Plasma Heparin-Precipitable Fraction in Leprosy Patients[1]

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The heparin-precipitable fraction (HPF) present in low concentrations in the plasma of normal individuals and elevated in some diseases was first characterized and studied by Smith ([15]) and Smith and Von Korff ([16]) in 1957. This fraction was demonstrated to be a "fibrinogen-like" protein that precipitated *in vitro* in cooled heparinized plasma. Smith reported significant increases of HPF in several inflammatory and necrotizing conditions, and more recently, Harville *et al.*([4]) found a correlation between the elevation of HPF and certain disease states involving a necrotizing vasculitis. Varying degrees of vasculitis and cutaneous necrosis are associated with reactions in leprosy ([8, 13, 19]), and for this reason a study of the occurrence of HPF in reactional episodes and its possible association with other phenomena was undertaken.

METHODS AND MATERIALS

Assay of heparin-precipitable fraction (HPF). The methods employed for the semiquantitative and quantitative assays of HPF were essentially those described by Harville *et* al.([4]) Venous blood (5 ml.) drawn from the antecubital vein (or occasionally from another arm vein), was added immediately to 0.1 ml. (100 units) of heparin, and the tube was inverted several times to effect mixing. The plasma was separated by centrifugation at 2,600 rpm at room temperature for 10 minutes. Two ml. of the supernate was then pipetted into a 10 mm. x 75 mm. test tube and placed in a refrigerator at 2°—4°C, usually for 24 hours, but on a few occasions for periods up to 72 hours. At the end of this incubation period, in the preliminary study, visual quantitation without centrifugation was made according to the following scale:

Negative	—No precipitate or only sufficient to provide a thin layer on the bottom of the tube.
Trace	—Precipitate amounting to less than one-fourth of the plasma volume.
1 +	—Precipitate amounting to between one-fourth and one-half of the plasma volume.
2 +	—Precipitate amounting to between one-half and three-fourths of the plasma volume.
3 +	—Precipitate amounting to three-fourths but not the total of the plasma volume.
4 +	—Precipitate occupying the entire plasma volume.

Chemical quantitation was carried out on precipitates obtained by the method described above, as follows. The tube containing the precipitate obtained at the end of the 24 to 72 hour incubation at 2°—4°C was centrifuged in a clinical angle-head centrifuge at 2,200 rpm for 15 minutes at 2°—6°C. After discard of the supernatant plasma, the precipitate was allowed to drain for several minutes. Precipitates were then washed twice with 2 ml. of 0.067 M phosphate buffer (pH 7.4) at 4°C, with care to effect a complete dispersion of the precipitate either by hand stirring or by use of an electrical vortex mixer. All pH values were obtained with a glass electrode using a Photovolt Model 125 meter.

The precipitated protein was assayed by a modified biuret test according to the method described by Henry ([5]). All pre-

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cipitates were first mixed with 2.5 ml. of 0.067 M phosphate buffer (PH 7.4) and incubated at 37°C for about 30 minutes. At this time 2.5 ml. of 6% NaOH and 1 ml. of biuret reagent were added. Occasionally it was necessary to continue the incubation at 37°C for about 30 minutes to obtain complete dissolution of the precipitate before reading in a Klett-Summerson Colorimeter (employing a no. 54 filter) against a biuret blank. A standard reference curve was prepared with a pooled normal human serum obtained from the local population. This pool had been standardized against a standard sheep serum obtained from Mayer and Myles Laboratories, Coopersburg, Pennsylvania, using an American Optical Company TS Meter (a model of the Goldberg Refractometer).

Cryoprecipitate assay. Two ml. of oxalated plasma was incubated at 2°–4°C for 24 hours and the resulting precipitate assayed for protein according to the methods described above for HPF.

Cryoglobulin assay. Two ml. of serum was incubated for 24 hours at 2°–4°C and the resulting precipitate analyzed for protein as described for HPF.

Sedimentation rates. Sedimentation rates were determined by the method of Westergren as described by Diggs ([3]), on freshly drawn oxalated blood (0.1 ml. of 0.1 M ammonium oxalate for each 0.9 ml. blood). Results are reported in millimeters of sedimentation at the end of one hour, without hematocrit correction. (Normal values: 0-15 mm. for men, 0-20 mm. for women.)

