesthesia into syringes containing 1 ml. of a high phosphate buffer (*vide infra*) and injected into recipients. This was done intraperitoneally at biweekly intervals.

Blood for preparation of quantitative inocula was obtained by cardiac puncture from infected rats under light ether anesthesia[2] at 60 to 65 hours after infection had been instituted.

[1] I wish to thank Dr. Quentin M. Geiman for the supply of this strain which was obtained in 1946 from Dr. Malcolm H. Soule of the University of Michigan, Ann Arbor. This strain, originally isolated in 1906 from the first case of relapsing fever on record in this country (6) and maintained since then in rats and white mice, is believed to be identical with *Spirochaeta obermeieri* (2).

[2] Ether anesthesia does not affect the course of *B. novyi* infection in experimental animals (7).

Blood was drawn into syringes containing 0.3 ml. of a heparinized Ringer's solution[3] per 10 ml. of blood. These bloods were pooled and diluted 1:200 with a sterile phosphate buffer devised by McKee and Geiman (8), which facilitates longer survival of the organisms by virtue of added glucose (9) and a higher than usual concentration of phosphate (7).[4]

0.005 ml. of the diluted blood was delivered on a clean slide beneath an 18 x 18 sq. mm. coverslip. The organisms were counted with a dark-field microscope, calibrated according to standard technic, and provided with wide-field oculars and oil immersion objective. The number of spirochetes per oil field was taken from the average of four 0.005 ml. preparations, and from this number the number of spirochetes/c.mm. of diluted blood was calculated. The counts indicated that from 0.15 to 0.25 ml. of the diluted freshly drawn blood would be needed to provide an infective dose of 1×10^8 spirochetes/kg. The required volume was brought to a final volume of 0.4 ml. with the phosphate buffer, and was then injected intraperitoneally into recipient rats. All procedures relating to the preparation of the quantitative inocula were carried out at 4°C.

Evaluation of Spirochetemia.—The course of the infection was determined in terms of the number of circulating spirochetes per cubic millimeter of blood at varying intervals after the inoculation as follows: Films of heart blood or tail venous blood were made and stained with Giemsa according to standard procedure. Thick films were prepared if dark-field inspection showed the number of organisms to be 1 per 1000 red cells or less. Thin films were prepared when the number was above this ratio. When the count in stained thick films was less than one spirochete per 100 white blood cells, the number of organisms per 600 white blood cells was determined. When the count was more than one spirochete per 100 white blood cells, the number per 200 white blood cells was determined. When no organisms were seen in counts of 600 white blood cells in consecutive fields (two readings per film), blood was considered negative.

Counts in stained thin films were made by Reed's method (10, 11), originally devised for the enumeration of malarial parasites in avian blood films. By using this method sufficient organisms and red cells are counted so that the probable error in evaluating the smears does not exceed 10 per cent.[5]

Because of the progressively severe anemia in this disorder (*vide infra*), calculation of spirochetal count per c.mm. of circulating blood required a redetermination of the red count each time a film for a spirochetal count was prepared.

EXPERIMENTAL

The Dose Response in Experimental Infection.—

In preliminary attempts to establish an infective dose of choice, inoculations in excess of 6×10^8 organisms/kg. produced too severe a spirochetemia to permit an accurate count. Study of the effect of smaller, graded infective doses $(1 \times 10^8, 1 \times 10^6, 1 \times 10^4)$ showed a decrease in the levels of spirochetemia and a delay in the onset and termination of the crisis

[3] 125 mg. of sodium heparin (u.s.p. 150 units/mg. 11.1 per cent H_2O, General Biochemical Co.) was dissolved in 25 ml. of modified Ringer's solution: NaCl 0.821 gm., KCl 0.3 gm., $CaCl_2 \cdot 2H_2O$ 0.27 gm., $MgCl_2 \cdot 6H_2O$ 0.1 gm., and aq. dist. to 10^3 ml., pH 7.0–7.2, and sterilized by autoclaving.

[4] $NaH_2HO_4 \cdot H_2O$ 0.7596 gm., Na_2HPO_4 3.1240 gm., NaCl 1.9685 gm., KCl 0.2050 gm., $MgCl_2 \cdot 6H_2O$ 0.0475 gm., $CaCl_2 \cdot 2H_2O$ 0.0371 gm., 32 mg. per cent solution of $MnCl_2 \cdot 4H_2O$ 0.5 ml., and distilled water to 500 ml. This buffer (pH 7.2) is isotonic with blood and is sterilized by autoclaving. 10 ml. of 10 gm. per cent solution of glucose in distilled water is added aseptically to give a final concentration of 200 mg. per cent glucose.

