Selective Collection of Granulocytes[1] (36325)

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(Introduced by G. E. Moore)

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The adherence of granulocytes to glass wool (1) or glass bead columns (2–4) is usually used as a method of isolating lymphocytes. The induction of granulocyte colonies *in vitro* (5–7) has increased the importance of isolating pure granulocyte populations under sterile conditions. The attachment of granulocytes to glass walls during the first 2 hr of leukocyte culture (8) can be quantitatively measured.

Method. Thirty-five to 100 ml of heparinized whole peripheral blood (70 units/ml) from ten healthy volunteers (4 males, 6 females, ranging in age from 21 to 50 years) were sedimented for not more than 4 hr. The buffy coat was washed once and the remaining erythrocytes were destroyed by hypotonic shock by suspension for 1 min in cold distilled water. The buffy coat was then resuspended in RPMI 1640 media with 20% fetal calf serum added (8). Five milliliters of the leukocyte suspension were placed in glass flasks with a 46 cm^2 bottom surface at a temperature of 37° and a pH between 6.9 and 7.1. Aliquots of 0.1 ml were taken for cell counting and differentiation. Cells were counted in eight fields of a Neubauer chamber; a sample was Giemsa stained. Another sample was peroxidase stained and counterstained with 1% safranin. Between 500 and 1500 cells were differentiated per sample.

Results. The attachment of granulocytes from ten leukocyte concentrations was determined during an incubation period of 120 min. The number of cells attached to the flask walls was calculated indirectly by measuring the loss of cells from the supernatant. It was found that in each sample most granulocytes were attached after 30 min and that this effect was independent of the cell concentration. Figure 1 illustrates this with plots of the attached percentage of the total cell count and of the granulocyte count versus incubation time. The two samples used were number one (4.90 $\times$ 10^6 cells/ml) and number ten (62.7 $\times$ 10^6 cells/ml).

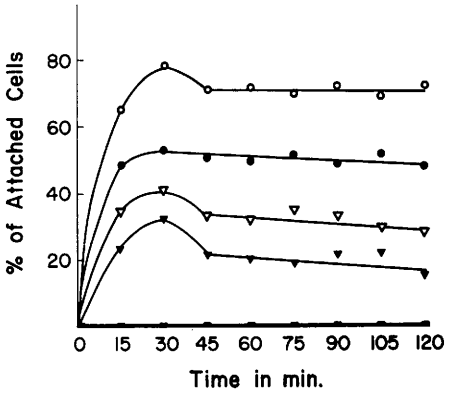

FIG. 1. Percentage of attached cells during incubation. Samples for counting and differentiation were taken every 15 min. Darkened points represent the percentage of the total cells which have become attached. Open points represent the percentage of granulocytes which have become attached. ○● is sample 1, ▽▼ is sample ten, and ■ represents an established lymphocytoid cell line with a concentration of 5 $\times$ 10^6 cells/ml. The cell viability in each sample was 99% as tested by trypan blue exclusion.

A saturation effect can also be demonstrated in this attachment process. In Table I it is shown that a maximum attachment of approximately 20 $\times$ 10^6 cells/ml is reached at an original concentration of about 37 $\times$ 10^6 cells/ml and is not exceeded although the

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TABLE I. Glass Wall Attachment of Granulocytes.

Sample no.		1[a]	2	3	4	5	6	7	8	9	10[a]
At time 0	cells × 10^6/ml	4.90	7.26	14.85	19.35	23.10	29.40	37.00	44.52	52.00	62.70
	granulocytes × 10^6/ml	3.23	5.08	11.43	12.00	16.90	22.05	27.25	29.40	36.40	38.54
	% granulocytes	66	70	77	62	73	75	73	66	70	61.5
Attached[b] after 30 min	cells × 10^6/ml	2.60	2.95	7.05	6.39	9.78	16.50	20.40	19.02	21.50	20.60
	granulocytes × 10^6/ml	2.57	2.86	6.76	6.40	9.14	14.70	16.75	15.60	17.40	16.20
	% granulocytes	98.8	96.9	95.8	100.0	93.4	88.9	82.1	82	89.9	78.7
% Enrichment[c]		96.5	98.7	81.7	100.0	75.6	55.6	33.7	47.0	36.3	44.6

[a] Sample used for Fig. 1.

[b] Attachment is assumed to be the difference in cell or differential counts in the medium at time 0 and after 30 min of incubation.

