Changes in the light transmission of suspensions of activated neutrophils are widely used to measure the dynamics of neutrophil aggregation. Such studies have suggested, for example, that aggregation is irreversible for human newborn neutrophils but fully reversible for adult cells. We have evaluated aggregation directly by microscopic particle counting and compared it with changes in light transmission (AT) and with release from three granule subsets for neutrophils activated with N-formyl-methionyl-leucyl-phenylalanine (FMLP). Maximal increases in %T in response to 0.5 μmol/L FMLP were ~ 25% larger for newborn than for adult neutrophils, and were only partially reversible by 8 minutes, while %T increases for adult neutrophils were fully reversible. However, measurements of neutrophil aggregation using light microscopy showed that both newborn and adult neutrophils fully disaggregated. A further independence of AT from aggregation was found by pretreating adult neutrophils with cytochalasin B (5 μg/mL) in the presence of 0.5% gelatin, a pretreatment that blocked FMLP-induced neutrophil aggregation while allowing large increases in %T and degranulation. In response to FMLP, newborn neutrophils released more enzyme from each granule subset than did adult neutrophils. Our results suggest that cellular events associated with neutrophil activation (other than aggregation) are implicated in light transmission responses and that these differ for adults and newborns. These results also suggest that reports of neutrophil aggregation should be based on direct particle counting methods rather than on %T responses.

MATERIALS AND METHODS

Reagents

FMLP, β-glucuronidase, human lactoferrin, trichloroacetic acid, tissue culture-grade dimethyl sulfoxide, 4-methyl-luminferal glucuronide, and Type I collagen (Sigma Chemical, St Louis, MO); Percoll (Pharmacia Fine Chemicals, Dorval, Quebec, Canada); hydroxyethylcellulose (Polysciences, Warrington, PA); cytochalasin B (Aldrich Chemical Co, Milwaukee, WI); electron microscopy-grade glutaraldehyde (J.B. Electron Microscopy Services, Pointe Claire, Quebec, Canada); (3H) acetic anhydride (Dupont, Mississauga, Ontario, Canada); 5% formic acid; monochloroacetic acid; and with release from three granule subsets for neutrophils activated with N-formyl-methionyl-leucyl-phenylalanine (FMLP).

From the Montreal Children’s Hospital Research Institute and the Department of Physiology, McGill University, Montreal, Quebec, Canada.

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Address reprint requests to Mony M. Frojmovic, PhD, Department of Physiology, McGill University, 3655 Drummond, #1102, Montreal, Quebec, Canada H3G 1Y6.

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sauna, Ontario, Canada); gelatin (Becton-Dickinson & Co, Cockeysville, MD); and sterile pyrogen-free water and saline (Baxter-Travelon, Pointe Claire, Quebec, Canada) were obtained commercially.

Isolation of Neutrophils

Citrated peripheral venous blood from healthy human adults and citrated venous umbilical cord blood from full-term healthy infants were obtained (1 part 3.8% citrate plus 9 parts blood), mixed with 1% hydroxyethylcellulose or 6% dextran (1:5 vol/vol), and allowed to sediment at room temperature. The resulting leukocyte-rich plasma was centrifuged over a 61% Percoll cushion (700g for 30 minutes at 4°C; 100% isotonic Percoll stock was prepared by mixing 9 parts Percoll, density ~ 1.129 g/mL, to 1 part 10x Tyrode’s solution). Neutrophil suspensions were obtained after erythrocyte lysis with ice cold water, and osmolality was restored by the addition of excess Ca²⁺-Mg²⁺-free Tyrode’s solution (pH 7.2 at 4°C). The cells were washed and resuspended at a concentration of 1.1 x 10⁶/mL in Ca²⁺-Mg²⁺-free Tyrode’s solution containing 10% heat-inactivated plasma (HIP; prepared by heating platelet poor plasma at 56°C for 30 minutes, followed by centrifugation at 16,000g for 15 minutes at 4°C) and maintained at room temperature. Platelet contamination was measured by staining isolated neutrophil suspensions (20 μL cell suspension in 1.98 cc 1% ammonium oxalate) and counting in a hemocytometer. The neutrophil suspensions contained 94% to 98% segmented neutrophils and less than 0.01% platelets.

