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Impairment of Plasma Fibrinolytic Activity in Leprosy Patients[1,2]

Wayne M. Meyers[3]

In a previous study ([14]) on the plasma heparin-precipitable fraction in leprosy patients, limited observations were made on alterations in euglobulin lysis times in the various clinical forms of leprosy and in patients undergoing reactions. Lepromatous patients not undergoing reaction showed mild reductions of plasma fibrinolytic activity, while those in reaction exhibited severe impairment. Audier ([2]), working in Congo, has recently presented data which tend to support these findings; however, Izaki and Kon ([12]), in a preliminary report from Japan, state that lepromatous patients with erythema nodosum leprosum (ENL) show marked increases in fibrinolytic activity in blood and affected tissue.

Fibrinolysis is an important homeostatic property in blood and other tissues, and the normal function of this mechanism is dependent upon the controlled regulation of the plasminogen-plasmin system through the action of activators and inhibitors ([18]). The resulting effective plasmin activity is a measure of fibrin digesting capability. Physiologic fibrinolysis serves to remove local fibrin deposits which occur in the healthy individual or in tissue repair. Pathologic enhancement of this activity may produce serious bleeding disorders either as a primary event or subsequent to disseminated intravascular coagulation. Impaired fibrinolysis has been related to progressive thrombosis in a few instances, and it has been suggested that a deficiency of plasminogen activator may exist in hyaline membrane disease of the newborn. The studies being reported here indicate that an impaired blood fibrinolysis exists in nontuberculoid forms of leprosy and that during reactional episodes there is a marked enhancement of this impairment. An attempt will be made to correlate this finding with the vascular involvement frequently observed in leprosy, and to compare it with changes seen in rheumatoid arthritis and cutaneous vasculitis.

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3 W. M. Meyers, M.D., Ph.D. Presently: Leonard Wood Memorial, National Institutes of Health Fellow in Research Pathology of Leprosy, Special Mycobacterial Diseases Branch, Armed Forces Institute of Pathology, Washington, D.C. 20305. *Permanent address:* Kivuvu Leprosarium, Institut Médical Evangélique, Kimpese via Kinshasa, Democratic Republic of Congo.

TABLE 1. *Euglobulin lysis times—controls and leprosy patients.*

Group	Individuals			Mean euglobulin lysis time (min.)			Range (min.)			Standard deviation of mean (min.)		
	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female
I. Controls	33	15	18	128.3	125.6	130.5	64–228	88–217	64–288	38.2	31.2	44.0
II. Nonlepromatous	20	13	7	190.0	187.7	194.3	98–307	82–251	118–307	63.4	63.5	68.0
III. Lepromatous	35	22	13	267.4	251.6	294.1	148–410	156–338	148–410	64.6	54.9	73.0
IV. Lepromatous in reaction	15	12	3	503.3	499.7	517.7	330–860	330–860	450–603	160.7	174.0	78.0

CLINICAL INFORMATION

The patients studied were under treatment at Kivuvu, the leprosarium of the Institut Médical Evangélique, Kimpese, Democratic Republic of the Congo. The controls and all but two patients were of the Bakongo tribe(s) with origin in either Congo or Angola. Estimated age limits were 18-45 years with sex distribution as indicated in Table I. The normal controls were spouses of leprosy patients and leprosarium employees or members of their families. Leprosy was excluded from the controls on the basis of a physical examination. Since the leprosarium is responsible for the medical care of both the patients and controls, a substantial medical history is available, and it may be assumed that, apart from leprosy and DDS therapy, both groups are comparable as concerns intercurrent diseases and diet. The diagnosis of leprosy is routinely based on clinical and histopathologic findings.

All leprosy patients were receiving DDS during the time of this study. Those with a history of reactions received 10 mgm. six times weekly, and the remaining patients 50 mgm. twice or thrice weekly.

MATERIALS AND METHODS

Fibrinolytic activity was determined using the euglobulin lysis time method of Buckell ([4]). Blood (4.5 ml.) was collected from the antecubital vein in a plastic disposable syringe, and added to 0.5 ml. of 0.1 M ammonium oxalate in a conical glass centrifuge tube. All samples were collected 7 a.m. and 9 a.m. following an approximative 12-hour fasting period. Total tourniquet application time to the arm did not exceed 30 seconds. Immediately after anticoagulation, the blood was placed in the refrigerator at about 4°C until euglobulin precipitation was performed (within 30 minutes). Plasma was separated by centrifugation at 2,600 rpm for 10 minutes at room temperature in a clinical angle-head centrifuge. Euglobulin was prepared in duplicate in 15 mm. x 125 mm. centrifuge tubes from 0.5 ml. of plasma by the addition of 9.4 ml. chilled, distilled de-ionized water and by adjusting the pH to 5.3 with 0.1 ml. of 1 per cent acetic acid. After

standing at 4°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. serologic tubes and, after the addition of 0.5 ml. of 0.025 M $CaCl_2$ the time of clotting was noted. The tubes were then placed in a water bath at 37° ± 0.5°C and observed for complete clot lysis time. Apart from the gentle raising of the test tube rack out of the bath for clot examination (approximately every 10 minutes) the test solutions were not agitated.