[c] The percentage of enrichment is calculated by: 100 (Granulocyte Percentage in Attached Cells—Granulocyte Percentage in Original Sample)/(100—Granulocyte % in Original Sample). This is illustrated for sample 1: 100 (98.8—66)/(100—66) = 96.5%.

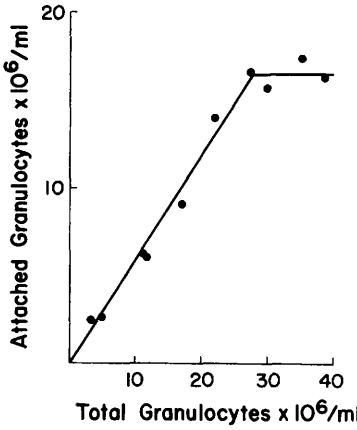

FIG. 2. Saturation curve of the glass wall (46 cm^2). Attached granulocytes × 10^6/ml medium after 30 min of incubation as a function of total granulocytes × 10^6/ml medium. Each point represents the data from one sample in Table I.

original concentration is increased to 62.7 × 10^6 cells/ml.

This saturation effect is also evident in the attachment of granulocytes when they are considered separately. Figure 2 shows a saturation plateau reached around 27 × 10^6 granulocytes/ml in the original sample. The same data is presented in tabular form in Table I.

The relatively specific attachment to glass walls by granulocytes was demonstrated by the finding that no attachment can be shown using normal established lymphocyte cell lines (8). This is shown in the bottom curve in Fig. 1. The same specific attachment and resultant increase in granulocyte percentage in the attached cells is shown in Fig. 3. This figure also shows that a relatively larger granulocyte attachment

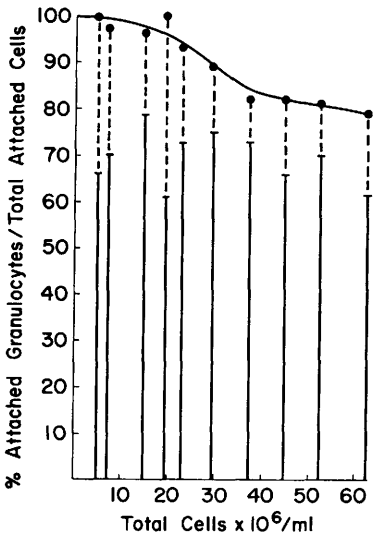

FIG. 3. Percentage of granulocytes attached to the glass wall per total attached cells plotted against total cells × 10^6/ml. — Relative percentage of granulocytes at time 0. - - - Gain in relative percentage of attached granulocytes after 30 min of incubation.

