Thromboplastic Activity of Leukemic White Cells.* (21226)

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This work was prompted by the observation that material aspirated from the bone marrow cavity of patients with acute monocytic leukemia clotted unusually rapidly. In contrast to the extreme thrombocytopenia exhibited by these patients, this finding suggested that a factor may be present in monocytes or their precursor cells with clot accelerating properties. The formation of thromboplastin in plasma is considered by most investigators to be due to the interaction of a platelet and several plasma factors. However, preformed thromboplastin is present in most tissues and cells(1). For this reason it appeared possible that thromboplastin might be found in monocytes, and preliminary experiments confirmed this assumption. In view, however, of marked differences in the chemical and enzymatic structure of leukocytes(2) this work was extended to the study of thromboplastic activity of various types of leukemic leukocytes, and striking differences were found.

Materials and methods. White cells were obtained from a total of 16 patients with various forms of leukemia (Table I), and a patient with periarteritis nodosa with a high eosinophile count. Only patients were studied in which white cell count exceeded 25,000/cu mm, to obtain a high yield of cells. The diagnosis of the type of leukemia was made after careful examination of the peripheral smear and bone marrow stained by the usual technics. All smears from patients with monocytic and myelo-monocytic leukemia were also examined after peroxidase staining. Thirty cc of blood were collected from each patient with 2-syringe technic. It was then transferred to Silicone-coated test tubes containing 1/10 volume of 1% Sequestrene-Na_2 solution (3). The tubes were centrifuged at 2,000 r.p.m. for 10 minutes, and supernatant plasma removed. The white cell layers were pipetted off, pooled into a glass test tube, and washed 3 times with normal saline. The white cells were finally resuspended in 3 ml of saline.‡ A white cell count was done on this preparation. The white cell preparation was then ground for 30 minutes in a mortar containing 5 g of sea sand. The resultant supernatant solution, after centrifugation at 2,000 r.p.m. for 15 minutes, appeared slightly turbid, but when examined microscopically contained no sediment (leukocyte extract). An unexpected difficulty was encountered in the preparation of extracts from lymphocytes, since these cells formed a viscous gel when washed in saline and could not be counted. The final extract was satisfactory. *Thromboplastic* activity of the leukocyte extract was determined by a modification of the method of Campbell and Stefanini for the platelet thromboplastic factor

TABLE I. Thromboplastic Activity of Leukemic White Cells Extracts.

Diagnosis	No. of cases	Thromboplastic activity/million cells (expressed as γ of brain tissue)*
Acute granulocytic leukemia	3	62.5 (75.5–50.0)
Chronic granulocytic leukemia	3	62.5 (78.0–55.0)
Acute monocytic leukemia	1	280.0
Acute monomyelocytic leukemia	3	30.0 (25.5–42.0)
Acute lymphocytic leukemia	1	0
Chronic lymphocytic leukemia	4	0
Erythroleukemia	1	traces
Eosinophilia (periarteritis nodosa)	1	0

* (See Fig. 1.)

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‡ Experiments were conducted using distilled water instead of saline solution, to facilitate lysis of the white cells and results similar to those described here were obtained.

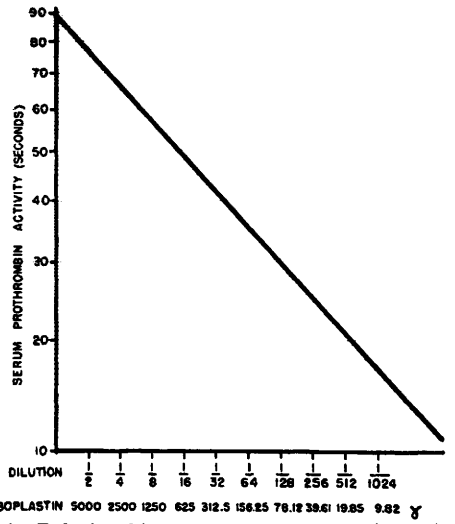

FIG. 1. Relationship of serum prothrombin activity of native human plasma to amount of rabbit brain thromboplastin.*

(4). Normal native human plasma was obtained by collecting blood from healthy individuals with the 2-syringe technic(5), transferring the blood into Silicone-coated test tubes which were then centrifuged at 3,000 r.p.m. at 4°C. All equipment was ice cold when used. While centrifugation proceeded, serial dilutions, in normal saline, of the leukocyte extracts were made. In a series of test tubes 0.9 ml of native plasma was added to 0.1 ml of each dilution of white cell extract. The mixture was rapidly transferred to a water bath at 37°C and allowed to clot. The clotting time was recorded and the prothrombin time of serum was determined one hour after completion of clotting, according to a standard technic(6). Similar experiments were conducted using serial dilutions of rabbit brain thromboplastin instead of leukocyte extract, and a curve obtained showing effect of various concentrations of thromboplastin on prothrombin conversion of clotting native plasma, determined with the one-stage method (Fig. 1). The thromboplastin activity of leukocyte extract was then compared directly to the activity of a definite amount of rabbit brain thromboplastin, as described in legend of Fig. 1.