Light Transmission Studies

Neutrophil suspensions (0.45 mL) were stirred in siliconized cuvettes with siliconized stir bars, and aggregation assays were done using a modification of the method of Craddock et al. A Payton Model 800 Dual channel platelet aggregometer (Buffalo, NY) was used with the sample cuvette containing undiluted neutrophil suspensions (1.1 x 10⁶/mL) and the reference cuvette containing neutrophil suspensions diluted with an equal volume of Tyrode’s buffer. Calibration with the Model 800 is automated such that full scale lies between 10% and 90% of the chart width. The reference neutrophil suspensions were always maintained in the 10% HIP-Tyrode’s medium, pre-stirred at 900 rpm, 37°C for 10 minutes before calibration of the aggregometer and then run in parallel with the test suspension. The reference suspension run against 10% HIP in buffer (stirring at 900 rpm, 37°C) showed unchanging light transmission over 30 minutes. The test neutrophil suspensions were allowed to equilibrate for 4 minutes at 37°C with a stir rate of 300 rpm; the stirring rate was increased to 900 rpm for 1 minute, and 30 μL of CaCl₂·MgCl₂ was then added (final concentration of 1.8 mmol/L Ca²⁺ and 0.8 mmol/L Mg²⁺) and stirred for an additional 1.5 to 2 minutes. When no significant increases in light transmission occurred during this time, 30 μL of the chemotactic agent was added to the test neutrophil suspension and light transmission was recorded. Baseline light transmission (%T = 0) was defined as the light transmission after the dilution effect of the added stimulant (FMLP), with increases in light transmission (ΔT) measured from this baseline value (Figs 1, 2, 4-6).

In some experiments, a fresh solution of cytochalasin B (5 μg/mL final) was added 5 minutes before the addition of the chemotactic stimulus. In experiments where cytochalasin B was added, the final test buffer (3.5% HIP in Tyrode’s) was supplemented with gelatin to give a final concentration of 0.5% gelatin.

Microscopic Analysis of Aggregates

Microscopic analysis of neutrophil aggregates was carried out using a modification of the method of Ringertz. While a light transmission recording was being made from one cuvette in the dual channel aggregometer, samples were taken from a duplicate cuvette. Ten microliters of the neutrophil suspension was fixed in 90 μL 25% glutaraldehyde (pH 7.2), stained with crystal violet and observed under light microscopy (Zeiss Microscope; bright-field; 400 × magnification). The number of individual neutrophils within each particle was counted, and the particle was classed into one of four categories using the method of Hoffsten et al; particles of one neutrophil, particles containing two or three neutrophils, particles containing 4 to 10 neutrophils, and particles containing more than 10 neutrophils. Between 150 and 250 particles were counted from samples obtained at 30 second or 1 minute intervals over 8 minutes. The number of particles in each size category was calculated as a percent of the total number of particles counted (method A; Figs 4A and 5A). For some experiments, the absolute number of neutrophils within each particle size class was counted, and the results were expressed as the number of neutrophils within each particle size class divided by the total number of neutrophils counted (method B; Figs 4B and 5B). We found that expressing the change in aggregate composition as the "percent of the total particles comprised by each particle size class" (method A) or as the "percent of cells within each particle size class" (method B) gave similar kinetic patterns. The data is represented by the two methods described in order to demonstrate that qualitatively, interpretation of the results by either method leads to similar conclusions. The rationale for using method A to calculate the distribution of aggregates is that counting by method A is much more rapid, allowing a greater number of cells or particles to be counted.