[5] The validity of Reed's formula for spirochetal counts has been verified (12).

phenomenon in proportion to dose (Table I). An inoculation of 1×10^8 spirochetes/kg. proved to be the optimal infective dose. With this dose the acute phase was relatively short and the spirochetemia could be quantitated with desirable accuracy. Observations on the course of untreated infections with this infective dose over a period of 18 months may be summarized as follows: (a) the primary infection lasts about 5 to 6 days, with the highest spirochetal count at about 72 hours (4 to 5 million/c.mm. blood); (b) crisis ends between 110 and 155 hours after inoculation; (c) the red blood cell count falls to 1.0 to 1.89×10^6 rbc/c.mm. blood[6]

TABLE I

The Effect of Graded Doses of Spirochetes on the Intensity and Duration of B. novyi infection

	Log No. of spirochetes/c.mm. blood*					
	Group 1		Group 2		Group 3	
Spirochetal dose/kg.....	1×10^8		1×10^6		1×10^4	
Rat No................	5	6	7	8	9	10
Time						
hrs.						
13	3.2	3.4	$-\infty$ ‡	$-\infty$	$-\infty$	$-\infty$
38	4.7	5.1	2.9	3.3	$-\infty$	$-\infty$
67	6.4	6.6	4.8	5.2	3.1	2.7
72	6.6	6.7	5.7	5.4	4.0	3.1
84	6.5	6.6	5.9	5.9	4.2	4.1
116	$-\infty$	$-\infty$	5.6	5.3	3.8	3.4
134	$-\infty$	$-\infty$	ND§	ND	ND	ND
139			$-\infty$	5.6	3.5	$-\infty$
157			$-\infty$	$-\infty$	$-\infty$	$-\infty$

* Blood samples from peripheral blood.

‡ No spirochetes (the log of zero is $-\infty$).

§ No data.

(12); (d) relapse starts from 30 to 70 hours after resolution of the primary infection. The highest count observed during the relapse (20 to 10,000 spirochetes/c.mm. blood) were 5×10^{-2} to 2.5×10^{-5} that of the primary infection. The duration of the relapse is usually 3 to 5 days, with maximal counts occurring from 1 to 4 days after its onset (12).

Since the response to a 1×10^8 dose was reasonably constant after repeated trials, we considered that this dose would provide crisis serum at about 100 hours after inoculation. Such serum was used for treatment of the primary infection in rats inoculated with 1×10^8 organisms.

Bio-assay of Crisis Serum.—After preliminary quantitative assays had suggested an appreciable inhibitory effect of crisis serum on the course of the induced infection, the following study was made.

---

[6] The normal range in the red blood cell counts in the rat has been variously reported to be 7.2 to 9.6 (13), 6.60 ± 0.76 (14), 8.50 ± 1.50 (14).

Thirty weanling rats were divided into three groups of ten each. Each rat received 1×10^8 spirochetes/kg. at 0 hour. One hour later those in Group 1 were treated with 10 ml./kg. of crisis serum. Those in Group 2 received 10 ml./kg. of normal serum. Those in Group 3 received no treatment. Two animals from each group were killed simultaneously at varying intervals after inoculation to provide heart blood[7] for determination of the spirochetemia.

TABLE II

The Effect of Crisis Serum and Normal Serum on the Course of B. novyi Infection

	Group 1		Group 2		Group 3	
Spirochetal dose/kg.....	1×10^8		1×10^8		1×10^8	
Serum treatment, 10 ml./kg..............	Crisis serum		Normal serum		None	
Time	Rat No	Log spiro*	Rat No.	Log spiro	Rat No.	Log spiro
hrs.						
54	11	1.8	21	6.2	31	6.08
	12	$- \infty$ ‡	22	6.3	32	6.01
78	13	$- \infty$	23	6.8	33	6.4
	14	1.8	24	$- \infty$	34	6.6
106	15	0.9	25	3.9	35	5.6
	16	3.9	26	3.9	36	$- \infty$
130	17	$- \infty$	27	$- \infty$	37	$- \infty$
	18	$- \infty$	28	$- \infty$	38	$- \infty$
183	19	1.6	29	1.4	39	4.1
	20	$- \infty$	30	6.1	40	0.9

* Log number of spirochetes.c.mm. heart blood.

‡ No spirochetes (the log of zero is $- \infty$).

Inspection of Table II shows that during the phase of active growth the spirochetemia was of about the same intensity in Groups 2 and 3, while in Group 1 there was no spirochetemia or one of far less intensity; *i.e.* from 10^{-5} to 10^{-6} that of the control group.

These data demonstrate the presence of inhibitors of the infection in crisis serum, and that this inhibition remains effective throughout an observation period lasting from 78 to 130 hours.

---

[7] Spirochetal counts from samples of heart blood were 25 to 40 per cent higher than counts from samples of tail blood. Analogous observations have been made in experimental malaria (7). Films from peripheral blood, nevertheless, were taken as well because they made possible the handling of all animals before being sacrificed. The differences between heart and peripheral blood do not appear in the table.

Biological Standardization of Crisis Plasma.—The next step was an effort to evaluate the protective titer of whole plasma and of fractions of plasma. The study was made with plasma instead of serum because plasma is as potent as serum (12), can be obtained in greater yield, and lends itself better to fractionation procedures (15).

Crisis blood was harvested from groups of 16 to 24 donor rats, which were bled by cardiac puncture from 91 to 93 hours after inoculation of 1×10^8 spirochetes/kg. The blood was heparinized as described above, and centrifuged at 4°C. for 1 hour. The pooled plasma was sterilized by passage through an ultrafine fritted glass filter at 4°C., and then diluted in phosphate buffer so as to provide 1:64, 1:16, and 1:4 dilutions. Five groups of four rats each

TABLE III

The Effect of Increasing Doses of Crisis Plasma on the Course of B. novyi Infection

