sory-motor overlap are considered to mediate 2 different cortical reflexes, the ES response of generalized alerting to unlocalized sound, and SS response one of oriented alerting to localized sound.

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Mechanical Fragility of Human Neutrophilic Erythrophagocytes. (22104)

CURTIS J. FLANAGAN. (Introduced by John B. Miale.)
Department of Clinical Pathology, University of Miami School of Medicine and Pathology Laboratories, Jackson Memorial Hospital, Miami, Fla.

It has been suggested that the erythrophagocyte may be a fragile cell(1) but controlled studies have not appeared in the literature. Studies presented here were designed to obtain quantitative data on this point as part of a study of properties of the erythrophagocyte and the nature of erythrophagocytosis. Erythrophagocytosis can be produced *in vitro*(2) by incubating phagocytic leucocytes with red cells sensitized by antibodies or other surface altering substances(3,4). A method for producing erythrophagocytes in high percentages in a uniform manner using iso-agglutinins was developed and used.

Definitions and method. For purposes of this experiment the erythrophagocyte is defined as any white blood cell which has ingested one or more red cells, red cell ghosts, or large fragments of either. Finely vacuolated cytoplasm in a leucocyte was not considered evidence for erythrophagia. White cells found erythrophagocytic under suitable conditions included neutrophiles, eosinophiles, and monocytes. This is in agreement with previous work(5,6). Of this group of cells only the neutrophile was present in appreciable percentages in the blood used, hence, our data applies to the neutrophilic erythrophagocyte. Cell survival time refers to *in vitro* period of morphologic integrity as indicated by presence of cell membrane or well defined cytoplasmic border seen by phase microscopy, chamber examination using special diluent, or on differential smear. Blood from normal, type A and O Rh negative white adult donors was used as source of red cells and anti-sera repectively. In plasma from type O donor, the anti-A titer was at least 1:512. The same red cell and anti-sera donors were used throughout. Anti-sera showed no agglutinating or lysing properties against the leucocytes used. White blood cells were obtained from normal type O adults and adult patients with elevated leucocyte counts and high neutrophile percentages. Blood was drawn in 5 cc samples to which 0.01 cc heparin (50 mg/cc) had been added. Erythrophagocytic activity does not appear to be altered by presence of anticoagulants (4,5). Blood from each donor was centrifuged 3 minutes at 2500 rpm promptly after drawing. The buffy coat from the leucocyte donor was removed with red cells and plasma to yield a mixture containing approximately 60% cells and 40% plasma by volume. Cells

TABLE I. Results of 14 Determinations in Normal Adults of 50% Survival Times (ST50) of Control and Experimental Neutrophiles Subject to Mechanical Trauma.

ST50 experimental	ST50 control	Ratio of ST50 control to ST50 exper.	% erythrophagocytes in total WBC	% neutrophiles erythrophagocytic	Total WBC
135	265	1.96	40	97	6850
144	289	2.01	48	100	6950
149	285	1.91	56	100	7400
135	255	1.89	60	99	6700
122	252	2.07	52	98	8050
108	250	2.31	66	96	7250
132	268	2.03	48	100	7250
144	295	2.05	58	100	8500
180	378	2.10	56	96	7650
180	360	2.00	68	99	6200
333	386	1.16	55	98	6950
144	351	2.44	64	100	7900
135	345	2.56	48	100	6950
117	304	2.60	56	96	6100

in this mixture were transferred to Wintrobe tubes placed in 37°C water bath, and tilted for rapid sedimentation by gravity to produce a leucocyte rich plasma. Final incubation mixtures placed in 5 cc test tubes consisted of 1.5 cc of anti-A plasma to which had been added 0.50 cc of leucocyte rich anti-A plasma and 0.025 cc of saline washed type A red cells. The leucocyte count in these mixtures varied from 8000-10000 cells/cu mm. When necessary, in samples from white cell donors with marked leucocytosis adjustment of cell count to this level was made by dilution with plasma from the same donor before adding the samples to incubation mixture. Mixtures were incubated 1 hour in 37°C water bath then transferred to 125 cc Erlenmeyer flasks, each containing six 3 mm round, glass beads, and placed on mechanical rotator at 180 rpm. The initial aliquot was taken prior to start of mechanical trauma. Temperature during period of trauma was maintained at approximately 35-37°C by placing flasks in a closed, insulated, electrically warmed box fastened to mechanical rotator. White cell counts and differential smears were obtained on initial sample and on each 0.15 cc interval aliquot. 300 consecutive white cells were counted for each differential. No attempt was made to differentiate between young and old neutrophilic forms of erythrophagocytes. Two separate pipettes and chambers were used for each cell count. All cells were counted using high power objective. A fresh solution of Giemsa stain in isotonic saline was employed as diluent. Turk's solution containing acetic acid or any hypotonic diluent tended to rupture erythrophagocytes. Our method left some red cells intact but their relatively small numbers did not interfere with leucocyte counts. Some clumping of white cells occurred after trauma began, as observed by others(7) but reproducible white counts were obtainable. Clumping was frequently marked if white cells other than those from type O donors were used. Comparison of differential counts made on smears with those made in a phase counting chamber using phase microscopy revealed close agreement showing that smearing did not measurably destroy erythrophagocytes. This was supported less directly by relatively slight decline in erythrophagocytic percentage in untraumatized control groups examined by smear. Control mixtures were employed with each series. The major control group contained anti-A plasma and white blood cells in the same quantity as in experimental group, however, type O red cells were substituted for type A to prevent their sensitization and subsequent ingestion by white cells. Other mixtures identical with those mentioned above but not subject to mechanical trauma were also used. In each instance, cells and plasma were obtained from the same donors as in experimental series.