RESULTS

Euglobulin lysis times in controls (Group I), nonlepromatous (4 tuberculoid, 16 borderline) (Group II), lepromatous not in reaction (Group III), and lepromatous in reaction (Group IV) are presented in Table 1.

The grouping of patients was established using the Ridley and Jopling [17] scheme of classification based on clinical, bacteriologic, hypersensitivity (lepromin reaction), and histopathologic criteria. All tuberculoid and lepromatous patients were designated (TT) and (LL) respectively, and the borderline (dimorphous) patients were distributed in Group II as follows: (BT) 5, (BB) 8, and (BL) 3.

Four of the Group III patients were bacteriologically negative at the time of the test, and five had a history of reaction, but not within one month prior to fibrinolysis assay. Three patients are represented in both Group III and Group IV. The 15 observations in Group IV were made on 10 different individuals, eight observations having been made on separate reactional episodes in three individuals.

Statistical comparison using an analysis of variance method were made on all four groups (Table 2). Within all groups, no sex effect was observed. Highly significant prolongations of euglobulin lysis times were observed on comparing all test groups with the controls and in the successively paired comparisons of Group II, III and IV. The p-values > 0.0005 indicate that the odds against the chance occurrence of such differences as found here are 1,999 to 1.

TABLE 2. *Statistical analysis of variance in euglobulin lysis times in controls and leprosy patients.*

Comparison	Variance ratio	Significance
Groups[a] I, II, III, IV	87.165	$p > .0005$
Groups I, II, III	58.282	$p > .0005$
Groups I, II, IV	106.672	$p > .0005$
Groups I, III, IV	115.411	$p > .0005$
Groups II, III, IV	56.437	$p > .0005$
Groups I, II	20.557	$p > .0005$
Groups I, II-T[b]	0.760	N.S.
Groups I, III	1,287.712	$p > .0005$
Groups I, IV	1,742.740	$p > .0005$
Groups II, III	24.003	$p > .0005$
Groups II, IV	67.750	$p > .0005$
Groups III, IV	60.875	$p > .0005$
Groups I-M[c], I-F[d]	0.138	$p > 0.5$
Groups II-M, II-F	0.005	$p < 0.5$
Groups III-M, III-F	4.063	$0.90 < p < 0.5$
Groups IV-M, IV-F	0.032	N.S.

[a] Group I—Controls; Group II—Nonlepromatous; Group III—Lepromatous; and Group IV—Lepromatous in reaction.
[b] 4 tuberculoid patients within the nonlepromatous group.
[c] M = males.
[d] F = females.

When the four tuberculoid patients (TT) of Group II were compared separately with the controls, no significant variation was detected. This finding may be explained by the fact that the total volume of involved tissue in these tuberculoid patients was less than in those with borderline forms. Alternatively, it may be that the "lepromatous component" of borderline leprosy is the determining factor.

In considering the impaired fibrinolytic activity seen during reactional episodes in lepromatous patients, several points may be emphasized. Plasma samples from three

patients with ENL were examined early in the reaction (the first morning of symptoms) and showed lysis times of 830, 524, and 381 minutes, indicating that in these cases an early increase in plasma fibrinolytic activity did not occur as had been described by Izaki and Kon [12] in Japan. Three patients with symptoms clinically limited to neuritis gave lysis times of 461, 364, and 348 minutes. Reactions in three patients accompanied by severe generalized edema and joint effusions demonstrated the longest lysis times recorded. One of these showed only 50 per cent lysis at 836 minutes (not included in the tabulated data for statistical reasons) and the two others at 860 and 830 minutes. Serial determinations were made on eight patients and the results are recorded in Table 3. Subjects 1-5 were followed through the same reactional episode while subject six had isolated assays done in reaction and not in reaction.