Group No...		1				2				3				4				5		
Crisis plasma treatment, 10 ml./kg..		1:64				1:16				1:4				1:1				None		
Rat No.	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Time																				
hrs.																				
13	3.2	2.9	−∞‡	2.9	2.9	2.7	2.2	2.7	2.7	−∞	−∞	−∞	−∞	−∞	−∞	−∞	2.4	3.3	3.4	3.2
37	4.5	4.6	1.3	4.8	4.8	4.4	4.1	4.6	4.5	−∞	−∞	−∞	−∞	−∞	−∞	−∞	3.9	4.8	4.8	4.8
76	6.4	6.4	0.9	6.8	6.7	6.2	5.5	6.5	1.2	2.5	2.2	2.6	−∞	−∞	−∞	−∞	6.6	6.5	6.7	6.5
120	−∞	−∞	3.8	−∞	−∞	−∞	−∞	−∞	−∞	3.6	3.2	3.9	0.9	1.8	3.7	−∞	6.2	5.7	−∞	−∞
155	−∞	−∞	3.6	−∞	−∞	−∞	−∞	−∞	−∞	−∞	1.0	−∞	−∞	1.0	2.0	1.2	−∞	−∞	−∞	−∞

*The header "Log No. spirochetes/c.mm. blood*" spans columns 1–5.*

* Blood samples from peripheral blood.
‡ No spirochetes (the log of zero is −∞).

received the infective dose of spirochetes (1×10^8/kg.). Four of the five groups were injected intraperitoneally with 10 ml./kg. undiluted plasma, or with one of the three dilutions of plasma. The fifth group received no plasma and served as a control.

The results (Table III) show that crisis plasma diluted 1:4, as well as undiluted plasma, has a high protective effect, with maximal spirochetal counts at 120 hours after treatment of 10^{-3} to 10^{-6} that of the control group (group 5). No suppression of spirochetemia occurred with crisis plasma of higher dilution.

The efficiency of the graded dose bio-assay in the standardization of crisis plasma is readily evident when the log of spirochetes/c.mm. blood is plotted as a function of the log dose of crisis plasma given (Fig. 1). The dose-response at 37 and at 76 hours after treatment (Fig. 1) reveals a significant difference in spirochetemia between the protected (1:4 dilution) and the unprotected (1:16 dilution) groups. This range in dilution is the protective titer according to the criterion for biological standardizations.
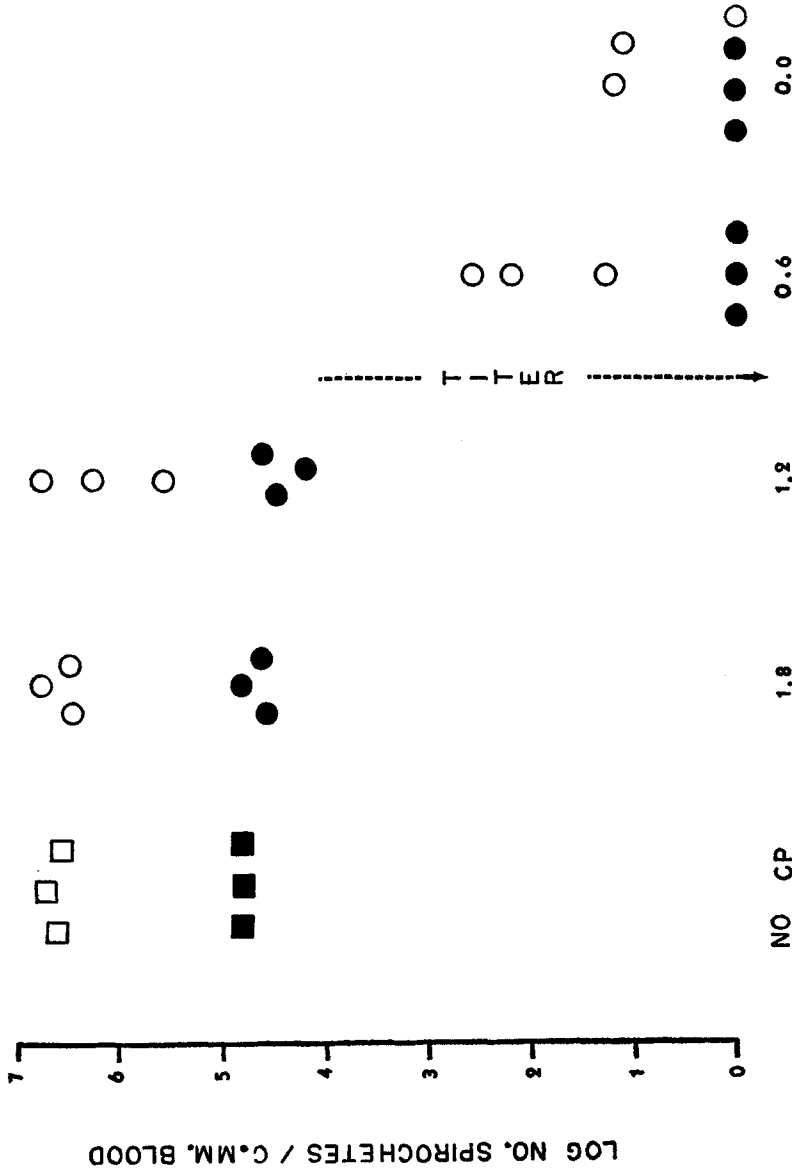
LOG DILUTION CRISIS PLASMA (CP)

FIG. 1. Biological standardization of crisis plasma (CP). Log number of spirochetes/c.mm. blood is plotted as a function of graded log doses of CP. Counts at 37 hrs.: ● treated; ■ untreated. Counts at 76 hrs.: ○ treated, □ untreated. The data show the level of the spirochetemia in control (untreated) group of 3 rats, and in four treated groups of three rats each, at 37 hours and at 76 hours after inoculation of spirochetes and treatment with CP. Graph shows a steep slope in dose-response curve between two protected and two unprotected groups. The arrow indicates CP protective titer falling between CP log doses 1.2 and 0.6, *i.e.* between dilutions 1:16 and 1:4 (see data in Table III).