Fibrinolytic activity. Buckell's method ([1]) for euglobulin lysis time was employed as a measure of fibrinolytic activity. Blood (4.5 ml.) was collected from an antecubital vein and added to 0.5 ml. of 0.1 M ammonium oxalate. Tourniquet application time to the arm was kept to a minimum, usually amounting to about 30 seconds. The specimen was then kept at 0°–4°C for up to 30 minutes, at which time the plasma was separated by centrifugation at 0°–6°C, immediately frozen, and kept at −20°C overnight. Euglobulin was prepared in duplicate in 15 mm. x 125 mm. centrifuge tubes from 0.5 ml. of freshly thawed plasma by adding 9.4 ml. of distilled de-ionized water and bringing the pH to 5.3 by the addition of 0.1 ml. of 1% acetic acid. After standing at 2°–6°C for 30 minutes, the euglobulin precipitate was collected by centrifugation and the supernatant liquid decanted. The precipitate was drained for about 10 minutes and then dissolved in 0.5 ml. of borate buffer (0.9% NaCl + 0.1% sodium borate) at pH 9.0. This solution was then transferred to 10 mm. x 75 mm. test tubes and, after the addition of 0.5 ml. of 0.025 M $CaCl_2$, the time of clotting was noted. Complete clot lysis time at 37°C was considered as the end point.

Fibrinogen assay. The method of Ware, Guest and Seigers as described by Henry ([6]) was employed. All plasma specimens had been sealed and stored at −20°C for periods of six to nine weeks prior to analysis for fibrinogen. This assay, performed in duplicate, employed 0.2 ml. of oxalated plasma. A mixture of 6 ml. 0.9% NaCl, 0.2 ml. of 0.2 M phosphate buffer (pH 6.4), and 0.1 ml. of 1% $CaCl_2$ was added to the plasma, followed by 0.1 ml. (20 units) of thrombin solution.[3] After mixing, the test tube was allowed to stand for 15 minutes to permit maximum clot formation. The clot was then partially disintegrated by mild vortex mixing, following which the tube was centrifuged at 2,600 rpm for 10 minutes. Clottable protein was readily separated by careful decanting. The residuum was washed once with 5 ml. of distilled water, recentrifuged, and allowed to drain for about 10 minutes. A mixture of 5.0 ml. of 3% NaOH and 1.0 ml. biuret reagent was then added and the tube placed in a 37°C water bath until fibrin dissolution was complete. Colorimetric analyses for protein content were made as described above for HPF, using the same standard curve.

CLINICAL MATERIAL

All data on leprosy patients have been collected from patients in residence at Kivuvu, the leprosarium of the Institut Médical Evangélique, Kimpese, Democratic Republic of Congo. Of the total of 48 individual patients studied all but four

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were BaKongo, with origin in either the Democratic Republic of Congo or Angola. Their estimated age range was 18 to 50 years.

A physician is in full-time residence at this leprosarium and patients in reaction are visited daily. Skin smears from eight sites are taken every three months in positive cases, and the bacillary indices (BI) are graded as outlined by Ridley [12] on a negative to 6 + scale. Morphologic indices (MI) are reported as the percentage of uniformly stained bacilli in the smear. All bacteriologic studies were performed by the same person.

All patients were on sulfone therapy—those with a history of severe and repeated reactions received 10 mgm. DDS six times weekly and all other lepromatous cases 50 mgm. DDS twice or three times weekly. Reactions were treated with various combinations of chloroquine, stibophen, and steroid therapy, with or without discontinuing sulfone.

Twenty-nine patients in the group studied had histopathologic diagnoses made at The Leprosy Study Centre, London, by Dr. D. J. Harman.

RESULTS

1. Studies on HPF

A. Preliminary semiquantitative assay of HPF in leprosy patients. This survey was made on a selection of 24 leprosy patients including the various clinical forms in various stages. Of 18 active lepromatous patients, four in acute reaction had 2 + or 3+ precipitates, two not in reaction had 1+, while the remaining twelve had trace quantities or were negative. Of six nonlepromatous patients, one had a trace of HPF precipitate and five were negative. Two normal persons were negative for HPF. These findings suggested that those cases in acute reaction showed elevations of HPF.

B. Assay of HPF in normal persons. Quantitative assay of HPF in the plasma of 16 normal persons was performed. Five of these were healthy leprosarium staff members and eleven were from unselected "healthy" blood donors at the adjacent general hospital of the Institut Médical Evangélique. This population could be assumed to be representative of that of the leprosy patients as concerns ethnic origin, dietary habits, and the occurrence of intercurrent illnesses. The statistical data on these assays are as follows:

Mean HPF—99 mgm./100 ml. plasma
Range—13 to 203 mgm./100 ml. plasma
Standard Deviation—16.3

This normal mean value of 99 mgm./100 ml. is somewhat below that given by Smith [15] (129 for males, 144 for females) for a generally comparable age group (16-45 years) of persons in the United States.

