Impairment of Plasma Fibrinolytic Activity in Leprosy Patients

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In a previous study (1) on the plasma heparin-precipitable fraction in leprosy patients, limited observations were made on alterations in eglobulin 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, Izuki and Kon (12), in a preliminary report from Japan, state that lepromatous patients with erythema nodosum lepromatosum (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 nontuberculous 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.
The patients studied were under treatment at Kivuvu, the leprosarium of the Institut Medical Evangelique, Kinshasa, Democratic Republic of the Congo. The controls and all but two patients were of the Baluba 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 approximate 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 2,000 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 5.5 with 0.1 ml. of 1 per cent acetic acid. After
standing at 4°C for 30 minutes the euglobulin precipitate was collected by centrifuga-

tion and the supernatant liquid decen-
dted. The precipitate was drained for

about 10 minutes and then dissolved in 0.5

ml. of borate buffer (0.06 M 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 CaCl₂, the time of clotting

was noted. The tubes were then placed in a

water bath at 37°C ± 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 tuberculous, 16 bor-
derline) (Group II), lepromatous not in

reaction (Group III), and lepromatous in

reaction (Group IV) are presented in Ta-

tle 1.

The grouping of patients was established

using the Ridley and Jopling (17) scheme

of classification based on clinical, bacte-

riologic, hypersensitivity (lepromin reac-

tion), and histopathologic criteria. All tuberculous

and lepromatous patients were designated

(TT) and (LL) respectively, and the bor-
derline line (dimorphous) patients were dis-

tributed in Group II as follows: (TT) 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 pro-

longations 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 dif-

ferences as found here are 1,999 to 1.

When the four tuberculoid patients

(TT) of Group II were compared sepa-

rately with the controls, no significant var-

iation was detected. This finding may be

explained by the fact that the total volume

of involved tissue in these tuberculoid pa-

tients was less than in those with border-

line forms. Alternatively, it may be that the

"lepromatous component" of borderline lep-

dray 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

| Table 2. Statistical analysis of variance in euglobulin lysis times in controls and leprosy patients. |
|---|---|---|
| Comparison | Variance ratio | Significance |
| Groups I, II, III, IV | 87.165 | p > 0.0005 |
| Groups I, II | 38.262 | p > 0.0005 |
| Groups I, III, IV | 38.262 | p > 0.0005 |
| Groups I, III | 115.411 | p > 0.0005 |
| Groups II, III, IV | 56.437 | p > 0.0005 |
| Groups I, II | 29.552 | p > 0.0005 |
| Groups I, II, TT | 0.760 | N.S. |
| Groups I, III | 1,287.712 | p > 0.0005 |
| Groups I, IV | 24.003 | p > 0.0005 |
| Groups II, III | 47.730 | p > 0.0005 |
| Groups III, IV | 60.875 | p > 0.0005 |
| Groups I, M- | 0.138 | p > 0.5 |
| Groups I, M- | 0.005 | p < 0.5 |
| Groups III, M-F, I-M | 4.063 | 0.90 < p < 0.5 |
| Groups IV-M, F | 0.632 | N.S. |

* Group I—Controls; Group II—Nonlepromatous; Group III—Lepromatous; and Group IV—Lepromatous in reaction.
* M = males.
* F = females.
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 800 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. \(^{(4)}\) 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 pheno-

### Table 3. Serial observations on euglobulin lysis times on patients in reaction.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Symptoms</th>
<th>Euglobulin lysis times (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. KT</td>
<td>9/7/68</td>
<td>ENL, joint effusion, edema—severe</td>
<td>836 (50% lysis)</td>
</tr>
<tr>
<td></td>
<td>18/7/68</td>
<td>Marked improvement (steroid therapy)</td>
<td>104</td>
</tr>
<tr>
<td>2. TP</td>
<td>9/7/68</td>
<td>ENL—severe</td>
<td>603</td>
</tr>
<tr>
<td></td>
<td>11/7/68</td>
<td>ENL—severe (predni­solone)</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>18/7/68</td>
<td>ENL subsiding (predni­solone)</td>
<td>189</td>
</tr>
<tr>
<td>3. TL</td>
<td>9/7/68</td>
<td>ENL—edema—mild</td>
<td>445</td>
</tr>
<tr>
<td></td>
<td>13/7/68</td>
<td>Reaction subsided (aspirin and chloro­quine)</td>
<td>200</td>
</tr>
<tr>
<td>4. JG</td>
<td>26/5/68</td>
<td>ENL, moderate</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>29/5/68</td>
<td>ENL, unchanged (aspirin and chloro­quine)</td>
<td>382</td>
</tr>
<tr>
<td>5. PS</td>
<td>5/6/68</td>
<td>Reaction subsided</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td>12/7/68</td>
<td>Neuritis, moderate</td>
<td>364</td>
</tr>
<tr>
<td>6. NN</td>
<td>30/7/68</td>
<td>Not in reaction</td>
<td>156</td>
</tr>
<tr>
<td>7. NA</td>
<td>18/7/68</td>
<td>ENL, severe</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>27/5/68</td>
<td>Not in reaction</td>
<td>203</td>
</tr>
<tr>
<td>8. PC</td>
<td>15/7/68</td>
<td>ENL, joint effusion, edema—severe</td>
<td>766</td>
</tr>
<tr>
<td></td>
<td>18/7/68</td>
<td>Reaction nearly terminated (DBI 50 mgm. t.i.d. + predni­solone 22.5 mgm.)</td>
<td>211</td>
</tr>
</tbody>
</table>
TABLE 4. Effect of DDS on euglobulin lysis time.

<table>
<thead>
<tr>
<th>DDS in test (µg/ml)</th>
<th>Euglobulin lysis time (Min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>163</td>
</tr>
<tr>
<td>16</td>
<td>155</td>
</tr>
<tr>
<td>24</td>
<td>149</td>
</tr>
<tr>
<td>0</td>
<td>160</td>
</tr>
</tbody>
</table>

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 euglobulin 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 (14) and rheumatoid arthritis (14).

Harville et al. (13) were able to correlate elevations in plasma heparin-precipitable reaction (HPF) and fibrinogen levels in necrotizing vasculitis and Cotton and Johnson (9) have found a similar association in rheumatoid arthritis. These findings, coupled with those of Cunliffe (1) and Chakrabarti et al. (9), 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 reactive 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 reactive 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 (15). 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 known to be a potent inducer of fibrinolytic activity in human plasma and operates through the inactivation of inhibitors of the fibrinolytic system. It also suppresses complement activity particularly the C5 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 (4) 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 relevance 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 Melanians and Caucasians (2) 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 (14). 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 fibrinolysis inhibition and "increased and accelerated" fibrinolysis 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 rheumatological arthritis and cutaneous vasculitis.
RESUMEN

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

RESUME

L'auteur a étudié les temps de lyse cuglobulique chez des groupes de lépreux atteints des formes suivantes de la maladie: lépre non-épromatose, lépre épromatose en rémission, lépre épromatose en évolution. Les trois groupes ont montré une diminution hautement significative de la fibrinolyse par rapport au groupe témoin. Les malades non-épromatose ont présenté les diminutions les plus faibles, tandis que les épromatose en évolution présentaient les diminutions les plus élevées des relations possibles entre cette anomalie et l'implication du système vasculaire dans la lépre sont discutées, spécialement en ce qui concerne certaines similitudes observées par d'autres auteurs dans l'arthrite rhumatoïde et la vasculite cutanée.

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