Statistical Analysis.—The results obtained from the biological standardization of crisis plasma were evaluated by the method of appropriate scores for linear bio-assays (16) to ascertain how much of the total variance in spirochetal counts can be explained by the protective dose of crisis plasma given. This fraction of the over-all score system in function of the dose of crisis plasma given is called the information (16).

Computation of appropriate scores throughout the course of the infection[8] (Table IV) shows that the maximum information obtained from dose and scores at 13, 37, and 76 hours is significant when the χ_c^2 test (16) is applied.

TABLE IV

Appropriate Scores (16) in the Biological Standardization of Crisis Plasma

Spirochetemia level at*	Total No. of rats	$\Sigma a_k(x' - \bar{x}')^2$‡	Appropriate scores§ $\Sigma_j \frac{(d_j)^2}{a_{.j}}$	Information‖	Significance of $a..\vartheta$ ¶
hrs.				*per cent*	
13	16	80	38.5	48.125	$\chi_c^2 = a..\vartheta = 7.68$ (DF = 2) $0.02 < P < .05$
37	16	80	59.1	73.875	$\chi_c^2 = a..\vartheta = 11.82$ (DF = 2) $0.001 < P < .01$
76	16	80	53.0	66.25	$\chi_c^2 = a..\vartheta = 10.6$ (DF = 4) $0.02 < P < .05$
120	16	80	14.4	18.0	$\chi_c^2 = a..\vartheta = 2.88$ (DF = 2) $0.20 < P < .30$

* Time in hours after inoculation of 1×10^8 spirochetes/kg. followed by administration of 10 ml./kg. of crisis plasma at the appropriate dilution.

‡ Sum of squares of deviations in response independent of crisis plasma dose given.

§ Sum of squares of deviations in response in function of crisis plasma dose given.

‖ Fraction of total variance in spirochetal counts in function of crisis plasma dose given.

¶ Maximum information extracted from dose and scores.

It will be noted that at 37 hours the score system shows the best fit with a larger χ_c^2 value ($\chi_c^2 = 11.82$), and with an associated probability of less than 1 chance in 100 that the maximum information extracted from dose and scores (73.875 per cent) is due to chance variation ($0.001 < P < 0.01$). It follows that the 37 hour readings under the specified conditions are the most significant and provide us with the maximum information from dose and scores.

Fractionation Procedures.—Following the demonstration of the potency of crisis plasma an effort was made to identify the fraction or fractions of plasma

---

[8] The essential data on the intensity of the infection under the specified conditions are taken from Table III.
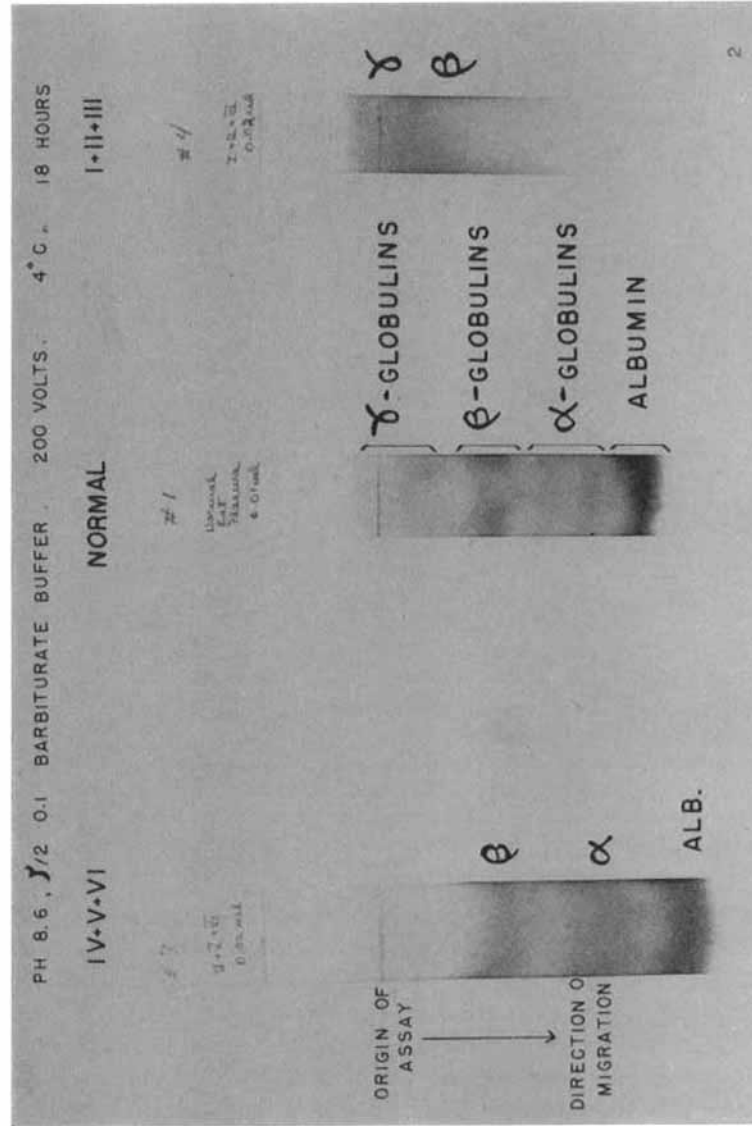
FIG. 2. Zone electrophoresis on paper of rat normal plasma and fractions from rat crisis plasma. Fractional precipitation of rat crisis plasma (method 10) allows a clear cut chemical separation of gamma globulin in fraction I + II + III. No trace of this fraction is detectable in fraction IV + V + VI. Conversely, none of the albumin content of fraction IV + V + VI is present in fraction I + II + III.