Enzyme Release

After the addition of the stimulus, the extent of degranulation was measured by assaying for enzyme content from the supernatant of the neutrophil suspension. Degranulation was stopped by immediately placing the samples on ice followed by centrifugation at 4°C. Enzyme release from primary (azurophilic) granules was measured as β-glucuronidase activity and assayed spectrometrically by the breakdown of phenolptalein glucuronide. Lactoferrin, a marker for secondary (specific) granules, was measured using an enzyme-linked immunosorbent assay (ELISA). Gelatinase activity, the marker for tertiary granules, was measured as the sum of granule markers found as &glucuronidase activity and assayed spectrometrically by the breakdown of phenolptalein glucuronide. The results were expressed as the percent of marker released as a function of the total releasable cellular marker; the latter was determined after stimulation of the reconstituted cell pellet with cytochalasin B and FMLP, in the presence of 0.1% Triton X-100 (Sigma). The total cellular content of the three granule markers studied was measured as the sum of granule markers found in the supernatant of stimulated neutrophil suspensions and that found in the remaining cell pellet.

Statistical Analysis

For comparisons between adult and newborn neutrophil samples, the Student’s t test was used. Using a two-tailed test, P values ≤ .05 were deemed significant. Correlation between changes in light transmission (ΔT) and the kinetics of particle size distribution, and correlation between changes in %T and release were carried out using linear regression analysis.

RESULTS

Light Transmission Changes During Adult and Newborn Neutrophil Activation

No significant increases in %T were seen with stirred neutrophil suspensions containing Ca²⁺-Mg²⁺ until FMLP was added. FMLP-induced changes in light transmittance were measured for both newborn and adult neutrophils (Fig...
NEUTROPHIL AGGREGATION IN ADULTS AND NEWBORNS

Fig 1. Light transmission curves from newborn (n = 6) and adult (n = 9) neutrophil suspensions stimulated with 5 × 10⁻⁷ mol/L FMLP and measured in a platelet aggregometer. Changes in light transmission are represented as the mean (± SD) ΔT after the dilution effect caused by the addition of the stimulus. *, P < .05; **, P < .01; †, P < .005; and ‡, P < .001 by Student’s t test.

1). Greater increases in peak light transmission measured at 2 minutes post-FMLP addition were observed when newborn neutrophils were stimulated with 5 × 10⁻⁷ mol/L FMLP than when adult cells were stimulated with similar concentrations of the chemoattractant: ΔT increase = 14.3% ± 1.9% (n = 6, newborns) versus 11.4% ± 2.4% (n = 9, adults), P < .01. The differences in light transmission between newborn and adult cells were significant at all time points measured between 1.5 and 8 minutes (Fig 1). These differences persisted over the concentration range of 10⁻⁸ mol/L to 10⁻⁴ mol/L, with neutrophils from both donor types yielding maximal %T increases between 10⁻⁷ and 10⁻⁴ mol/L (Fig 2). After maximal light transmission at 2 minutes in both cell populations, the decrease in light transmission over time was slower and significantly less in the samples from the newborn compared with those of adults. Thus, the relative decrease in light transmission from peak values at 2 minutes was 56.8% ± 17.7% for newborns versus 86.5% ± 14.4% for adults (P = .005) at 8 minutes after FMLP addition. The light transmission returned to near baseline values at 8 minutes (%T = 1.7 ± 1.5) in adult neutrophil suspensions, whereas the light transmission remained elevated in suspensions from newborns (%T = 6.2 ± 2.6; Fig 1).

