

MONOCYTE CHEMILUMINESCENCE:  
PHYSICAL AND BIOLOGICAL PARAMETERS

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*The use of liquid scintillation counting systems to measure the generation of photons of energy (chemiluminescent events) via metabolic oxidation by cells has become increasingly useful. This technique provides a rapid non-destructive method for assessing this type of metabolic activity as a function of time.*

*Our experience in measuring chemiluminescent events by peripheral blood monocytes stimulated with phagocytizable particles has shown that consistent and precise data can be acquired if technical aspects are given special attention. The failure to acclimate monocytes to the media and to the dark, and the presence of phenol red in the media, will result in suboptimal results. The suspension of cells in gelatin and the addition of luminol to amplify the response are unnecessary. Glass vials have lower background chemiluminescence and provide more consistent data than that obtained with plastic vials.*

*Peaks in chemiluminescence by monocytes are noted at two times following exposure to phagocytizable particles; the first is within 5 seconds; the second, of greater magnitude, occurs between 45 and 60 minutes. Data indicate that in preparations of monocytes and lymphocytes, monocytes are responsible for both peaks.*

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*This technique has been used to explore the biochemical nature of chemiluminescence of intact monocytes to biological (zymosan) and non-biological (latex) particles, as well as the response of cell membranes, via freeze-fracturing, to these agents.*

## I. INTRODUCTION

Monitoring metabolically generated flashes of light (chemiluminescence - CL) emitted by cells, with a liquid scintillation spectrophotometer, has gained attention because these events can be monitored without destroying the cells. Chemiluminescent events appear linked to the intracellular generation of unstable oxygen intermediates (Cheson et al, 1976; Nelson et al, 1976; Sagone et al, 1977; Johnston, 1978). The exact source of CL, as well as the exact location of the enzyme(s) responsible, are still under investigation (Badwey et al, 1979; Curnutte et al, 1979; McPhail et al, 1979, Dewald et al, 1979; Babior et al, 1976). Currently, this technique is being used to monitor the metabolic generation of unstable oxygen intermediates over time as a function of various biologic activities of neutrophils, e.g. phagocytosis, chemotaxis, and microbial killing (Johnston, 1978; Tsan, 1977; Stevens et al, 1978; King et al, 1977).

Monocytes have many functions which are similar to neutrophils; however, many functions are unique to monocytes or their tissue counterpart, the macrophage. Examples of the latter include antigen presentation to lymphocytes in immune responses, and the specific killing of tumor cells in vitro. Because of insights gained in understanding neutrophil function, we, as well as other investigators, have used the generation of CL events to study oxidative metabolism of monocytes (Johnston, Lumine and Guthrie, 1976; Nelson et al, 1976; Johnston, 1978, King et al, 1977; Jederberg and Krueger, 1979; Krueger and Jederberg, 1979; English et al, 1976). Unfortunately, a great deal of variability, relative to neutrophils, is noted in the data generated by studies of CL by monocytes and macrophages. We, and others (English et al, 1976; Nelson et al, 1977; Andersen and Brendzel, 1978), have studied some of these variables. By controlling those variables which can be controlled, and being aware of others, we demonstrate that monitoring CL generated by monocytes, in response to challenge by biologic and non-biologic substances, will provide further insight into membrane and cytoplasmic physiology of such cells.

## II. MATERIALS AND METHODS

### A. *Isolation of Cells*

Heparinized venous blood from human volunteers (10 units/ml preservative-free heparin, Hepathrom, Chromalloy Pharmaceuticals, Inc., Anaheim CA) is diluted with an equal volume of Hank's Balanced Salt Solution (HBSS, Gibco, Grand Island NY) and carefully layered over Ficoll-Paque (Pharmacia, Piscataway NJ). After centrifugation at ambient temperature at 400 xg for 20 minutes, the cells at the Ficoll-Paque, HBSS interface are collected and washed. Red cells contaminating the preparation are lysed by suspending the mononuclear cell (lymphocytes, monocytes and platelets) pellet in 2 ml of 0.85%  $\text{NH}_4\text{Cl}$  for 20 minutes as described by Weeming, Wever and Roos (1975). Neutrophil contamination ranges from 0.1-2.0% in these preparations. The resulting cell suspension is washed twice in HBSS and once in phenol red free RPMI-1640 (Gibco, Grand Island NY) containing 0.025 M HEPES (h-2-Hydroxyethylpiperazine-H-2-ethanesulfonic acid, Sigma Chemical Co., St. Louis MO) and 100 units penicillin and 100  $\mu\text{g}$  streptomycin/ml (Gibco, Grand Island NY) (RPMI-PS). The cells are counted and suspended in RPMI-PS at  $2 \times 10^6$ /ml. Smears are made from each preparation for differential counts and determination of non-specific esterase activity (specific for monocytes) by the method of Yam, Li and Crosby (1971). This specific stain permits the adjustment of the peripheral blood mononuclear cell (PBMC) suspension to a concentration where the monocyte concentration per volume can be made constant from individual to individual.

To permit the cells to adapt to the media and reduce the spontaneous background CL, the cell preparation is held for 1 hour in a dark humidified incubator at 37°C.

### B. *Preparation of Zymosan*

Zymosan (100  $\mu\text{g}$ , Sigma Chemical Co., St. Louis MO) washed in 0.025 M phosphate buffered saline is opsonized with 10 ml of pooled fresh serum from two normal, healthy individuals for 1 hour at 37°C in a shaking water bath. Aliquots of this opsonized zymosan in serum are frozen at -70°C until use.