which contain the inhibitor(s). Fractionation was done within 4 hours after collection of the blood as follows:—

Pooled, heparinized crisis plasma from 24 donor rats was treated by method 10 of Cohn *et al.*, (17, 18) to the end of the first step, which yields the two main fractions (I + II + III and IV + V + VI). Fractional precipitation was carried out in a −5°C. agitated bath containing 20 per cent ethylene glycol by adding one volume of crisis plasma to four volumes of reagent solution (18) freshly prepared from 95 per cent ethanol water and stock solution of C.P. sodium acetate buffer. The precipitate (I + II + III), con-

TABLE V

The Effect of Crisis Plasma and of Fractions I + II + III and IV + V + VI from Crisis Plasma (CP) on the Course of Bor. novyi Infection

Group No.	Log No. of spirochetes/c.mm. blood*																			
	1				2				3				4				5			
Spiro-chetal dose/kg.	1×10^8				1×10^8				1×10^8				1×10^8				1×10^8			
Crisis plasma treat-ment, *10 ml./kg.*	I + II + III				IV + V + VI				CP −55°C.				CP 4°C.				None			
Rat No. ...	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Time																				
hrs.																				
26	−∞‡	−∞	−∞	−∞	−∞	−∞	−∞	3.9	−∞	−∞	−∞	−∞	−∞	−∞	−∞	−∞	4.2	4.2	4.3	4.0
51	−∞	−∞	−∞	−∞	0.9	−∞	1.2	4.8	−∞	−∞	−∞	−∞	−∞	−∞	−∞	−∞	5.9	6.0	5.9	6.0
76	−∞	−∞	−∞	ND§	2.6	−∞	2.0	3.0	−∞	−∞	−∞	−∞	−∞	−∞	−∞	−∞	6.7	6.6	6.7	6.7
120	0.9	3.4	4.0	ND	−∞	3.1	−∞	−∞	3.0	−∞	3.5	3.0	0.9	0.6	−∞	2.8	−∞	5.3	4.9	2.3

* Blood samples from peripheral blood.
‡ No spirochetes (the log of zero is −∞).
§ No data.

taining the gamma globulin fraction, was separated from the supernatant (IV + V + VI), containing the albumin fraction, by centrifugation at 4000 R.P.M. at −5°C. for 30 minutes. The two main fractions were dried from the frozen state and were dissolved to plasma volume for electrophoretic analyses and bio-assays. The protein concentrations of fraction I + II + III and fraction IV + V + VI from 50 ml. of pooled plasma dissolved to plasma volume were 1.181 gm. per cent and 4.377 gm. per cent, respectively.

Electrophoretic Procedure.—

Dried fractions were weighed out and dissolved in barbiturate buffer (pH 8.6, Γ/2 0.1 sodium diethyl barbiturate), clarified by centrifugation for 30 minutes at 18,000 R.P.M. at 4°C., resuspended to plasma volume in the barbiturate buffer and applied to the origin line of paper strips. A sample from normal rat plasma was included. The strips, after treatment with different ranges of plasma protein concentration (0.01 ml., 0.02 ml., and 0.03 ml.), were

saturated with barbiturate buffer and inserted into the apparatus.[9] The apparatus and its contents were allowed to equilibrate for at least 1 hour. Runs were completed after 18 hours at 4°C. with voltage adjusted (5.5 ma., 200 volts). The strips were then removed, dried, stained
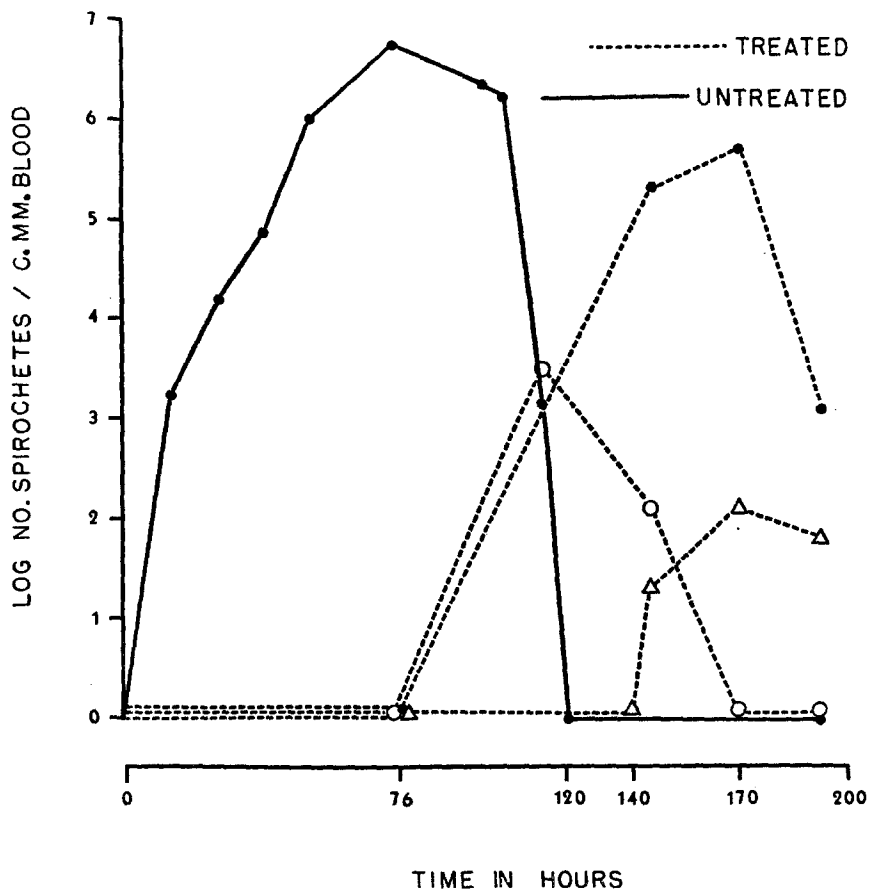