C. Assay of HPF in patients in reaction. Twenty lepromatous patients were followed with serial determinations of HPF during the course of 22 separate reactional episodes. Pre- and/or postreactional determinations were also made in 21 of these episodes. From Table 1 it may be observed that HPF levels were elevated in all 16 patients in severe reaction, and in two patients with mild reactions. Increased HPF levels were seen in both ENL and neuritic forms.

In four mild ENL reactions the HPF was not significantly elevated, 200 mgm. HPF/100 ml. plasma being considered the upper limit of normal. In one of these, Patient No. 15 (J.G.), no elevation was observed in a second mild ENL reaction, which followed one week after a severe episode of ENL during which HPF levels had reached 763 mgm./100 ml. plasma. Patients No. 17 (T.A.) and No. 20 (N.H.) were of special interest. The ENL in Patient No. 17 was limited to a few nodules that subsided rapidly, and in Patient No. 20 only a bilateral swelling of the pinnae was noted. These two patients at this time showed low fibrinogen levels, 300 mgm./100 ml. and 205 mgm./100 ml. respectively. Patient No. 19 (D.M.) experienced only a few ENL nodules on the arms.

Three cases (Patients No. 9, 11, 12) were biopsied during the acute stage of the reaction. Tissue necrosis was noted in all three and a marked vasculitis was reported in No. 11. All of these patients had HPF levels of greater than 500 mgm./100 ml. at

some time during the reaction.

Although this study was not made to assess the effect of specific drug therapy on the HPF plasma concentration, it should be noted that in Patients No. 5 (T.J.) and No. 13 (K.T.), in severe reaction, the administration of heavy doses of prednisolone may account for the relatively moderate elevations of HPF. However, conversely, Patient No. 10 (N.A.), who had been on long term steroid therapy, showed the highest HPF recorded in this study, 1,370 mgm./100 ml. plasma. To assess the effect of various programs of medical management of HPF levels in reactions would require additional studies directed at this point.

Joint effusions have not received much attention as a manifestation of reactions in leprosy; nonetheless it has been observed among patients here with some degree of

TABLE 1. *Heparin-precipitable fraction levels in plasma of lepromatous patients in reaction.*

Patient	Sex	Type and degree of reaction	Reaction duration (days)	HPF (mgm./100 ml. plasma)		
				Pre-reaction	Reaction[a]	Post-reaction
1 (N.V.)	M	Neuritic, severe	13	60[b]	289, 255	135
2 (P.F.)	M	Neuritic, severe ENL, mild	12	—	310	75, 137
3 (P.S.)	M	Neuritic, severe	11	—	475	70, 128
4 (D.E.)	M	Neuritic, severe	5	—	820	287
5 (T.J.)	M	Neuritic, severe ENL, mild	9	—	245, 260	75
6 (M.G.)	M	Neuritic, severe ENL, severe Iridocyclitis, mild	12	—	330	155
7 (P.C.)	M	ENL, mild Joint effusion, severe	35	—	225, 463	—
8 (M.M.)	M	ENL, severe	18	—	300, 258	137, 90, 131
9 (T.P.)	F	ENL (with skin necrosis), severe	14	—	300, 588	175, 85, 187
10 (N.A.)	M	ENL (with skin necrosis), severe Joint effusion, severe	12	75	1370, 515, 700	212
		ENL, severe Joint effusion, severe	13	212	465, 312, 763	—
11 (N.S.)	F	ENL (with skin necrosis), severe	14	100[b]	450, 515, 225	—
12 (N.N.)	M	ENL (with skin necrosis), severe	56	—	200, 487, 465, 412, 670, 400, 325	87, 155
13 (K.T.)	M	ENL, severe	7	60[b]	195, 212	135
14 (N.S.)	M	ENL, severe	15	—	363, 237	87, 65
15 (J.G.)	M	ENL, severe	14	110	763, 237	130, 105
		ENL, mild	18	105	55, 100, 100	137
16 (N.Se.)	M	ENL, mild	7	80	287	—
17 (T.A.)	M	ENL, mild	5	100[b]	87, 112	88
18 (M.S.)	F	ENL, mild	7	100[b]	131, 240	—
19 (D.M.)	F	ENL, mild	6	—	100, 130	85
20 (N.H.)	F	Swelling of ears, mild	10	—	25, 37, 25	63, 25

[a] Determinations made at approximately evenly spaced intervals throughout the duration of the reactio
[b] Approximate values assigned to semiquantitative determinations.