### C. *Measurement of Chemiluminescence*

In a darkened room with a photo red safety light, 2.5 ml of dark-adapted RPMI-PS and 2.5 ml of the above PBMC suspension are added to glass scintillation vials (20 ml, Kimble,

Division of Owens Illinois, Toledo OH), also previously dark-adapted for 24 hours. The vials are sequentially loaded in a liquid scintillation spectrometer (LSC-8100, Beckman Instruments, Irvine CA) with the coincidence circuit disabled. The lid of the spectrophotometer is closed, and the PBMC are monitored until a stable baseline is established. Once the baseline is established, test material (generally 100  $\mu$ l of dark-adapted zymosan, or other test preparations) is added 5 seconds prior to the vial being counted. After adding the experimental component, the lid of the spectrophotometer is closed, and each sample recounted at predetermined intervals until the end of the experiment. This is facilitated in the Beckman LS-8100 because the computer can signal the conveyor to reverse, and recount samples.

### III. RESULTS - DISCUSSION

#### A. *Physical Parameters, Media, Vials, Etc.*

Early studies clearly demonstrate that the baseline CPM of CL events, emitted by dark-adapted components of the test system (vials, media, cells, and phagocytizable particles) are highly constant, i.e. individual samples on different days varied by less than 5%. To prevent quenching of CL by gravitational settling of the cells, the PBMC suspension was suspended in various concentrations of gelatin. This procedure did not enhance either baseline or peak CL generated by monocytes phagocytizing opsonized zymosan particles. Similarly, keeping the cells suspended by gentle shaking between counts to again avoid quenching actually caused a decrease in CL.

Luminol will potentiate both background and generated CL. Experiments demonstrate that the presence of luminol does not increase the difference between baseline and generated CL enough to justify its use in this system. Keeping test vials at 37°C between counts, i.e. removing them and placing them in a water bath under red safety light conditions, again causes elevated baselines, as well as higher peak CL. The difference between peak and baseline as a percentage is not different, 37° vs ambient temperature. Glass vials have more consistent and lower background spontaneous CL than do plastic liquid scintillation vials.

#### B. *Variations of Zymosan Dose and Opsonizing Sera*

Figure 1 presents the results of various doses of opsonized

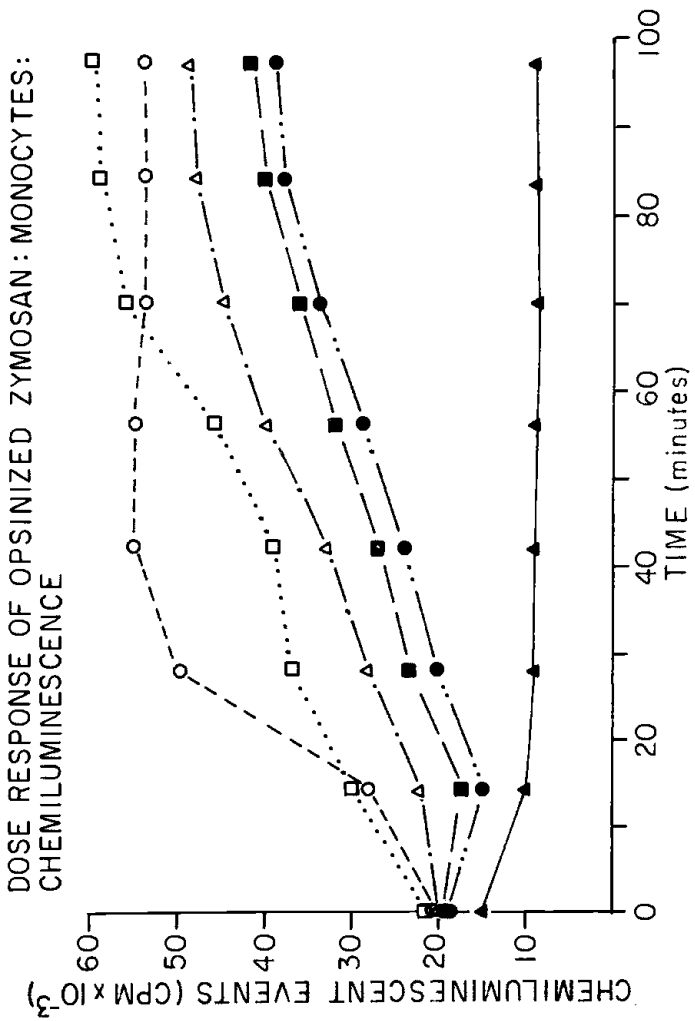


FIGURE 1. Effect of various doses of zymosan on monocyte chemiluminescence. Each point is the mean of triplicate samples. ▲ = PBMC  $5 \times 10^6$  /vial, ● = 25  $\mu$ l, ■ = 50  $\mu$ l, △ = 100  $\mu$ l, □ = 200  $\mu$ l, ○ = 400  $\mu$ l opsonized zymosan/ $5 \times 10^6$  PBMC

zymosan on CL generated by monocytes as a function of time. PBMC ( $5 \times 10^6/5$  ml) from one individual are incubated with 25, 50, 100, 200 and 400  $\mu$ l of opsonized zymosan (100 mg/ml). Each assay is performed in triplicate. Standard deviations are rarely greater than 5%, and if are, indicate an error in technique. Using 100  $\mu$ l of this concentration of opsonized zymosan with  $5 \times 10^6$  PBMC/5 ml gives a particle to cell ratio of 50:1. This ratio leads to an optimum peak response of CL at about 40-60 minutes. This observation is supported by other investigators (Nelson, 1976; Anderson and Brendzel, 1978).

The variability induced by the opsonization of zymosan with fresh vs previously frozen serum, as well as the effect of freezing, storing and thawing opsonized zymosan, was explored. Freezing is a necessity if a constant phagocytizable particle is needed. Figure 2 presents the results of an experiment which determined whether fresh serum is necessary, and whether large amounts of opsonized zymosan can be prepared and frozen in small aliquots. Sera were collected from two healthy individuals, one-half frozen for 1 hour at  $-70^\circ\text{C}$ , thawed and then used to opsonize zymosan in parallel with fresh sera. As shown, cells exposed to fresh or frozen sera alone do not generate CL events, and zymosan opsonized with frozen sera is as good as that opsonized with fresh sera. Further, there is no difference between the CL response to opsonized zymosan never frozen, vs that which has been frozen.