Results. A total of 17 white cell preparations were studied with regard to their thromboplastic activity, as outlined in Table I. Extracts of cells from patients with acute and chronic lymphocytes leukemia, marked eosinophilia (and from one preparation of pure erythrocytes) were found to have no effect on the clotting time or the serum prothrombin activity of human native plasma. Extracts from cells from patients with both acute and chronic granulocytic leukemia were found to accelerate clotting of native plasma, and increase the prothrombin consumption of native plasma (although they did not influence the extent of clot retraction). It was estimated by comparison with the thromboplastin dilution curve of Fig. 1, that one million granulocytes contained the activity of 62.5 γ of rabbit brain thromboplastin. Cells from patients with monomyelocytic leukemia were also found to be active (average thromboplastin activity: 30 γ/million cells). Finally, extract of cells from one patient with acute monocytic leukemia exhibited extremely high activity (thromboplastin activity: 280 γ/million cells). There appeared to be no appreciable relationship between degree of maturation of granulocytes and their thromboplastic activity, that was indistinguishable whether the cells were obtained from patients with the acute (predominantly immature) or the chronic (predominantly more mature cells) form of the disease. One patient with erythroleukemia (diGuglielmo's disease) was studied to investigate thromboplastic activity of immature red cells. This was very low, probably due to

* 10 mg of rabbit brain thromboplastin suspended in 1 ml of physiologic saline. Suspension incubated at 45° for 10 min. Tubes left standing 15 min. at 37°C to separate heavy particulate matter. It was arbitrarily assumed that supernatant contained entire thromboplastic activity; thus the presumptive weight in thromboplastin is indicated under each dilution. The serum prothrombin time of clotted native human plasma was then determined after addition of each dilution, one hr after completion of clotting (see text). The curve was used for calculation of thromboplastin activity of white cells. To translate thromboplastic activity of white cells into thromboplastic activity of brain tissue, the serum prothrombin time of native plasma containing the extract from 10 million cells was first determined (100000 cells/mm³). With use of graph it was observed to which concentration and weight of thromboplastin this result could be related. The figure obtained was then divided by ten, to express the thromboplastic activity of the leukocyte extract from 1 million cells.

contamination of the extract with substances from granulocytes, usually increased in number in the disease, and could not be separated from the normoblastic elements.

The ability of monocytic or granulocytic cells to correct the coagulation defect of hemophilia was also tested, since this disease is considered to be a classical example of thromboplastin deficiency. 0.1 ml of leukocyte extract from a patient with mono-myelocytic leukemia was mixed with 0.8 ml of fresh citrated hemophilic plasma and the mixture recalcified with 0.1 ml $CaCl_2$ 0.2 M. The serum prothrombin activity of hemophilic plasma (determined by a one-stage technic) was reduced from 89% to 12% by addition of a leukocyte extract at dilution 1/4 with saline and to 43% by the addition of the same extract at dilution 1/16.

Discussion. There are only a few and contradictory results in the literature concerning the effect of white cells on the coagulation mechanism. Thus, Lenggehager(7) found that lysed leukocytes from normal and hemophilic blood shortened the clotting time of normal plasma. Martin and Roka(8) found that lysed white cells from patients with chronic granulocytic leukemia prolonged the recalcification time and the one-stage prothrombin time of normal plasma. These, however, were accelerated by lysed lymphocytes from patients with chronic lymphocytic leukemia.

Like the preceding observations, our results should be considered, qualitative or semiquantitative, since little is known of the solubility and stability of the thromboplastic agent or agents in the white cells. Even more arbitrary, but convenient, is the reference of the thromboplastic activity of white cells to that of rabbit brain thromboplastin, since one assumes the complete solution or suspension of the thromboplastin in the supernatant saline. The results obtained indicate unequivocal thromboplastin activity of some of the white cells and also point to basic differences between their various types. Monocytes and granulocytes supply thromboplastin; eosinophiles and lymphocytes do not. It appears probable that the hypercoagulability of bone marrow material collected from patients with monocytic leukemia, may be explained by the release of thromboplastin from these cells. It should be pointed out, however, that the presence of thromboplastic material in leukocytes in no way improves the bleeding tendency of these patients. This remains closely dependent on the severity of the thrombocytopenia.

The possibility of correcting the hemostatic defect of hemophilia by addition of white cell extracts raises another interesting problem, perhaps related to the observation that administration of histamine to hemophiliacs may result in amelioration of the bleeding tendency and temporary shortening of the clotting time (9); one wonders whether the thromboplastin activity of granulocytes may not be linked to their high content in histamine(10).

Summary. Some types of white cells collected from leukemic patients (granulocytes, monocytes) but not others (lymphocytes, eosinophiles) release thromboplastic material. The extracted material is able to correct the coagulation defect of hemophilic plasma *in vitro*.

1. Howell, W. H., *Bull. Johns Hopkins Hosp.*, 1945, v76, 295.

2. Rheingold, J. J., and Wislocki, G. B., *Blood,* 1948, v3, 361.

3. Dillard, G. H. L., Brecher, G., and Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 796.

4. Campbell, E. W., and Stefanini, M., *ibid.*, 1953, v83, 105.

5. Stefanini, M., *Bull. New England Med. Center,* 1950, v12, 102.

6. Stefanini, M., and Crosby, W. H., *Blood,* 1950, v5, 964.

7. Lenggenhager, K., *Weitere Fortschritte in der Blutgerringslehre,* Ge. Thieme, Stuttgart, 1949.

8. Martin, H., and Roka, L., *Klin. Wschnschr.*, 1951, v29, 510.

9. Sanford, H. W., Hall, F. R., and Butler, S., *Pediatrics,* 1952, v9, 212.

10. Valentine, W. N., Pearce, M. L., and Lawrence, J. S., *Blood,* 1950, v5, 623.

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