TABLE 2. *Heparin-precipitable fraction levels in lepromatous patients with high bacterial indices.*

Patient	BI[a]	MI[b] %	HPF (mgm./ml. plasma)
1 (L. M.)	4.85	45	62
2 (M. P.)	4.75	10	135
3 (M. M.)	4.50	50	80
4 (N. S.)	4.50	25	80
5 (B. P.)	4.25	30	27
6 (L. E.)	3.75	15	87
7 (M. Pa.	3.50	3	80
8 (L. Mb.)	3.12	3	87
9 (L. A.)	2.85	30	137
10 (Mv. M.)	2.12	2	105

[a] BI = Bacillary Index (Maximum 6.0, Ridley[12]) Mean: 88.0
[b] MI = Morphologic Index (per cent uniformly staining bacilli).

frequency. In all three episodes reported here with this involvement the HPF was elevated.

D. Assay of HPF in lepromatous patients with high bacterial indices. Even though there was no evidence from the data in Table 1 to indicate that a correlation existed between bacterial positivity *per se* and elevated HPF levels, this relationship was assessed in a separate study (Table 2). All 10 selected cases had been admitted to the leprosarium within the previous nine months and had no discernible history of reactions under our observation, with the exception of Patient No. 8 (L.Mb), who had several episodes of joint effusion during this period, the most recent episode having been four months prior to this assay.

From these data it would seem possible to exclude bacterial positivity as playing a direct rôle in the elevation of plasma HPF. The mean HPF concentration of 88 mgm./100 ml. is not significantly at variance ($P>0.4$) with that of the local normal sample (99 mgm./100 ml.) using the t-distribution analysis.

2. Correlation of HPF and cryoprecipitate in lepromatous patients

Although it was evident from semiquantitative observations on oxalated plasma that cryoprecipitate could account for only a small part of the total HPF, a group of seven patients (9 assays) was studied quantitatively and simultaneously for these two components. From this limited series, the following observations may be made (Table 3); (1) That no correlation exists between high HPF concentrations and cryoprecipitate levels; (2) that no correlation is evident between reactional status and cryoprecipitate levels; and (3) that only a small portion of the total measured HPF could have its origin in the cryoprecipitate fraction *per se*.

Cryoglobulin was not assayed simultaneously on these samples; therefore the composition of the precipitate as to cryoglobulin and cryofibrinogen concentrations cannot be ascertained.

3. Correlation of HPF with cryoglobulin levels

The relatively consistent finding of cryoproteins in the serum of lepromatous patients by Matthews and Trautman ([10]), and its association with a nodose type of cutaneous response to cold by Tilley ([18]), prompted a simultaneous assay of HPF and cryoglobulin in 15 selected patients (12 lepromatous and 3 nonlepromatous). No direct correlation between high HPF levels and cryoglobulin is apparent (Table 4). Cryoglobulin was interestingly absent or present in low titer in the three patients undergoing or recovering from severe ENL reactions. Conversely, Patient No. 9 (M.P.), who had the highest concentration of cryoglobulin, has been singularly free of reaction during the four years of treatment here for active leprosy. Patient No. 14 (L.M.), with 50 mgm. cryoglobulin/100 ml. serum, has had one mild ENL episode during one year of treatment. Although this survey study was not designed to establish this fact, a causal relationship between cryoglobulin and reactional status is not evident in the patients studied nor is it associated with elevated HPF in those patients examined. It must be noted, however, that cryoglobulins were detected in 12 of 15 leprosy patients.

TABLE 3. *Comparison of plasma heparin-precipitable fraction and cryoprecipitate levels.*

Patient	HPF (mgm./100 ml.)	Cryoprecipitate (mgm./100 ml.)	Remarks
1a[a] (N.A.)	700	15	ENL, severe
1b[a]	515	42	ENL, severe
2 (N.S.)	450	0	ENL, severe
3 (N.V.)	289	30	Neuritis, severe
4 (P.F.)	253	12	ENL and neuritis, mild
5 (D.M.)	100	27	ENL, mild
6 (N.Se.)	80	25	Not in reaction
7a[a] (N.H.)	27	10	Not in reaction
7b[a]	25	0	Not in reaction

[a] Separate determinations on the same patient.