### *c. Monocytes as the Primary Source of Chemiluminescence*

In a cell suspension of PBMC, only the monocytes appear capable of carrying out physiologic phagocytosis. Further, they are felt to be the major source of CL events in a suspension of PBMC undergoing phagocytosis. Monocytes, as well as some lymphocytes, will attach to glass and plastic surfaces. In our laboratory, incubation of PBMC on a clean glass surface in the presence of 10% sera at  $37^\circ\text{C}$  for 1 hour will deplete a PBMC suspension of 80-96% of its monocytes. Figure 3 presents the results of an experiment to determine which cells in the PBMC preparation are responsible for the measured CL response. PBMC are harvested from an individual and placed in scintillation vials, as described above. Serum, 10% AB+, is added to one-half of the vials; all vials are incubated in a tilted position to increase PBMC contact with the surface, and rotated  $45^\circ$  in a clockwise direction (relative to the long axis of the vial) every 5 minutes for 1 hour. Vials containing AB+ sera are rinsed 3 times with RPMI-PS; the cells in these washings are adjusted to the original concentration ( $1 \times 10^6/\text{ml}$ ) and 5 ml are placed in new vials. Vials enriched

OPSONIC ACTIVITY, FRESH vs FROZEN SERUM : FRESH vs  
FROZEN OPSONIZED ZYMOSAN

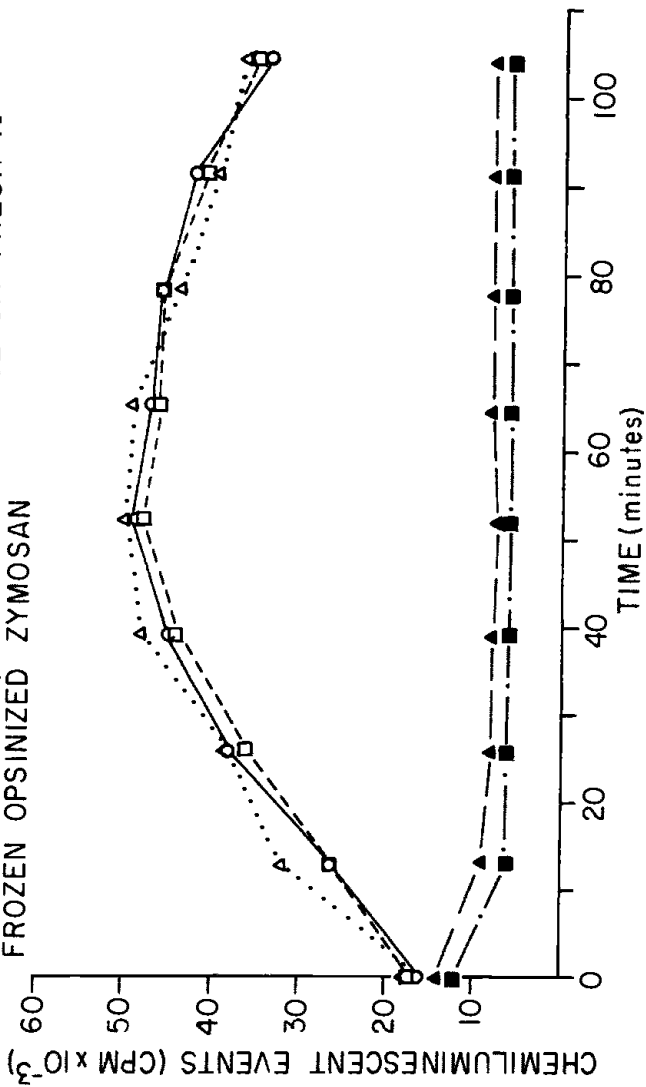


FIGURE 2. Comparison of various types of sera relative to opsonic activity and baseline chemiluminescence. PBMC  $5 \times 10^6$  /vial with: ■ = 100  $\mu$ l fresh serum, ▲ = 100  $\mu$ l frozen serum, □ = 100  $\mu$ l frozen opsonized zymosan, ○ = 100  $\mu$ l zymosan opsonized with frozen serum, △ = 100  $\mu$ l zymosan opsonized with fresh serum.

### CHEMILUMINESCENCE OF ADHERENT PBMC vs NONADHERENT PBMC

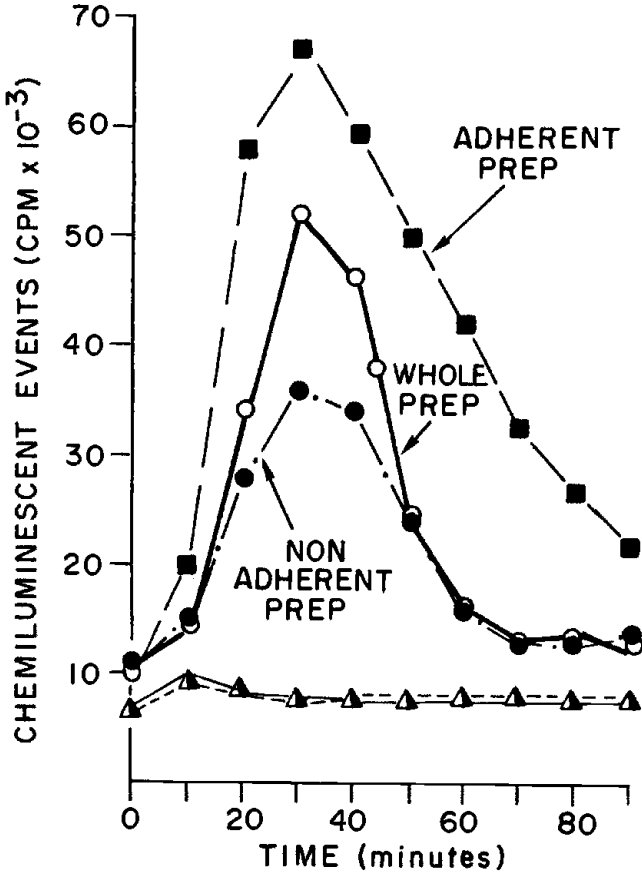


FIGURE 3. Adherent vs non-adherent fraction of PBMC and CL generated when incubated with 100  $\mu$ l of opsonized zymosan.  $\Delta$  = Adherent fraction and whole PBMC without zymosan.  $\bullet$  = non-adherent PBMC adjusted to  $5 \times 10^6$ /vial (monocyte depleted) with zymosan 100  $\mu$ l.  $\circ$  = whole PBMC with zymosan 100  $\mu$ l.  $\blacksquare$  = PBMC adherent fraction with zymosan 100  $\mu$ l.