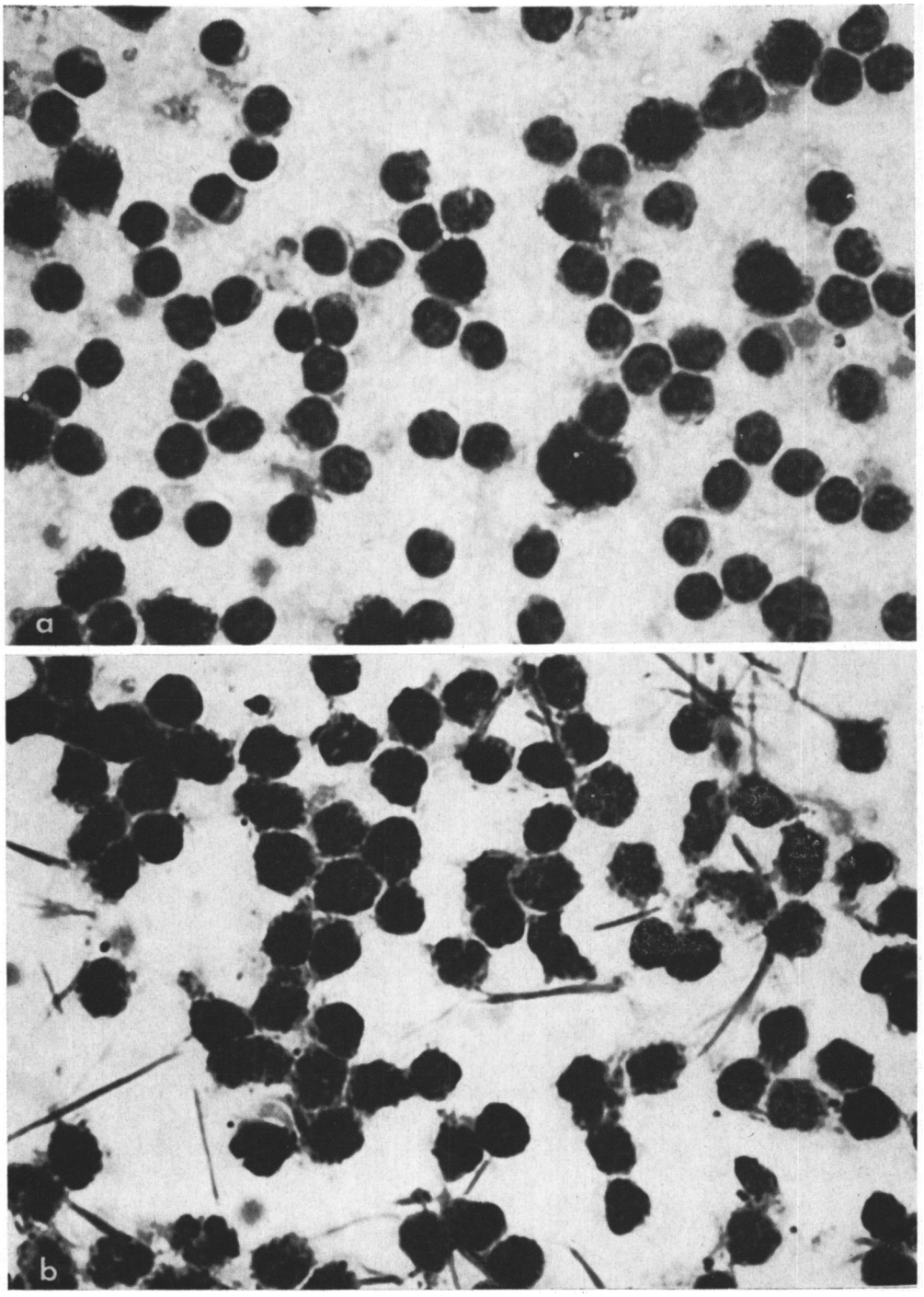

FIG. 4. Photomicrographs of Peroxidase-stained cells: (a) primarily peroxidase negative cells remaining in the medium and (b) primarily peroxidase positive cells attached to the glass wall. After 30 min of incubation medium was taken from the culture bottle and the washed attached cells were scraped from the glass wall into fresh added medium. Both cell populations were concentrated, smeared, and stained. Enlargement ×2000. Cell concentration at time 0 was 4.8 × 10^6/ml.

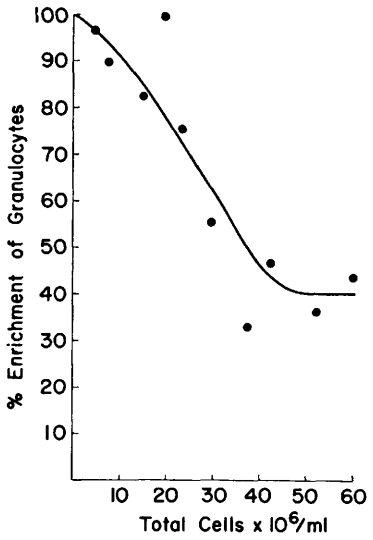

FIG. 5. Enrichment of granulocytes by glass wall attachment after 30 min of incubation plotted against total cells $\times$ 10^6/ml medium. Each point represents the data from one sample in Table I.

results when the leukocyte concentration is low than when it is increased.

To further illustrate this concentration of granulocytes in the attached cells ten cultures of 5 ml each were initiated from seven different healthy donors at concentrations below 5×10^6 cells/ml. After 30 min of incubation the cultures were gently shaken. The supernatant and washed attached cells were then treated as described in the legend of Figs. 4a and 4b.

These figures show the concentration of peroxidase-negative (lymphocytic) cells in the supernatant and of peroxidase-positive (granulocytic) cells in the attached cells. In each sample the attached cells were primarily granulocytes (89–96%) and the supernatant cells were primarily lymphocytes.

Conclusions and Summary. Glass wall attachment can be used as a method of concentrating granulocytes. Enrichment of granulocytes can be calculated from the data given in Table I and is shown in Fig. 5. The enrichment is shown to be dependent on total cell concentration and to increase with a decrease in cells/ml in the culture.

Under the given condition of a 5-ml-incubation volume and a glass surface of 46 cm^2, quantitative granulocyte enrichment of over 95% is possible if the total cell concentration is under 5×10^6/ml. The specificity of granulocytic attachment decreases with an increase in cell concentration, perhaps because an increase in lymphocytes interferes with granulocytic attachment. One centimeter square of glass wall can be saturated with 1.7×10^6 granulocytes.

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