TIME IN HOURS

FIG. 3. Effect of crisis plasma (CP) stored 24 hours at −55°C. on the course of *B. novyi* infection in rats. The solid curve represents the course of the untreated infection (counts are averages from four animals, see data in Table V). The broken line curves show the course of the infection in three rats which received this plasma 2 hours after inoculation of spirochetes. The curves show that the maximum suppressive activity of frozen CP lasts from 76 to 140 hours and compares well with the activity of plasma stored 24 hours at 4°C. (see data in Table V) and with the activity of fresh undiluted CP (see data in Table III).

by immersing them for 15 to 20 minutes in a saturated solution of naphthol blue black[10] in 10 per cent acetic acid and 3 per cent phenol, rinsed in 10 per cent acetic acid and 3 per cent phenol, and allowed to dry at room temperature.

---

[9] The apparatus used for electrophoresis was patterned after that of Latner (19).

[10] Howe and French, Inc., Laboratory Supply Division, Boston.

Results of Fractionation Studies.—

Electrophoretic analyses (Fig. 2) demonstrated the absence of gamma globulin in fraction IV + V +VI. Conversely, no albumin was present in
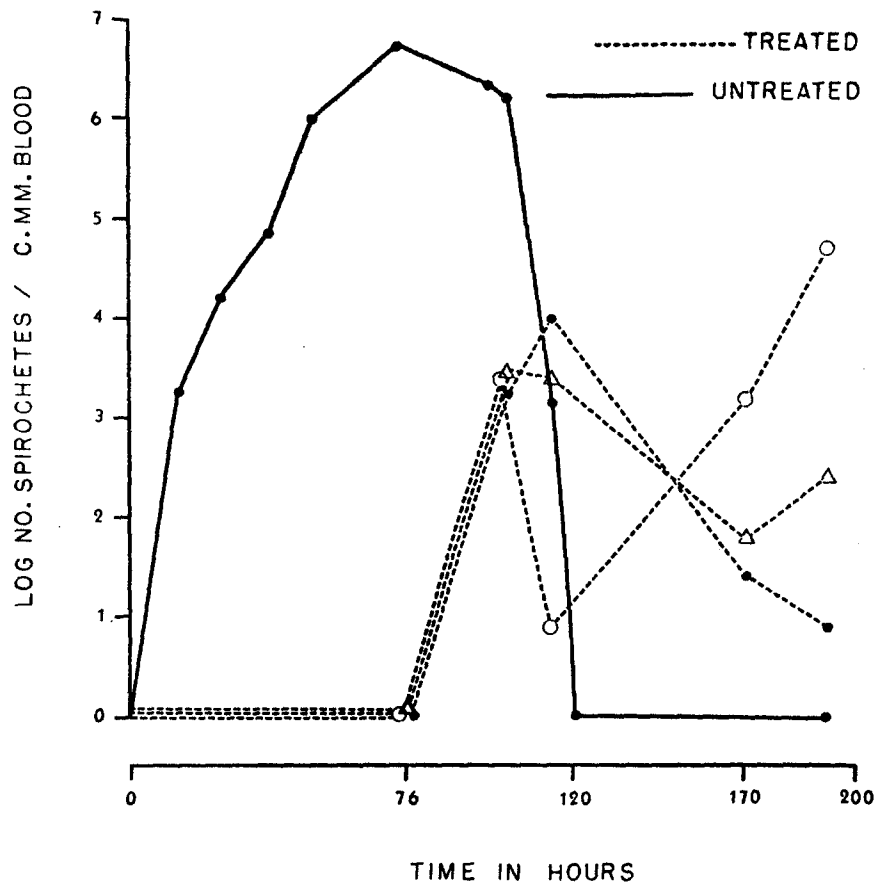

TIME IN HOURS

FIG. 4. The effect of fraction I + II + III from crisis plasma on the course of *B. novyi* infection in rats. The solid curve represents the course of the untreated infection (counts are average counts from four animals, see data in Table V). The broken line curves show the course of the infection in three rats which received this fraction 2 hours after inoculation of spirochetes. They show that this fraction suppresses the infection for 76 hours.

fraction I + II + III. As in human plasma, proteins with the mobility of beta globulins were found in both fractions. Although some of the alpha globulins in human plasma are known to be destroyed by drying fractions from the frozen state, a component with the mobility of alpha₂ globulins (glycoproteins) was present in fraction IV + V + VI as a distinct band.