Comparison of Light Transmission Changes and of Microscopically Determined Neutrophil Aggregation

Effects of extracellular calcium in the absence of FMLP. Suspensions of neutrophils from both newborns and adults formed spontaneous aggregates when warmed from room temperature to 37°C and formed additional aggregates at 37°C after the addition of Ca²⁺ and Mg²⁺ (Fig 3). Under these conditions, neutrophils are recruited into aggregates of up to 10 cells, yet no increase in light transmission occurs (data not shown). Adult neutrophils had a tendency to form more spontaneous aggregates, but the difference between the two populations was not significant: 66.4% ± 24.0% of all particles were singlets at t = 0 (addition of stimulus) for adults versus 74.3% ± 15.8% for newborn. At t = 0, the percentage of particles containing two to three cells was virtually identical in samples from adults and newborns, whereas adult neutrophils were more readily recruited into particles of 4 to 10 cells before stimulation (Fig 3). Thus, before activation, up to 40% to 50% of neutrophils may form aggregates of two or more cells with no measurable change in %T. However, these neutrophils remain spherical throughout the spontaneous stir-associated aggregation (≥7 minutes), whereas 8 minutes after FMLP, the deaggregated neutrophils remain shape-changed. Enough Ca²⁺ may be present in our test system (10% HIP) to allow for the unstimulated non–shape-changed aggregation to occur. Cells resuspended in Tyrode’s in the absence of HIP, or in 10% HIP/Tyrode’s with the addition of 5 mmol/L EDTA, showed no aggregate formation when stirred at 300 or 900 rpm (37°C) for 5 minutes (data not shown).

Effects of FMLP. Assuming that changes in light transmission measured in the aggregometer are due to cell-cell aggregation and deaggregation, a correlation between light transmission and the extent of aggregation could be expected. We therefore determined the number and size of neutrophil aggregates per unit volume as a function of known changes in light transmission. Representative experiments, along with light transmission tracings, are shown for adult (Fig 4) and newborn neutrophils (Fig 5). Surprisingly, we found that for newborn neutrophils, although light transmission tracings remained significantly above baseline (Fig 1) suggesting irreversible aggregation, in no case (n = 6) was irreversible aggregation observed by microscopic assessment (Fig 5). In fact, after stimulation with FMLP, the neutrophils of both adults and newborns were found to deaggregate and return within 8 minutes to the particle distribution found immediately before the addition of the stimulus (Fig 3).

For adult neutrophils, increases and decreases in light transmission correlated most closely with parallel changes in singlets and in aggregates of 4 to 10 cells (Table 1). The percentage of particles containing two to three or more than

Fig 2. Relationship between increasing concentrations of FMLP and peak increases in light transmission for newborn and adult neutrophil suspensions. Each point is represented as the mean (± SD) of six to nine experiments, with the exception of the 10⁻⁴ mol/L points, for which the mean value of two experiments is given. Concentrations of FMLP are expressed as the final molar concentration in the neutrophil suspension.
The percentage of large particles (greater than 10 cells) was not found to correlate. Light transmission increases with selective blockage of adult neutrophil aggregation. The temporal correlation between increases in %T and in the formation of aggregates of 4 to 10 cells, obtained for both adult and newborn neutrophils (Table 1), could potentially arise from cellular changes temporally associated with the aggregation, rather than with the aggregation itself. We therefore evaluated %T.

Fig 3. Distribution of particle sizes before and 8 minutes after the addition of $5 \times 10^{-7}$ mol/L FMLP. (A) Mean (± SD) of nine individual experiments using adult neutrophils. 25°C represents the neutrophil suspension at room temperature before incubation at 37°C; 37°C 300 rpm represents the neutrophil suspension after 4 minutes of incubation with a stirring speed of 300 rpm (the stirring speed is increased to 900 rpm at this point); Ca$^{2+}$/Mg$^{2+}$ was added at the open arrow; FMLP represents the suspension immediately before the addition of the chemoattractant; and 8 minutes represents the suspension 8 minutes after the addition of FMLP. Time of addition of the FMLP is represented by the arrow. (B) Mean (± SD) of six individual experiments for newborn cells. In no instances were particles containing greater than 10 cells observed before FMLP addition, or 8 minutes after stimulation.