for monocytes (those cells which remain adherent) have the most dramatic CL response following a challenge with 100  $\mu$ l of opsonized zymosan. Note that the whole PBMC preparation has less CL activity, but is more than that seen in the non-adherent

fraction. Non-specific esterase staining demonstrates that approximately 5% of the cells in the non-adherent fraction are monocytes (no neutrophils are present). This demonstrates that adherent cells, primarily monocytes, generate most of the CL response in the PBMC suspension. The shape of the vial does not permit microscopic analysis of adherent cell type and number. Similar adherent washing experiments with tissue culture slides reveal  $88 \pm 8$  of the adherent cells of the PBMC suspension are monocytes. The amount of CL generated by monocytes is more apparent when it is appreciated that the adherent fraction was only 18% of the total PBMC suspension. As noted in Figure 3, this number of cells,  $1.8 \times 10^5$ /ml generates more CL than either the whole suspension or the non-adherent fractions adjusted to  $1 \times 10^6$  cells/ml. It is also apparent that the non-adherent cells of the whole cell suspension quenched the CL response to some degree, i.e. CL generated by the adherent fraction is greater than that seen with the whole suspension.

#### *D. Effect of Agents Which Generate Chemiluminescence*

Phenol-red is a useful indicator of the pH of media; however, it has the potential to quench CL events. In that other substances in complete media, e.g. some amino acids, may also quench CL, an experiment was carried out in the presence of media, either containing or not containing phenol-red. These data are displayed in Figure 4. Note that the presence of phenol-red does not quench baseline, spontaneous, CL by PBMC.

#### *E. Reproducibility, Sample to Sample Variability*

The question of reproducibility of a given individual on different dates has been assessed by comparing CL assays of PBMC of 6 individuals on several occasions. Experiments separated by several weeks to months show that a specific individual's monocytes display a rather consistent CL response (see Table 1); however, the variability between the individuals can be quite large. Nonetheless, the range of peak CL response to opsonized zymosan by a group of normal subjects is fairly predictable. In a study looking at this, the variance in peak CL by PBMC in response to opsonized zymosan among 17 normal subjects was 20% (Krueger and Jederberg, 1979).

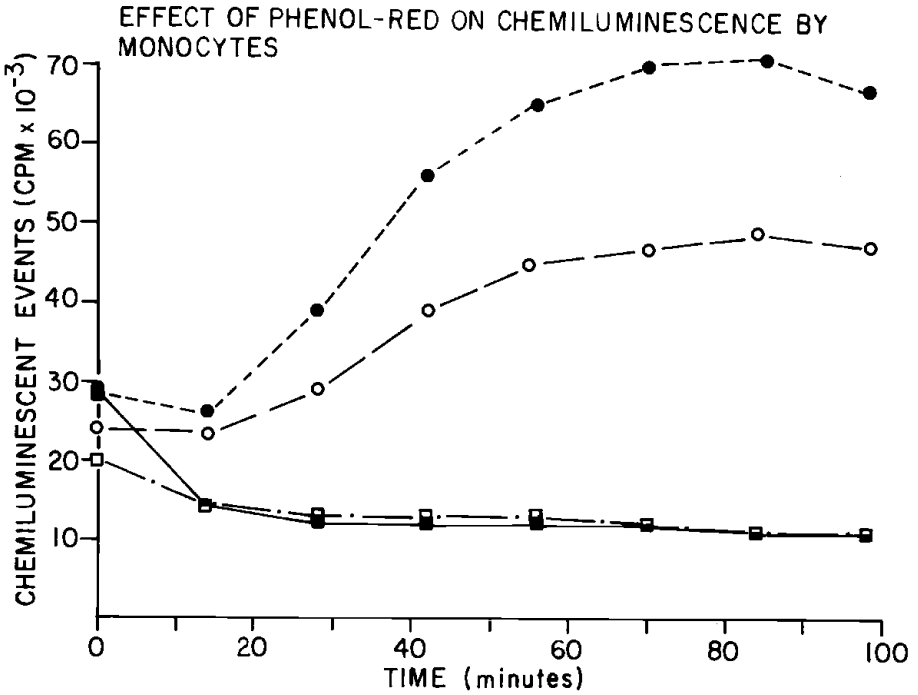


FIGURE 4. CL in the presence and absence of phenol-red in complete media, RPMI-1640.  $\square$  = PBMC in media with phenol.  $\blacksquare$  = PBMC in media without phenol.  $\circ$  = PBMC in media with phenol and opsonized zymosan.  $\bullet$  = PBMC in media without phenol and opsonized zymosan.