Bio-assay of Whole Plasma, Fractions I + II + III and Fractions IV + V + VI.—

The dried frozen fraction IV + V + VI was resuspended to plasma volume in distilled water. The dried frozen fraction I + II + III, containing no salts, was resuspended to plasma
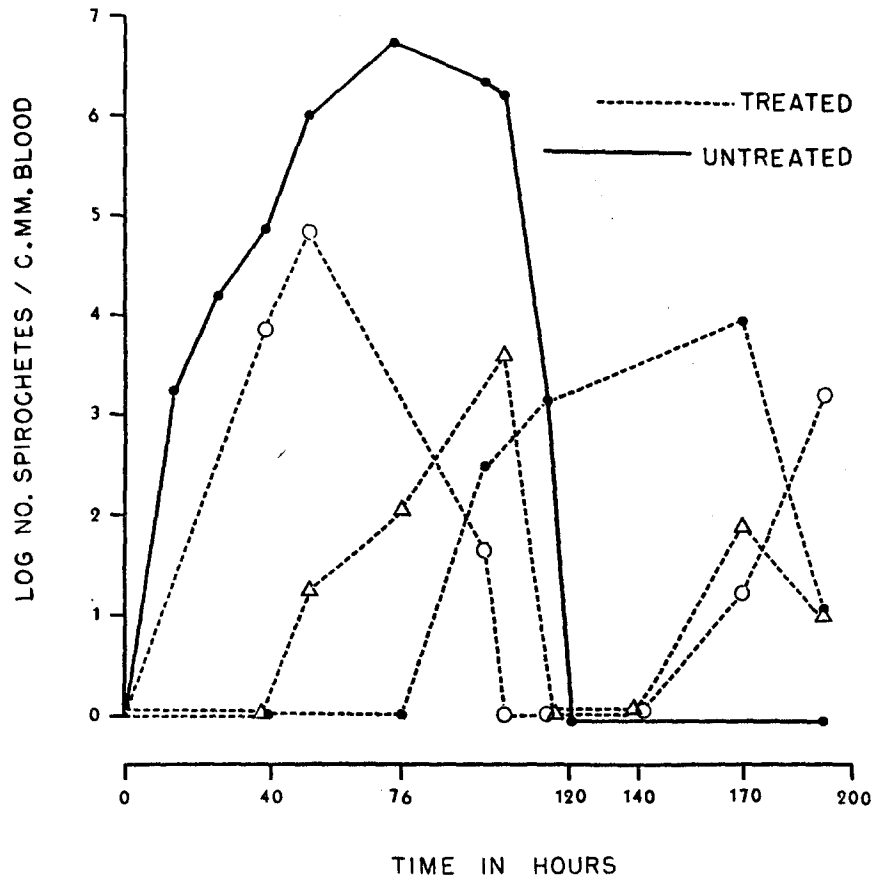

Fig. 5. The effect of fraction IV + V + VI from crisis plasma on *B. novyi* infection in rats. The solid curve represents the course of the untreated infection (counts are average counts from four animals, see data in Table V). The broken line curves show the course of the infection in three rats treated with this fraction 2 hours after inoculation of spirochetes. They show that the magnitude of the spirochetemia is reduced and that the delay in its onset is of variable length, up to a maximum of 76 hours.

volume in phosphate buffer to provide a solution isotonic with blood. These fractions were centrifuged at 4°C. for 30 minutes at 18,000 r.p.m. for the removal of denatured proteins which might be present. Dialysis, therefore, was not considered necessary.

Twenty weanling rats were divided into five groups of four animals each. At 0 hour all received the infective dose of 1 × 10⁸ spirochetes/kg. Two hours later groups 1 and 2 received 10 ml./kg. of fraction I + II + III and fraction IV + V + VI respectively,

dissolved to plasma volume and sterilized by filtration through an ultrafine fritted filter. Group 3 received the same dose of whole plasma, which had been quick frozen and stored at $-55°C$. for 24 hours as a control for the effect of freezing, which is a step in fractional precipitation. Group 4 received whole plasma, stored at 4°C. for 24 hours. Group 5 received no plasma and served as control for the infection.

The results show that freezing to $-55°C$. did not destroy the inhibitor(s), because the suppressive activity of such plasma preparations persisted from 3 to 6 days after injection (Table V, column 3, and Fig. 3), and compares well with the activity of plasma stored 24 hours at 4°C. (Table V, column 4) and with the activity of fresh undiluted crisis plasma (Table III, column 4).

Both fractions were also effective in suppressing the acute spirochetemia, but differed in duration and effectiveness (cf. Fig. 4 with Fig. 5). Fraction I + II + III was about as effective as whole plasma, for the infection was suppressed for 76 hours and at 120 hours the spirochetal counts were only 10 to 10^4/c.mm. (cf. Table V, column 1, with Table III, column 4). Fraction IV + V + VI was less effective, for spirochetes were demonstrable after 14 to 97 hours; but maximal counts were from 10^{-2} to 5×10^{-3} the count of the controls. In terms of potency relative to plasma, this fraction at 76 hours was roughly equal to a 1:4 dilution of whole plasma (cf. Table V, column 2, with Table III, column 3).