10 neutrophils did not correlate with increases in light transmission, although the dispersion of large aggregates (greater than 10 neutrophils) followed similar kinetics to that of decreasing light transmission. Newborn neutrophils differed from adult neutrophils only in that the deaggregation of newborn neutrophil particles containing two to three cells was also related to the reduction in light transmission, while the percentage of large particles (greater than 10 cells) was not found to correlate.

Fig 4. Microscopic analysis of aggregates formed after stimulation of adult neutrophils with $5 \times 10^{-7}$ mol/L FMLP. The data from a representative experiment is reported as the number of aggregates within each particle size class as a percentage of the total particle population (A) and as the percent of neutrophils found within each particle class (B). The light transmission tracing for the representative experiment is shown on the same time scale as the particle data. The increase in light transmission due to the addition of the stimulus has not been shown. The dashed line represents %T = 0.
changes with FMLP stimulation of adult neutrophils pre-treated with 0.5% gelatin and cytochalasin B, conditions that essentially blocked aggregation (Fig 6). Under these conditions it appears that changes in %T are largely independent of neutrophil aggregation per se. It is noteworthy that, although no significant aggregation occurred, the slower secondary increase in %T observed for the cytochalasin B-gelatin–treated suspension seemed identical to that of the HIP-treated or the HIP-gelatin–treated suspensions, where aggregation did occur (Fig 6). This suggests that the entire light transmission response of FMLP-treated neutrophil suspensions (10% HIP in Tyrode’s) may be due to activation responses other than aggregation.

Comparison of Granule Release and Light Transmission for Adult and Newborn Neutrophils

**Granule release.** We found that the total cellular content of lactoferrin and β-glucuronidase was similar in samples from newborns and adults (lactoferrin, 51.3 ± 10.1 μg/10⁷ cells for newborn versus 50.7 ± 16.1 μg/10⁷ cells for adults; β-glucuronidase, 6.8 ± 1.1 μg/10⁷ cells for newborn versus 7.6 ± 1.3 μg/10⁷ cells for adults). In contrast, total cellular gelatinase activity was found to be diminished in samples from newborns (74% ± 14% of adult values, P < 0.005). However, a significantly greater percent of granule content release was seen for newborn than for adult neutrophils, particularly for secondary and tertiary granule types (Fig 7). We found similar time courses for both adult and newborn neutrophil release (t½ = 30 seconds), and as previously described, very little β-glucuronidase release from primary granules occurred when FMLP was used as the stimulus.¹

Granule release and light transmission changes for adult and newborn neutrophils. We next evaluated the relationship between the percent release from secondary (lactoferrin) or tertiary (gelatinase) granules and the changes in %T measured in parallel for adult and newborn neutrophils. We could not find a significant relationship between granule release and light transmission at any time point ranging from 30 seconds to 2 minutes after FMLP stimulation of adult neutrophils. For newborns, granule release correlated with the extent of light transmission increase at 2 minutes after FMLP addition (gelatinase: r = .711, P < .05; lactoferrin: r = .846, P < .005, nine observations), with no correlation,
Changes in light transmission with enhanced degranulation of adult neutrophils using cytochalasin B and FMLP. These results suggested to us that increased granule release from newborns might be implicated in the differences in light transmission observed during deaggregation of newborn neutrophils. In order to evaluate more directly the effect of degranulation on changes in light transmission, we devised experimental conditions that led to large releases of granule contents but little or no aggregation. Pretreatment of neutrophils with cytochalasin B followed by a stimulus such as FMLP is known to cause markedly enhanced release of granule contents, and the formation of very large aggregates, while inhibiting cellular shape-change. We found that supplementing an adult neutrophil suspension with 0.5% gelatin in Tyrode's (3.5% HIP) resulted in inhibition of aggregation but intact degranulation responses (Fig 6). With the use of 0.5% gelatin, pretreatment of adult neutrophils with cytochalasin B followed by FMLP resulted in very rapid and large increases in light transmission (%T = 32.0% ± 5.4%; n = 3) but little cell-cell aggregation when assessed microscopically (Fig 6). Three minutes after stimulation, the mean FMLP-stimulated primary granule release from neutrophils pretreated with cytochalasin B in the presence of 0.5% gelatin was 46% (n = 3) of the total releasable enzyme, and more than 10-fold that of neutrophils suspended in Tyrode's buffer (10% HIP) and stimulated with FMLP alone (3.6% ± 0.7% of the total releasable enzyme; n = 7). Thus, enhanced release was associated with further changes in %T, observed during the initial rapid burst in light transmission increase, in the absence of significant aggregation.