TABLE I. Chemiluminescence - PBMC; Normal Subjects - Reproducibility

Subject	# Times Tested	Spont CPM $\times 10^{-3} \pm SD$	Peak CPM $\times 10^{-3} \pm SD$	Index <sup>1</sup>
SC	4	8 $\pm$ 3	55 $\pm$ 7	5.8 $\pm$ 1
PG	4	13 $\pm$ 3	89 $\pm$ 15	7.2 $\pm$ 0.3
JP	3	12 $\pm$ 4	91 $\pm$ 44	7.4 $\pm$ 3.4
BL	2	10 $\pm$ 1	66 $\pm$ 4	6.7 $\pm$ 0.5
TT	2	10	53 $\pm$ 4	5.3 $\pm$ 0.4
WJ	2	13 $\pm$ 4	88 $\pm$ 4	7.1 $\pm$ 2

$$\bar{x}^2 = 11 \pm 2 \quad 74 \pm 18 \quad 6.6 \pm 1$$

<sup>1</sup> Peak cpm/spontaneous cpm

<sup>2</sup> arithmetic mean  $\pm$ SD

Sample to sample variability of PBMC stimulated with opsonized zymosan from 1 individual is very low, and is demonstrated in Figure 5. The points on the figure represent the mean of 5 replicates along with the standard deviations. Appreciate that the counts do not vary by more than 500-2000 counts on either side of the mean, generally less than 5%. The time to peak CL in 11 normal subjects was also quite predictable; the mean is 62 minutes, 1 standard deviation = 10.6 minutes. Monitoring CL for prolonged periods of time, such as that shown in Figure 5, in 5 normal subjects, demonstrates that the time from peak CL back to baseline can be extremely variable, i.e. 40 minutes to over 12 hours (compare Figures 3 and 6). Reasons for this are unknown. Refeeding with opsonized zymosan, when CL returns to baseline, does not initiate a new response. However, PBMC held in culture for 24, 48 and 72 hours prior to feeding does increase the time to peak CL, but not the peak of the CL response.

#### *F. Static Buildup*

The effects of moving samples over a large area between counts are presented in Figure 6. Rather than reversing after counting the test samples through 10 cycles, the samples were caused to traverse the entire conveyor chain before recounting. The points on the figure represent the mean of triplicate samples. The sharp jump between the first and second sets of counts probably represents static buildup, caused by traversing the entire 300 position conveyor. In our standard assay, the sample sets are reversed following counting without traversing the entire conveyor.

#### *G. Effect of Other Substances on Monocyte Chemiluminescence*

Other biologic and non-biologic substances, e.g. latex beads and peptides, have been reported to stimulate CL in neutrophils (Hatch *et al*, 1978; Bass *et al*, 1977; Curnutte *et al*, 1979). To test the ability of these substances to induce CL in PBMC cultures, latex beads (.81  $\mu\text{m}$  in diameter, Gibco, Grand Island NY) were diluted 1:6 in RPMI-PS and varying doses used to stimulate PBMC. As is demonstrated in Figure 7, latex initiated a very rapid and brief CL response that is dose dependent. Analysis of these data demonstrate that 100 and 200  $\mu\text{l}$  of this latex suspension caused a significant ( $p < 0.02$ , student t test, 2 tail) stimulation. Microscopic examination demonstrated that 90% of the monocytes (none of the lymphocytes) had phagocytized 3 or more particles of latex. When compared with other CL responses, the decrease in CL by cells

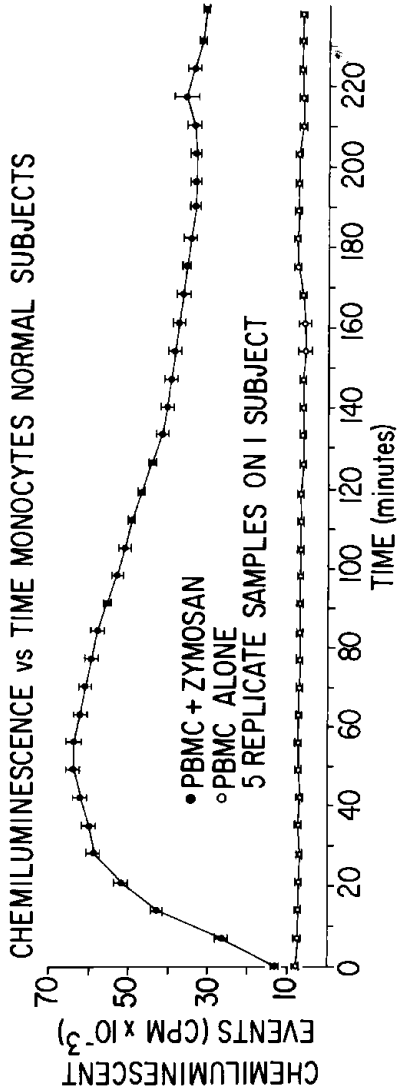


FIGURE 5.  $\circ = \bar{x}$  CL of PBMC alone, 5 replicate samples  $\pm 1$  SD.  $\bullet = \bar{x}$  CL of PBMC with opsonized zymosan, 5 replicate samples  $\pm 1$  SD. Representative curve from 1 individual.