Results. The results of 14 determinations using white blood cells from normal adult type O donors are shown in Table I. Total

white blood count of this group varied from 6100 to 8500 leucocytes/cu mm. In each case the number of stab and juvenile forms was less than 6%. Neutrophilic erythrophagocytes contained an average of over 2 ingested red cells, ghosts, or large fragments and at times as many as 9. About ¾ of these appeared to be ghosts. Following a Lepehne (8) stain for hemoglobin varying amounts of this pigment could be demonstrated in many red cell ghosts. Donor No. 1 provided antisera for the entire group as well as white cells for the first determination. Survival times of 50% of cells (ST50) were obtained from curves plotting percentage of remaining cells against time. In every instance ST50 of the control neutrophiles exceeded that of erythrophagocytic neutrophiles. To provide a measurement of relative fragility of cells under those experimental conditions the ratio of ST50 of the control to ST50 of experimental groups was tabulated (column 3, Table I). The average value of this ratio equaled 2.08 with standard deviation of 0.34.

In patients with leucocytosis and neutrophilia with varying degrees of "shift to the left" similar survival times were done. These are shown in Table II. The ratios for relative fragilities in this group were somewhat higher than for normals but of the same general order of magnitude.

The disappearance of experimental neutrophilic erythrophagocytes and control neutrophiles from 14 normal donors are shown in Fig. 1, based on average values obtained from determinations of absolute neutrophilic erythrophagocyte and neutrophile numbers derived by multiplying total white cell count of an aliquot with percentage of these cells in the differential smear of the same aliquot. Significant differences in disappearance of neutrophilic erythrophagocytes and neutrophiles were not found in those systems which did not receive mechanical trauma.

Discussion. Results indicate that within the artificial system employed, neutrophilic erythrophagocyte is about 2 times more fragile than neutrophile which has not ingested red cells or red cell ghosts. This means that antibody which is active against

TABLE II. 50% Survival Times (ST50) of Experimental and Control Neutrophiles Subject to Mechanical Trauma in 6 Patients with Leucocytosis and Neutrophilia.

ST50 experimental	ST50 control	Ratio of ST50 control to ST50 exper.	% erythrophagocytes in total WBC	% neutrophiles phagocytic	% juveniles and stabs in neutrophile group	Total WBC (× 1000)	Disease
117	380	3.26	92	99	42	24.5	Bronchiectasis
104	348	3.34	90	100	36	18.5	Cerebral hemorrhage & bronchopneumonia
108	290	2.69	90	98	50	16.5	? Pyelonephritis
126	370	2.94	86	97	36	21.0	Carcinoma of cervix with metastasis
147	384	2.62	88	100	64	28.0	Carcinoma of prostate and bronchopneumonia
117	450	3.84	88	98	32	14.5	Cirrhosis of liver

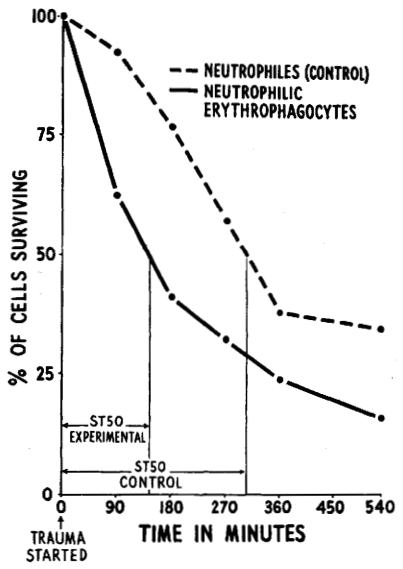

FIG. 1. Disappearance of neutrophilic erythrophagocytes and control neutrophiles subject to mechanical trauma with comparison of 50% survival times.

red blood cells can shorten survival time of neutrophiles present in the same system by promoting their conversion to fragile erythrophagocytic forms. This occurs in the absence of anti-leucocytic activity in the antibody. An exact measurement of this enhanced fragility, under these special circumstances, may be less important than the fact that fragility appreciably increases. Jordan (9) has summarized the theories relating frequent association of leucopenia and especially neutropenia with erythrophagocytosis *in vivo*. There are two main concepts: (1) entrapment of large erythrophagocyte in capillaries of the lung, liver, and spleen and (2) action of leucocytic lysins. It is suggested from our results that leucopenia from enhanced neutrophile fragility should be investigated as a possible explanation of this association.

Further, this enhanced fragility may help explain other findings associated with some acquired hemolytic anemias. It has been observed that frequently erythrophagocytosis is absent or minimal in films of peripheral blood in cases which have demonstrable red cell sensitization (positive direct anti-globulin test). However, if blood from the same sources is incubated *in vitro* at 37°C, large numbers of erythrophagocytes may be produced(1), suggesting that removal of circulatory trauma allows these mechanically fragile forms to survive, although other explanations can be given. While considerable emphasis previously has been on the role of phagocytes in reducing the number of circulating red blood cells, this study gives evidence for the importance also of the reverse consideration, namely, possible role of the red cell in reducing the number of circulating neutrophilic phagocytes.

Summary. Neutrophilic erythrophagocytes were produced in high percentages by sensitizing type A human red cells with anti-A isoagglutin in the presence of viable leucocytes. These erythrophagocytes were subject to mechanical trauma and found appreciably more fragile than neutrophiles which had not ingested red cells but which were treated in an identical manner. The possible significance of this decreased survival time is briefly discussed.

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