DISCUSSION

Changes in light transmission through suspensions of stirred neutrophils have been widely used as an indicator of

![Diagram 1](image1.png)

Fig 6. Light transmission tracings and microscopic analysis of aggregates formed following stimulation with 5 x 10^-7 mol/L FMLP of suspensions of adult neutrophils (a) pretreated with cytochalasin B (5 μg/mL final) in the presence of 0.5% gelatin and 3.5% HIP (--); (b) made up in 0.5% gelatin and 3.5% HIP (---); or (c) made up in 3.5% HIP alone (------). The light transmission tracings for a representative experiment are shown on the same time scale as the particle data. The increase in light transmission due to the dilution occurring with the addition of the stimulus has not been shown. The aggregate data is reported as in Figs 3 and 4 for particles containing one neutrophil (○), two to three neutrophils (●), or 4 to 10 neutrophils (△).

![Diagram 2](image2.png)

Fig 7. Time course for the release of gelatinase (○, ●), lactoferrin (△, ▼), and β-glucuronidase (□, ■) from newborn (open symbols; n = 4 to 6) and adult (closed symbols; n = 4 to 6) neutrophils stimulated with 5 x 10^-7 mol/L FMLP. The plots for lactoferrin released have been skewed slightly to the right to allow clear delineation of the SD bars. The released activity is represented as the percentage of the total cell enzyme content as mean ± SD. * P < .05; ** P < .01; † P < .005; by Student's t test.
neutrophil aggregation. When adult neutrophils are stimulated with certain chemoattractants such as FMLP or the activated complement component C5a, they undergo reversible light transmission responses, which have generally been interpreted as representing neutrophil aggregation and subsequent deaggregation.16-18,26-30 This reversible aggregation of adult neutrophils has been confirmed using electronic particle counting and particle volume analysis39 or visual counting of neutrophil aggregates.8,36 However, after activation with FMLP, FMLP, or C5a, newborn neutrophils have been reported to undergo an irreversible or only partially reversible increase in %T, which has been ascribed to irreversible aggregation.7,16 To our knowledge, there are no reports that confirm irreversible aggregation of newborn neutrophils by analysis of aggregate distribution over time using light microscopy. We confirmed the reported observations of %T changes for both adult and newborn neutrophils. However, microscopic analysis of aggregate formation demonstrated that newborn neutrophils deaggregated to levels found before the addition of FMLP. When adult neutrophils occasionally showed partially reversible %T increases with FMLP activation (even for the same donor on some occasions), fully reversible aggregation was observed (individual data not shown).

The complex relation between changes in light transmission and particle size for both adult and newborn neutrophils (Table 1 and Figs 4 through 6) suggest that ΔT may reflect activation events paralleling aggregation but not necessarily aggregation per se. It therefore appears that neutrophil microaggregation must be directly evaluated by particle counting techniques, including microscopy or resistive particle counting, as reported for similar studies of human platelets.30 The present studies demonstrate that for both adult and newborn neutrophils, stimulation with 5 × 10^-7 mol/L FMLP results in recruitment of singlet neutrophils into microaggregates with a t½ = 15 to 30 seconds, with maximal recruitment by ≈ 90 seconds (Figs 4 and 5). Unexpectedly, it was found that both adult and newborn neutrophils deaggregated to the same pre-FMLP activation levels within 8 minutes after FMLP activation (Figs 3 through 5). Further kinetic and log-dose response studies with direct particle counting will establish if any significant differences do exist in aggregatory responses of newborn versus adult neutrophils.