DISCUSSION

To our knowledge this study demonstrates for the first time the presence in plasma of the rat at the time of crisis of spirochetal inhibitor(s) which delays the onset of the spirochetemia for at least 76 hours, and reduces its intensity. It is present in fraction I + II + III in greater potency than in fraction IV + V + VI. Since proteins with the mobility of beta globulins did occur in both fractions, their role deserves more attention in the further study necessary to identify the nature of the inhibitor(s).

If the inhibitor(s) is an antibody it is remarkable that the rat should be able to develop it within 76 hours after inoculation of the spirochetes.

SUMMARY

The intensity and duration of *Borrelia novyi* infection in rats depends upon the dose of spirochetes administered. The spirochetemia in response to an inoculum of 1×10^8 spirochetes/kg. characteristically reaches a peak of 4 to 5 million organisms per c.mm. of blood at about 72 hours. Resolution of the primary infection occurs within 48 hours after the peak counts have been observed, with crisis ending at 100 to 120 hours after inoculation.

Treatment of the acute infection, so standardized, by 10 ml./kg. of crisis plasma intraperitoneally delays the onset of the spirochetemia 76 to 140 hours, and reduces the maximal spirochetal count 10^{-3} to 10^{-6} that of the unmodified controls. This is evidence that inhibitor is present in the plasma at the time of crisis and plays a role in limiting the primary infection and subsequent relapses.

The activity of crisis plasma is not destroyed by freezing, or after storage at 4°C. for 4 days. Fractions I + II + III and IV + V+ VI from crisis plasma, obtained by method 10 of Cohn *et al.*, were also effective in suppressing the acute spirochetemia, but differed in duration and effectiveness. The first of these was about as potent as the undiluted whole plasma, and the second about as potent as plasma diluted 1:4.

BIBLIOGRAPHY

1. Norris, C., Pappenheimer, A. M., and Flournoy, T., Study on a spirochete obtained from a case of relapsing fever in man, with Notes on morphology, animal reactions, and attempts at cultivation, *J. Infect. Dis.*, 1906, **3,** 266.
2. Novy, F. G., and Knapp, R. E., Studies on *Spirillum obermeieri* and related organisms, *J. Infect. Dis.*, 1906, **3,** 291.
3. Schuhardt, V. T., The serology of the relapse phenomenon in relapsing fever, *in* A Symposium on Relapsing Fever in the Americas, (F. R. Moulton, editor), Washington, D. C., American Association for the Advancement of Science, Monograph 18, 1942, 58.
4. Kemp, H. A., von Haam, E., Wilton, F. M., and Evans, H. L., Pathology and immunity in American relapsing fever, unpublished observations quoted by the authors *in* A Symposium on Relapsing Fever in the Americas, (F. R. Moulton, editor), Washington, D. C., American Association for the Advancement of Science, Monograph 18, 1942, 117.
5. Davis, G. E., Species unity or plurality of the relapsing fever spirochetes *in* A Symposium on Relapsing Fever in the Americas, (F. R. Moulton, editor), Washington, D. C., American Association for the Advancement of Science, Monograph 18, 1942, 41.
6. Carlisle, R. J., Two cases of relapsing fever; with Notes on the occurrence of this disease throughout the world at the present day, *J. Infect. Dis.*, 1906, **3,** 233.
7. Geiman, Q. M., personal communication, 1954.
8. McKee, R. W., and Geiman, Q. M., unpublished data.
9. Geiman, Q. M., Metabolism of spirochetes, *Ann. Rev. Microb.*, 1952, **6,** 299.
10. Reed, L. J., quoted by Hartman, E., *in* Certain interrelations between plasmodium and its host, *Am. J. Hyg.*, 1927, **7,** 407.
11. Reed, L. J., quoted by Gingrich, W., *in* Immunity to superinfection and cross-immunity in malarial infections, *J. Prev. Med.*, 1932, **6,** 197.
12. Calabi, O., Quantitative studies on the crisis phenomenon in experimental relapsing fever (*Borrelia novyi*), Doctoral Dissertation, Boston, Harvard School of Public Health, 1956.
13. Standard values in blood, (E. C. Albritton, editor), Wakefield, Mass., McGregor and Werner, Inc., United States Air Force Technical Report No. 6039, 1952, 42.

14. Wintrobe, M. M., Clinical Hematology, Philadelphia, Lea and Febiger, 1956, 1129.
15. Surgenor, D. M., 1955, personal communication.
16. Ipsen, J., Appropriate scores in bio-assays using death-times and survivors symptoms, *Biometrics*, 1955, **5**, 465.
17. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, D., Mouton, F. F., Schmid, K., and Urema, E., A system for the separation of components of human blood: quantitative procedures for the separation of the protein components of human plasma, *J. Am. Chem. Soc.*, 1950, **72**, 465.
18. Lever, W. F., Gurd, F. R. N., Urema, E., Brown, R. K., Barnes, B. A., Schmid, K., and Schultz, E. L., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XL. Quantitative separation and determination of the protein components in small amounts of normal human plasma, *J. Clin. Inv.*, 1951, **30**, 99.
19. Latner, A. L., An apparatus for electrophoresis on filter paper, *Biochem. J.*, 1952, **51**, xii (abstract).