TABLE 4. *Comparison of plasma heparin-precipitable fraction and cryoglobulin levels.*

Patient	Sex	Diagnosis	HPF (mgm./100 ml.)	Cryoglobulin (mgm./100 ml.)	Remarks
1 (N.A.)	M	Lepromatous	425	0	Severe ENL
2 (T.P.)	F	Lepromatous	275	15	Severe ENL, recovering
3 (P.C.)	M	Lepromatous	155	6	Severe ENL, recovering
4 (M.M.)	F	Lepromatous	120	17	Not in reaction
5 (M.C.)	F	Lepromatous	107	32	" " "
6 (F.)	M	Borderline	105	33	" " "
7 (L.E.)	M	Lepromatous	100	26	" " "
8 (N.T.)	F	Lepromatous	82	25	" " "
9 (M.P.)	M	Lepromatous	80	105	" " "
10 (K.S.)	M	Tuberculoid	70	15	" " "
11 (K.V.)	M	Tuberculoid	70	0	" " "
12 (N.Nd.)	M	Lepromatous	68	15	" " "
13 (C.G.)	M	Lepromatous	55	17	" " "
14 (L.M.)	F	Lepromatous	37	50	" " "
15 (B.P.)	M	Lepromatous	25	0	" " "

4. Correlation of HPF and fibrinogen in lepromatous patients

A total of 32 patients were examined simultaneously for plasma fibrinogen and HPF levels. The results have been divided into eight arbitrary categories to demonstrate the degree of correlation between the levels of these two plasma fractions (Table 5). High levels of HPF are seen to be associated with increases in fibrinogen concentration. This agrees well with the findings of Smith ([15]) and Harville *et al.* ([4]), whose reports involved patients with inflammatory and necrotizing lesions.

Studies were performed to establish the electrophoretic identity of HPF and fibrinogen fractions in leprosy patients. Four individual plasmas (oxalated) from patients in reaction were studied, using a cellulose polyacetate (Gelman Sepraphore III) supporting medium. Electrophoretic separation was carried out in veronal buffer at pH 8.6 for 75 minutes at 1.5 milliamp. per strip (1″ x 6¾″) in a Gelman Rapid Electrophoresis Chamber (No. 51101) at room temperature (26°–28°C). After electrophoresis the strips were stained with Ponceau S, oven-dried, cleared by the acid-

methanol method, and plotted on the Gelman Manual Scanner. Separate electrophoretic studies were made on HPF obtained from heparinized blood drawn at the same time as the oxalated sample. The HPF was washed twice with 0.067 M phosphate buffer at 4°C as previously outlined and then dissolved in veronal buffer prior to electrophoresis. On comparison of each plasma pattern with that of its corresponding HPF fraction, the major component of the HPF was found to be consistently coincident with the plasma fibrinogen peak. However, variable quantities of protein contaminants were found in the separated HPF; these amounted to between 5 per cent and 20 per cent of the total HPF. The contaminating proteins migrated mainly in the albumin and alpha-2 globulin fractions. Smith and Von Korff ([16]) reported an electrophoretic homogeneity of HPF carried out on paper at 37°C. It is not known whether the electrophoretic heterogeneity reported here is a result of variations in technic or undetermined factors in plasma from leprosy patients.

Although the electrophoretic heterogeneity of the HPF introduces an element of possible error in the assay of total HPF, this would not materially affect the conclusions drawn from the results reported. However, it may provide some explanation, for example, for the exceedingly high HPF of 1370 mgm./100 ml. found in one sample from Patient No. 10 (N.A.) (Table 1). Nevertheless, it should be noted that Smith ([15]) reported similar levels of HPF on occasion.

5. Correlation of HPF with erythrocyte sedimentation rate

A total of 24 simultaneous determinations of HPF and erythrocyte sedimentation rate (ESR) were made on a total of 18 individual bacteriologically active lepromatous patients. The results are depicted in the scattergram of Figure 1. Since all patients had active disease, nearly all the ESR are above normal limits; however, two observations may be made: (1) that HPF levels greater than 200 mgm./100 ml. consistently produced marked ESR elevations, but (2) marked ESR elevations occur regularly in the presence of normal HPF levels. Therefore, a relationship does exist between high HPF levels and elevated ESR, but other factors are also operative, since ESR elevations are common in active lepromatous leprosy not in reaction, at which time the HPF is usually within normal limits.