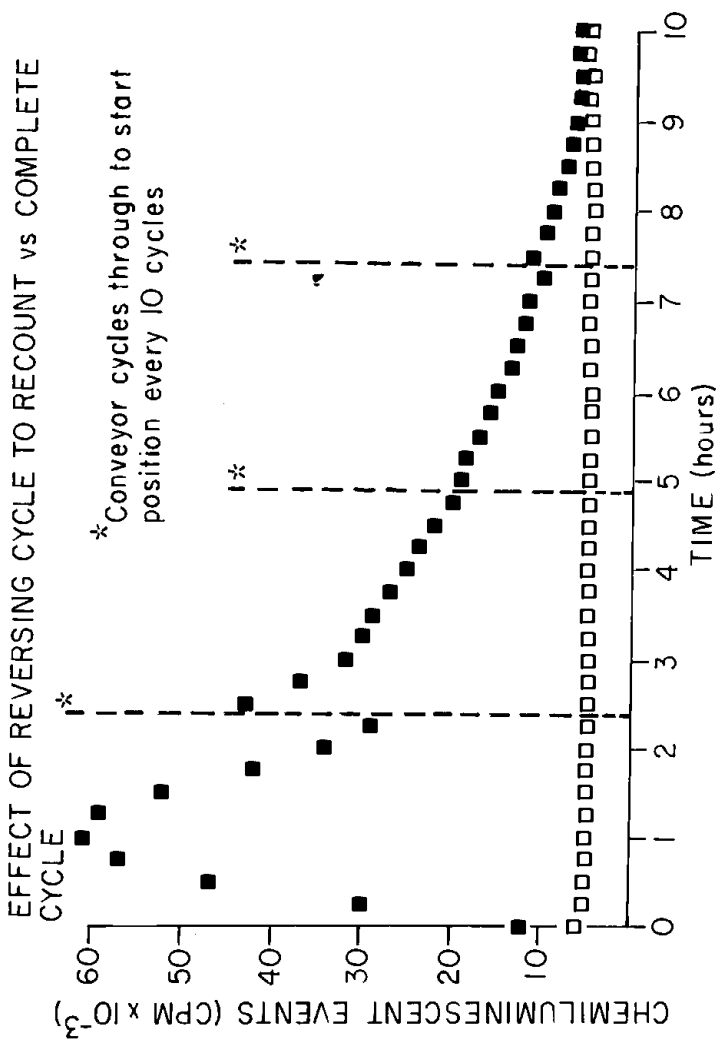


FIGURE 6. After counting 3 opsonized zymosan stimulated and 3 non-stimulated samples for 10 cycles, the vials are moved completely around a 300 position conveyor before counting another 10 cycles. □ = FBMC alone. ■ = FBMC with opsonized zymosan.

EFFECT OF VARIOUS RATIOS OF  
LATEX: PBMC ON EARLY CHEMILU-  
MINESCENCE

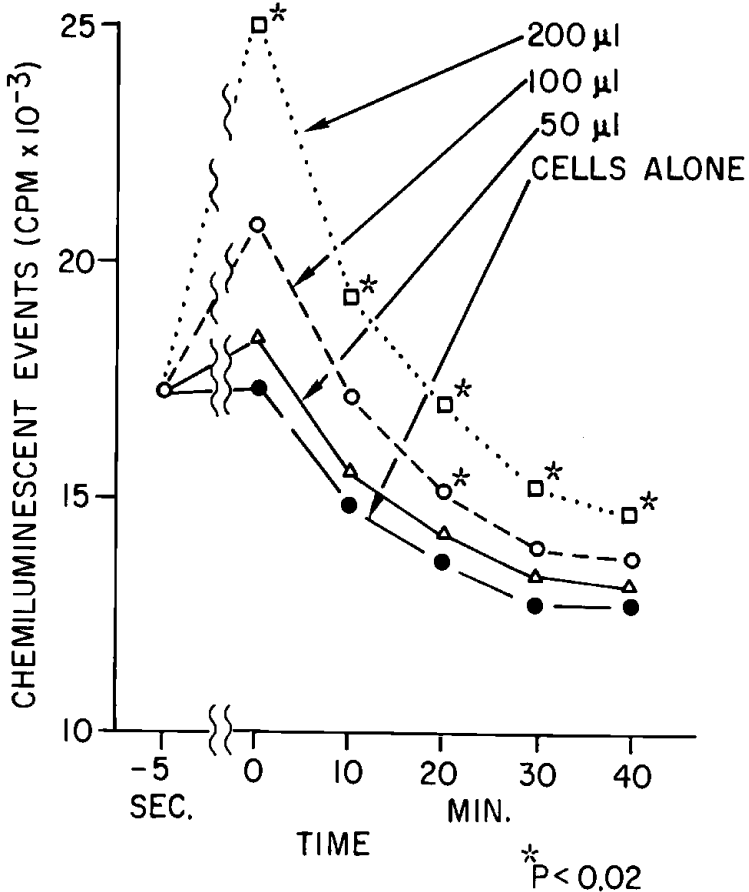


FIGURE 7. Various doses of latex beads added to  $5 \times 10^6$  PBMC approximately 5 seconds prior to first count.

alone appears dramatic. However, this is secondary to the scale used to display these responses, and probably represents CL that the cells acquire, via the mechanism of pipetting into the vials. Opsonization of the latex particles did not change the CL response.

Formyl-Methionyl-Phenalanine (f-Met-Phe) peptides, unique to procaryocytes, and Formyl-Methionyl-Alanine (f-Met-Ala) (Sigma Chemical Co., St. Louis MO) 20 µm in RPMI-PS were used,

100  $\mu$ l, to stimulate the PBMC preparation (see Figure 8). Again, a burst of CL is noted; however, only f-Met-Phe caused a significant ( $p < 0.02$ , student t test, 2 tail) increase in CL. This discriminatory response to different formyl-methionyl peptides by monocytes is similar to that seen with neutrophils (Hatch et al, 1978).