Since Fearnley *et al.* [8] reported some correction of impaired fibrinolytic activity in rheumatoid arthritis by phenformin therapy, this approach was attempted on two patients in acute severe reaction (Table 3). High dosages of phenformin (DBI-TD 50 mgm. t.i.d.) accompanied by prednisolone therapy (22.5 mgm. daily) resulted in a very rapid resolution of reactional symptoms in two patients. Previous observations in a controlled study had suggested that phenformin at lower dosages (DBI-TD 50 mgm. q.d. or b.i.d.) was probably ineffectual in preventing reactions when given over periods of up to two months. In these two patients the resolution of the joint effusions and marked edema was accompanied by a return to normal euglobulin lysis times. This degree of response had not been seen during previous similar reactional episodes in the same two patients with prednisolone therapy alone, and may indicate a salutary effect of phen-

TABLE 3. *Serial observations on euglobulin lysis times on patients in reaction.*

Patient	Date	Symptoms	Euglobulin lysis times (min.)
1. KT	9/7/68	ENL, joint effusion, edema—severe	836 (50% lysis)
	18/7/68	Marked improvement (steroid therapy)	104
2. TP	9/7/68	ENL—severe	603
	11/7/68	ENL—severe (prednisolone)	500
	18/7/68	ENL subsiding (prednisolone)	189
3. TL	9/7/68	ENL—edema—mild	445
	13/7/68	Reaction subsided (aspirin and chloroquine)	200
4. JG	26/5/68	ENL, moderate	381
	29/5/68	ENL unchanged (aspirin and chloroquine)	382
	5/6/68	Reaction subsided	189
5. PS	12/7/68	Neuritis, moderate	364
	20/7/68	Not in reaction	156
6. NN	27/5/68	ENL, severe	524
	18/7/68	Not in reaction	203
7. NA	15/7/68	ENL, joint effusion, edema—severe	766
	18/7/68	Reaction nearly terminated (DBI 50 mgm. t.i.d. + prednisolone 22.5 mgm.)	211
8. PC	15/7/68	ENL, joint effusion, edema—severe	820
	18/7/68	Reaction much improved (DBI 50 mgm. t.i.d. + prednisolone 22.5 mgm.)	127

TABLE 4. *Effect of DDS on euglobulin lysis time.*

DDS in test (μgm./ml.)	Euglobulin lysis time (Min.)
8	163
16	155
24	149
0	160

formin when used in this manner. Additional studies to establish or refute the therapeutic efficacy of phenformin in reactions are planned.

The only known variable, other than leprosy, between the controls and the patients was DDS therapy, hence the possible effect of this drug on euglogulin lysis time was determined (Table 4). Crystalline DDS was dissolved in distilled water and incorporated into the euglobulin clot lysis assay employing a pooled plasma sample from normal controls. No significant drug effect was noted and it may be assumed that DDS does not alter euglobulin lysis times *in vitro* at up to 4-8 times usual therapeutic blood levels. Control patients treated with DDS prior to euglobulin lysis assay were not studied.

DISCUSSION

In this study the prolonged euglobulin lysis times previously noted (14) in lepromatous leprosy patients in reaction have been confirmed in an enlarged survey. Less pronounced impairments of fibrinolysis were also found in lepromatous and borderline forms of the disease without clinical evidence of reaction. At this time a precise explanation cannot be offered for these findings, since disease states involving reductions in fibrinolytic activity are not well documented in the literature (18). This is especially true of nonthrombotic diseases; however, prolongations of fibrinolysis times have been noted in cutaneous vasculitis (7) and rheumatoid arthritis (5).

Harville *et al.* (11) were able to correlate elevations in plasma heparin-precipitable reaction (HPF) and fibrinogen levels in necrotizing vasculitis and Cotton and Johnson (6) have found a similar association in rheumatoid arthritis. These findings, coupled with those of Cunliffe (7) and Chakrabarti *et al.* (5), suggest a relationship between impaired blood fibrinolytic activity and increases of HPF and fibrinogen concentrations in these two conditions. The prolonged euglobulin lysis times being reported here, especially with regard to acute reactional states, combined with the previous findings (14) of elevated plasma HPF and fibrinogen concentrations among essentially the same patient population would suggest that a similar triad of findings can exist in leprosy.

Whether there is a common denominator in the pathology of these disease states or not, or if a causal relationship exists between reduced fibrinolysis and elevated HPF and fibrinogen concentrations has not been established. It is possible that the vascular damage occurring in reactional episodes in leprosy, in rheumatoid arthritis, and in cutaneous vasculitis is an element common to all three. If so, then a certain degree of vasculitis could be anticipated in borderline (dimorphous) and lepromatous patients not in reaction, to explain the impaired fibrinolysis occurring in these forms. Such vascular changes in lepromatous leprosy are well known (9). Considerable numbers of bacilli are often seen in all layers of the vessels including the endothelium, and may be found in the small arteries, arterioles, capillaries, and venules. Endothelial swelling and intimal thickening may not only seriously compromise the hemodynamics of the skin locally, but may play a role in altering circulating plasminogen activator levels.

Plasminogen activators serve to activate the enzyme precursor plasminogen to form plasmin which is capable of digesting fibrinogen and fibrin. Such activators are found in trace amounts in body fluids, in urine (urokinase), and in many body tissues (18). The tissue activator is apparently most abundant in lysosomal granules and in vascular endothelium. Activator from the latter source probably plays a more important role in controlling blood fibrinolytic activity than the lysosomal source as it is more soluble and can be readily released into the blood stream. Since lepromatous

leprosy and even borderline (dimorphous) forms can involve extensive amounts of body tissue, it is conceivable that the activator supply could be compromised even in uncomplicated forms of leprosy, and the superimposition of an acute vasculitis in reactional states may aggravate this deficiency profoundly.