Partial stir-associated spontaneous neutrophil aggregation of both adult and newborn neutrophils occurred with the use of our 10% HEP-Tyrode’s buffer, estimated to contain greater than 4 amol/L free ionized calcium. Up to 50% of neutrophils could be recruited into aggregates of two or more cells, with no detectable change in %T. These results are consistent with reports that the formation of platelet microaggregates are not detectable by %T changes for up to ≈ 7 to 10 platelets per aggregate.39 However, our stir-associated spontaneous aggregation does not represent “physiologic” aggregation and is distinct from FMLP-induced aggregation in that before activation, neutrophils appear passively recruited as spherical, non-shape-changed neutrophils. Conversely, the reversion of aggregates induced by FMLP to levels comparable with those present before FMLP addition was associated with neutrophils that were still shape-changed. Since for adult neutrophils, %T essentially returned to baseline within 8 minutes of activation, and at this time the cells were still shape-changed, it follows that shape change per se makes a negligible contribution to changes in %T, particularly in the reversible phase of %T measurements.

Our results with enhancement of %T increases without reversion seen when maximal release is induced by a combination of cytochalasin B and FMLP (Fig 6) suggest that release or events associated with release may be major determinants of the %T increases seen with both adult and newborn neutrophils; blocking degranulation with the anion channel blocker DIDS has been reported to block greater than 85% of light transmission increases stimulated by FMLP.31,32 The time course for granule release found in our studies generally parallels that seen for the initial %T increases in FMLP-activated adult or newborn neutrophils (compare Figs 1 and 7), with t½ ~ 30 seconds in all cases. However, release per se does not reverse, while %T shows partial to complete reversal for newborn and adult neutrophils, respectively. Analyses of the relationship between the extent of increases in %T and increases in % release suggest that (1) changes in %T only correlate with changes in % release by ≈ 2 minutes after FMLP stimulation for newborn neutrophils, which are insensitive at earlier times; and (2) the degree of irreversibility of %T measured at 5 minutes post-FMLP activation in newborn neutrophils correlates directly with the maximal extent of gelatinase release seen between 2 and 5 minutes post-FMLP. It is therefore suggested that events associated with release of intracellular granules, such as membrane and cytoskeletal reorganization, such as microaggregation could account in large part for the changes in %T seen with FMLP activation of both adult and newborn neutrophils. Such a dependency has been reported for platelets.36 It is suggested that these events are likely to return to pre-activation levels for adult neutrophils, but only partially so for newborn neutrophils. It should be noted in this regard that adult neutrophil cytoplasts, which are devoid of granules, show irreversible light transmission increases after stimulation with chemoattractants such as FMLP; ie, “uncharacterized” irreversible cellular changes are occurring in the absence of actual granule secretion.

These studies clearly demonstrate that newborn neutrophils do not undergo irreversible aggregation in response to the chemoattractant FMLP in vitro. However, it has yet to be demonstrated whether irreversible aggregation occurs in vivo, thereby contributing to known defects in neutrophil migration into infected tissue or neutrophil depletion in the septic newborn.14 Furthermore, studies of the kinetics and sensitivity of aggregation for both adult and newborn neutrophils must be conducted with more direct particle counting methods. The further characterization of the contributions of membrane and intracellular changes in activated neutrophils to measured changes in light scattering can also be expected to point to the nature of differences that must exist between activated adult and newborn neutrophils, as reflected in the distinct light transmission curves.
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Comparative studies of microscopically determined aggregation, degranulation, and light transmission after chemotactic activation of adult and newborn neutrophils

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