In the two reactional patients presented in this study with HPF below 200 mgm./100 ml., the reactions were mild and of ENL type.

6. Correlation of HPF with euglobulin lysis time

Simultaneous measurements of euglobulin lysis times and HPF were performed on 23 patients (Fig. 2). A positive correlation between elevated HPF levels and increased lysis time may be observed, but delayed lysis may be seen in a few cases with normal HPF levels.

An analysis of the data on euglobulin lysis time alone reveals the following relationships in the three groups of patients represented here:

A. Six nonlepromatous patients (2 borderline (dimorphous), 4 tuberculoid)—mean 220 minutes; range 125-280 minutes.
B. Eleven lepromatous patients (not in reaction)—mean 287 minutes; range 215-430 minutes.
C. Six lepromatous patients in reaction—mean 449 minutes; range 315-550 minutes. (Two patients, recorded as 550 minutes, showed about 75 per cent lysis of the clot at the end of this period.)

A statistical comparison of the means of the above group by the t-distribution method gave the following results:

Nonlepromatous *vs.* lepromatous not in reaction—$P<0.1$
Lepromatous *vs.* lepromatous in reaction $P<0.05$
Nonlepromatous *vs.* lepromatous in reaction—$P<0.001$

Thus lepromatous patients in reaction demonstrated significant reductions in euglobulin lysis times in comparison with both nonlepromatous patients, and lepromatous patients not in reaction. In the case

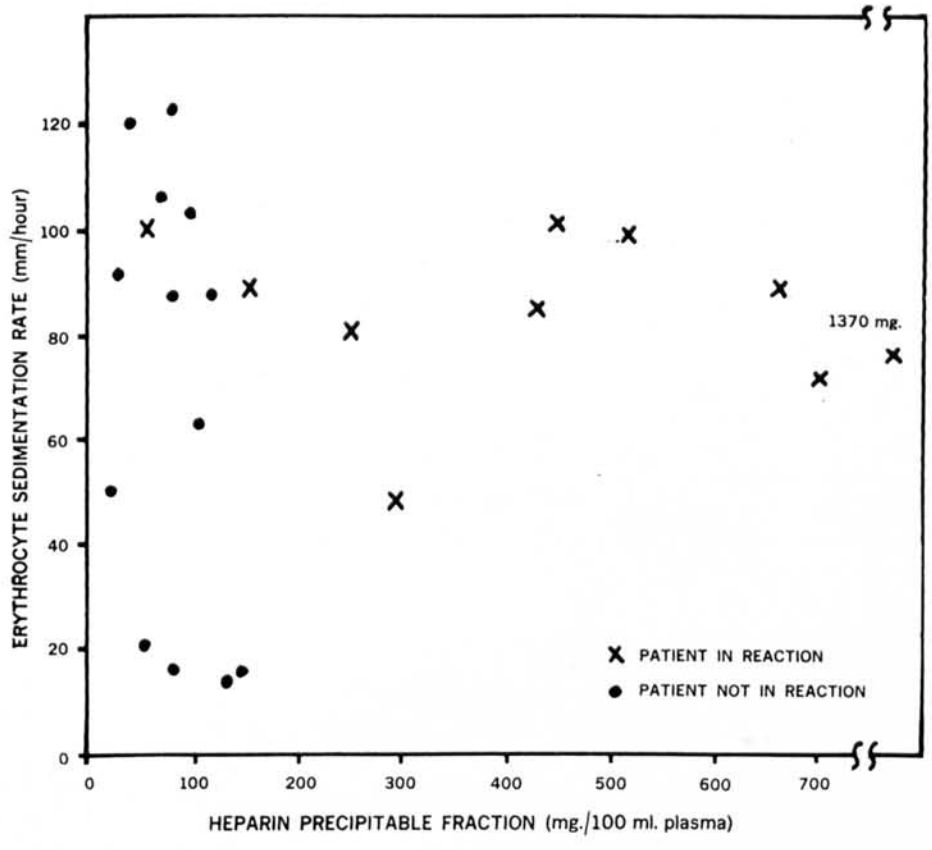

FIG. 1. Correlation scattergram of heparin-precipitable fraction and erythrocyte sedimentation rate.