It must be emphasized that this explanation is only speculative. Considerable investigation on the actual levels of plasminogen activator in the blood and tissues of leprosy patients needs to be carried out to assess the role of this factor in the observed impairments of fibrinolysis. The same may be said for other factors which regulate the fibrinolytic mechanism, such as plasminogen levels and plasmin inhibitor concentrations.

Recently Goodwin [10] reported the therapeutic efficacy of Arlef (flufenamic acid) in the treatment of reactions in leprosy; however, no mechanism apart from an anti-inflammatory action was suggested. This drug is know to be a potent inducer of fibrinolytic activity in human plasma and operates through the inactivation of inhibitors of the fibrinolytic system. It also suppressses complement activity particularly the C'3 component, or B,C-globulin [1]. Possible relationships between these activities of flufenamic acid and the reported therapeutic effect in leprosy remain to be investigated.

Major vessel thrombosis in leprosy patients is not known to occur with abnormal frequency, but the increased fibrinogen concentrations and decreased fibrinolysis would be expected to predispose to such an event. A similar set of circumstances along with an increased antiheparin activity exists in rheumatoid arthritis where thrombosis of large vessels is likewise not enhanced. Cotton and Johnson [6] have postulated that in rheumatoid arthritis a potentiation of the reticuloendothelial phagocytic capacity may account for the removal of fibrinogen-fibrin intermediates, thus minimizing the expected tendency to thrombosis. The relevancy of this concept to the leprosy patient has not been established.

No definitive explanation can be offered for the opposing nature of the findings of Izaki and Kon [12], who studied Japanese patients, and those reported here on Bantu patients. Methods differed somewhat, but these differences are not thought to account for the disagreement. Ethnic origin may have contributed to the variations noted since comparisons of the Bantu and Caucasians [13] and Melanesians and Caucasians [3] have revealed differences in blood fibrinolysis capability. South African Bantus exhibit significant increases in fibrinolytic activity over South African Caucasians, as a result of elevated levels of plasminogen activator. (Preliminary comparisons of the Bakongo Bantu and Caucasians living in Congo indicates that the same situation may exist in the population where this present study was performed [16]. New Guinea Melanesians were found also to possess a more active fibrinolytic system than Caucasians residing locally and this was explained on the basis of a decreased fibrinolysin inhibition and "increased and accelerated" fibrinolysin activation. The literature is not known to contain any reports on comparative fibrinolytic activities in the Japanese and Bantu ethnic groups, thus no valid comment can be made on the relevance of this as a factor in explaining the differences in the experimental findings of Izaki and Kon [12] and those reported in this communication. However, it is important to recall that the clinical manifestations of leprosy differ strikingly from one geographic area to another.

SUMMARY

Euglobulin lysis times have been studied in the following groups of leprosy patients: nonlepromatous, lepromatous not in reaction, and lepromatous in reaction. Compared with healthy controls, all three groups showed highly significant impairments of fibrinolysis. Nonlepromatous patients showed the mildest impairments and lepromatous patients in reaction the most marked. Possible relationships between this derangement and vascular involvement in leprosy are discussed, especially with regard to certain similarities observed by others in rheumatical arthritis and cutaneous vasculitis.

RESUMEN

Se estudió el tiempo de lisis de euglobulina en los siguientes grupos de enfermos: no lepromatosos, lepromatosos sin reacción y lepromatosos con reacción. Al compararlos con controles sanos, los tres grupos mostraron trastornos de la fibrinolisis altamente significativos. Los enfermos no lepromatosos mostraron los trastornos menos acentuados y los lepromatosos en reacción los más marcados. Se discute la posible interelación entre este trastorno y el ataque vascular en la lepra, especialmente con respecto a ciertas ciertas similitudes observadas por otros en artritis reumatoide y vasculitis cutánea.

RÉSUMÉ

L'auteur a étudié les temps de lyse euglobulinique chez des groupes de lépreux atteints des forms suivantes de la maladie; lèpre nonlèpromateuse, lèpre lépromateuse en rémission, lèpre lèpromateuse en évolution. Les trois groupes ont montré une diminution hautement significative de la fibrinolyse par rapport au groupes tèmoin. Les malades nonlépromateuse ont présenté les diminutions les plus faibles, tandis que les lépromateux en évolution présentaient les diminutions les plus élevées des relations possibles entre cette anomalie et l'implication du système vasculaire dans la lèpre sont discutées, spécialement en ce qui concerne certaines similitudes observées par d'autres auteurs dans l'arthrite rhumatoide et la vasculite cutanée.

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