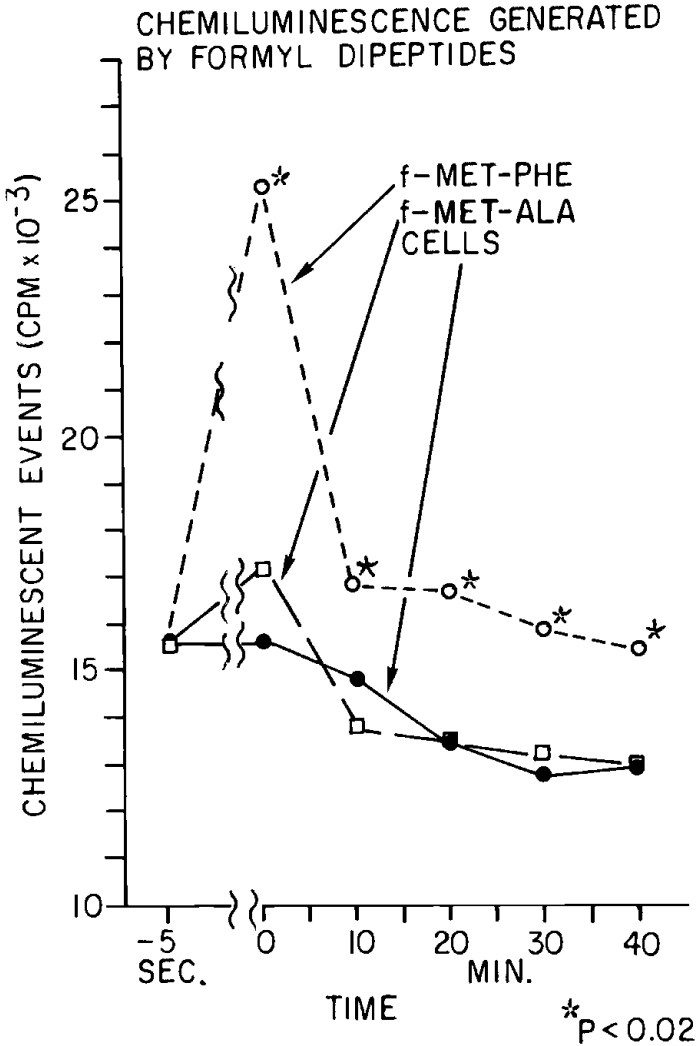
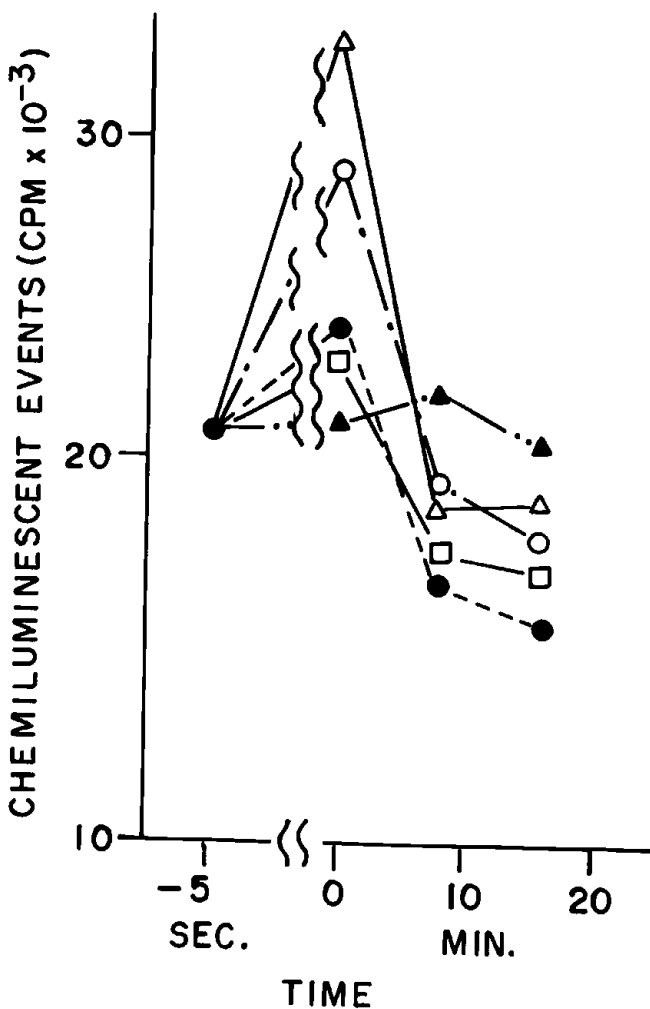


FIGURE 8. Chemiluminescence generated by the addition of 100  $\mu$ l of formyl dipeptides (20  $\mu$ m) to PBMC as a function of time, mean of triplicate samples.

### H. Whole Cell vs Cell Wall in the Generation of Chemiluminescence

The question has been raised as to whether whole cells are necessary for the CL response, or if the cell components contain the necessary enzymes to produce this type of response (Bass *et al.*, 1978; Dewald *et al.*, 1979; McPhail *et al.*, 1978; Badwey *et al.*, 1979). Figure 9 presents data from an experiment

## CHEMILUMINESCENCE OF FREEZE FRACTURED CELLS



designed to begin to explore this question and to look more closely at the differences in the CL response to zymosan, latex and f-met peptides. PBMC are suspended in the usual manner, snap frozen in liquid nitrogen, and thawed in hot water 3 times. Microscopic evaluation and trypan blue exclusion demonstrates that this membrane-rich preparation has very few intact cells, viability of intact cells being less than 1%. The suspensions were stimulated with 100  $\mu$ l of the 1:6 dilution of latex, opsonized zymosan, f-Met-Phe, or f-Met-Ala, and CL activity monitored as with whole cell preparations. Microscopically, intact non-viable cells did not phagocytize either particle. Latex and f-Met-Phe caused a response similar to that seen when whole cells are exposed to these substances (see Figures 8 and 9). The response to zymosan was minimal. These data suggest that CL generated by phagocytosis of a non-biodegradable particle, such as latex, is almost entirely secondary to the effect of such particles on PBMC membranes. The significance of the early membrane associated CL in monocytes is unknown, but appears to be specific, in that one of these unique peptides (f-Met-Phe) does cause CL and the other does not (f-Met-Ala). Support for specificity of this discriminatory response is provided by the data of Schiffman *et al* (1975), which point out that f-Met-Phe is more chemoattractive for granulocytes than f-Met-Ala. This selectivity of response to dipeptides, the failure of zymosan to cause a burst of CL, and the dose response by latex particles, all suggest that early CL events are not spurious. On the other hand, their significance is currently only speculative.

#### *I. Effect of Fluoride and Superoxide Dismutase on PBMC Generated Chemiluminescence*

The generation of unstable oxygen intermediates, superoxide, and others, has been reported to occur in neutrophils stimulated with fluoride ion (Harvath *et al*, 1978; Curnutte *et al*, 1979; Andersen *et al*, 1978; Johnston and Leymeyer, 1976). Hence, the effect of fluoride ion on CL by PBMC was addressed. Sodium fluoride (Sigma Chemical Co., St. Louis MO) 0.01 M, 100  $\mu$ l, was added to a monocyte suspension alone, as well as with zymosan and latex. In the presence of sodium

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FIGURE 9. CL generated after adding various agents (see text for details) to freeze-fractured PBMC preparations.