TABLE 5. *Correlation of plasma heparin-precipitable fraction and fibrinogen in lepromatous patients.*

Number of patients	HPF (mgm./100 ml.)	Fibrinogen (mgm./100 ml.)
1	25	205
13	55–88 (mean 71)	300–595 (mean 422)
8	100–140 (mean 110)	435–705 (mean 522)
3	212–245 (mean 223)	530–845 (mean 725)
2	260–275 (mean 267)	690, 845 (mean 767)
1	312	820
3	400–425 (mean 415)	565–875 (mean 725)
1	475	860
32		

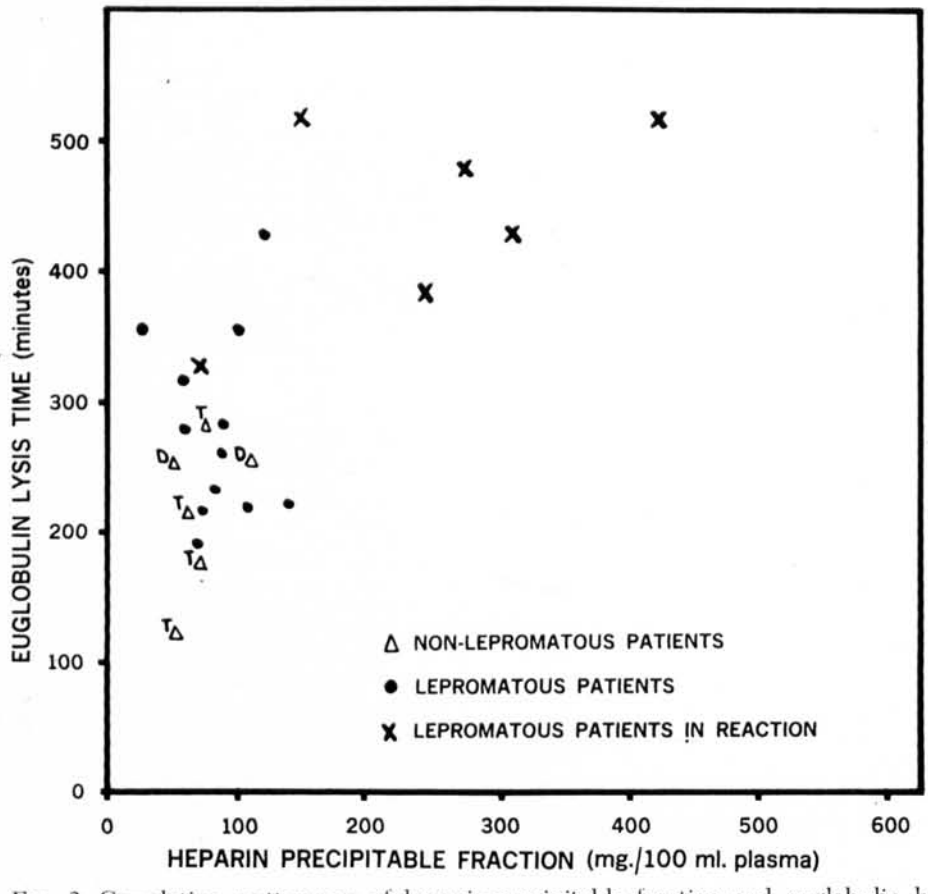

FIG. 2. Correlation scattergram of heparin-precipitable fraction and euglobulin lysis time.

of nonlepromatous *vs.* lepromatous patients not in reaction, the significance of the difference of the means indicates a possible reduction of euglobulin lysis activity in the latter group, making this point worthy of further study.

DISCUSSION

Heparin precipitable fraction is thought to represent fibrin monomers that are bound to circulating fibrinogen and can be produced experimentally by the intravenous perfusion of small amounts of thrombin. On this basis HPF assay has been considered a sensitive measure of thrombin action on fibrinogen *in vivo* [9]. An increased concentration of HPF may therefore probably be closely identified with abnormal intravascular coagulation or fibrin formation, rather than a general or unspecified response to inflammation [14].

It may be reasoned that the marked increases in HPF found rather consistently in leprosy patients undergoing reactional episodes have their source in an "allergic" vasculitis that provokes intravascular fibrin formation. Vasculitis has been observed with some frequency in biopsied ENL lesions [8, 13, 19], and tissue necrosis is a well established sequela in some leprosy reactions. In the patients reported here, acute panniculitis and tissue necrosis have been accompanied by high HPF levels.

It has been generally considered that in reactional episodes in leprosy pathologic changes have been largely limited to a few anatomic regions, *e.g.*, skin, subcutaneous tissue, and nerves. Moschella [11], for example, was unable to establish the presence of a systemic vasculitis in a study of six leprosy patients in reaction with resulting cutaneous necrosis. Since Harville *et al.* [4]

reported that cutaneous vasculitis did not produce elevations in HPF concentration unless accompanied by a systemic vasculitis, some explanation must be offered for the observed increase of HPF in leprosy reactions. Either changes other than vasculitis taking place in the skin and nerves of leprosy patients produce increased HPF levels, or there is a systemic vasculitis that has remained thus far undetected.

The finding that reactions apparently clinically confined to nerves were accompanied by increased HPF levels is of interest. Histopathologic studies on neural tissue in reaction in leprosy have of necessity not been extensive; however, Callaway *et al.* ([2]) made no comment on vasculitis in a study of 100 cases in which small branches of major nerves (usually the ulnar) were biopsied at surgery. HPF increases could point to a neural vasculitis in the pathogenesis of neuritic leprosy reactions, but this obviously remains to be confirmed.

Observed diminutions in fibrinolytic activity in patients undergoing reactions present other interesting considerations. The fibrinolytic system functions as an efficient mechanism for the rapid removal of formed fibrin. Whether or not impaired fibrinolysis in reactions is a primary or secondary event has not been established, but it may contribute to increased fibrin deposition, which could interfere with cutaneous and neural microcirculation, provoking tissue ischemia, hypoxia, and necrosis.

Waters and Ridley ([19]) commented on the presence of fibrinoid deposits in and about blood vessels in necrotizing reactions in leprosy. The relationship of HPF increases and fibrinoid deposition in cutaneous vasculitis is not clear. The occurrence of these two factors seems to be correlated in the generalized Shwartzman reaction ([17]), but Harville *et al.*([4]) could not establish such an association in cutaneous vasculitis.

The etiology of secondary amyloidosis in leprosy remains obscure, and although the experimental data presented here do not bear directly on this point, some speculation may be offered as to possible relevance of the findings. One theory holds that amyloid deposits are derived from any plasma protein present in abnormally high concentration (*e.g.*, hypergammaglobulinemia). Hyperfibrinogenemia has been proposed by Horowitz *et al.*([7]) as one possible cause of secondary amyloidosis. In this study, employing immunofluorescent technics on a case of familial Mediterranean fever with marked hyperfibrinogenemia, a preponderance of fibrinogen was found in the amyloid deposits. Since repeated or prolonged reactional states in leprosy seem to lead to an increased incidence of amyloid disease, it may be that the presence of elevated fibrinogen and HPF occurring during these episodes could result in amyloid formation. The deposition of fibrin and/or HPF could be enhanced by the observed impaired fibrinolysis and possibly by disturbances in the reticuloendothelial phagocytosis of these aggregated proteins. Such deposits may eventually be transformed into amyloid.

Whether or not the alterations reported in this communication present a new rationale in the therapy of reactions has not been established. Further investigations on the coagulation and fibrinolytic systems in leprosy are necessary before this can be assessed.

SUMMARY

The concentration of heparin-precipitable fraction (HPF) in the plasma of lepromatous patients undergoing reactions of both erythema nodosum and neuritic forms is increased. This elevation is usually accompanied by prolonged euglobulin lysis times, increases in erythrocyte sedimentation rate, and elevated plasma fibrinogen concentrations. Possible relationships between HPF levels, vasculitis, and intravascular coagulation are discussed.

RESUMEN

La concentración de la fracción heparina-precipitable (HPF) en el plasma de enfermos lepromatosos que experimentaron reacciones de erythema nodosum y formas neuríticas, está aumentada. Esta alza está corrientemente acompañada por una prolongación del tiempo de lysis de euglobulina, aumento de la tasa

de sedimentación de eritrocitos, y elevación en la concentración del fibrinógeno del plasma. Se discute una posible relación entre niveles de HPF, vasculitis, y coagulación intravascular.

RÉSUMÉ

La concentration de la fraction précipitable par l'héparine (HPF) est augmentée dans le plasma des malades lépromateux présentant des réactions, tant formes névritiques qu'érythéme noueux. Cette augmentation est généralement accompagnée par une prolongation des temps de lyse des euglobulines, une augmentation de la vitesse de sédimentation des érythrocytes, et par un accroissement des concentrations plasmatiques en fibrinogène. On discute des relations possibles entre les niveaux de HPF, la vasculite, et la coagulation intravasculaire.

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