▲ = PBMC alone. □ = PBMC with 100  $\mu$ l f-Met-Ala, 20 m.  
○ = PBMC with 100 ml f-Met-Phe, 20  $\mu$ m. ● = PBMC with 100  $\mu$ l opsonized zymosan. Δ = PBMC with 100  $\mu$ l latex 1:6.

fluoride, zymosan induced CL was dramatically inhibited (see Figure 10). The early latex response, not pictured, was not inhibited. Sodium fluoride appears to stimulate CL events

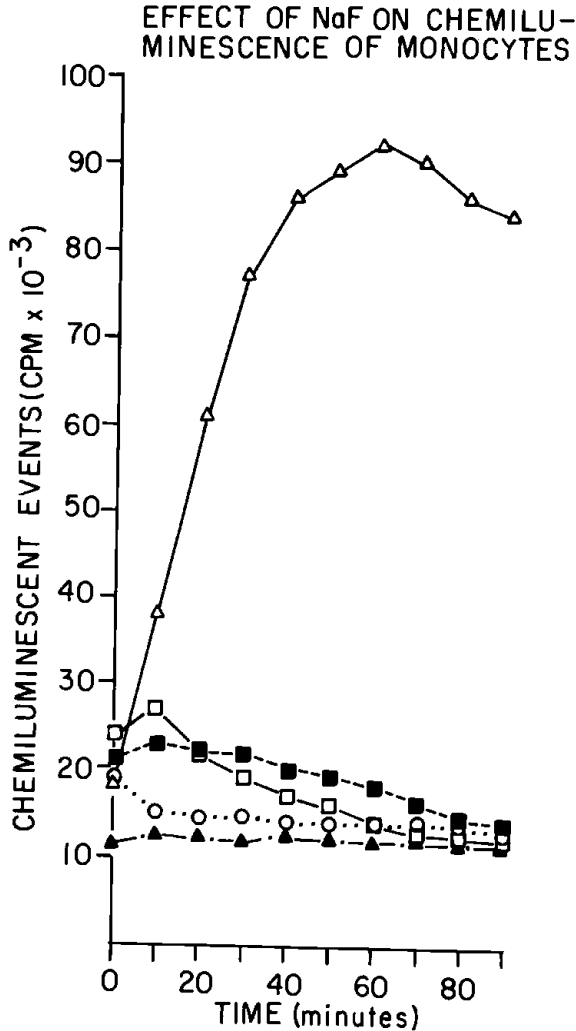


FIGURE 10. Sodium fluoride to PBMC with and without opsonized zymosan. ▲ = PBMC alone. ○ = PBMC with latex. △ = PBMC with zymosan. ■ = PBMC with zymosan and 100 µl NaF 0.01 M. □ = PBMC with NaF, 100 µl, 0.01 M.

early in the time course of these experiments, similar to that seen with f-Met-Phe, and latex, and inhibit the response to zymosan. Reasons for this are unclear. Experiments assessing the effect of sodium fluoride on the freeze/thawed membrane preparations have not been carried out.

This system permits other specific types of interference of the generation of CL. Although not all CL is generated by the degradation of superoxides, a major component is. This is evidenced by the following experiment, which demonstrates that the enzyme which speeds the enzymatic degradation of superoxide (superoxide dismutase) will blunt CL generated by monocytes stimulated with opsonized zymosan. In this experiment (see Figure 11), superoxide dismutase (SOD) (Sigma Chemical Co.,

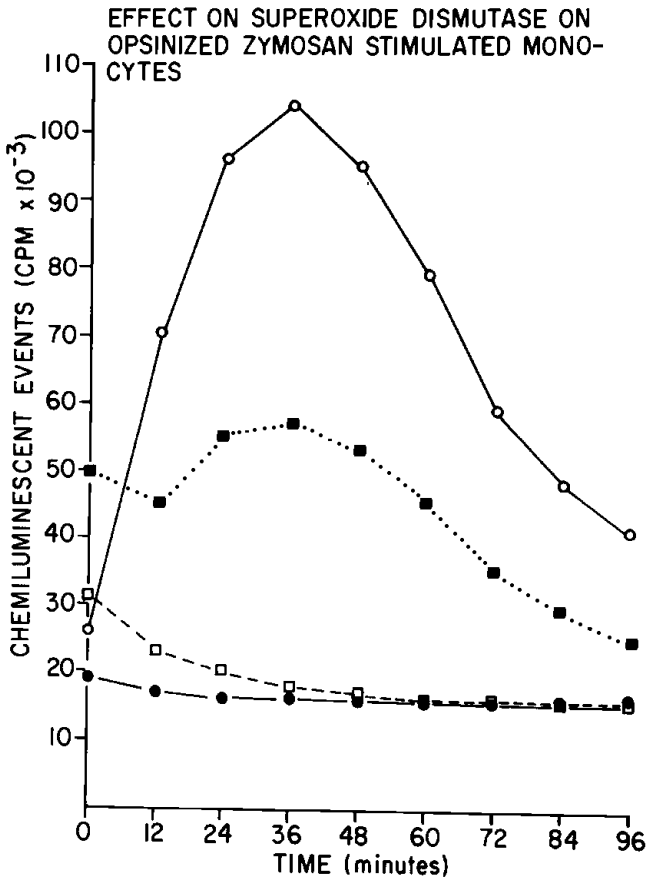


FIGURE 11. SOD 110  $\mu\text{g/ml}$  added to  $1 \times 10^6$  PBMC/ml under various conditions. ● = PBMC alone. □ = PBMC with SOD alone. ○ = PBMC with zymosan. ■ = PBMC with zymosan and SOD.

St. Louis MO) 100  $\mu\text{g ml}$ , was added, 100  $\mu\text{l}$ , to the standard assay. Note that while superoxide dismutase clearly blunts the response to zymosan, it does not totally abrogate it. There are a number of interpretations of this data: 1) that not enough enzyme was added to totally abrogate the response, 2) that this is the expected response (CL does not measure increased flux, i.e. more in, more out in a given time, giving rise to an apparent decreased response), 3) that contaminants in the enzyme preparation contribute to the high baseline activity (see Figure 11, cells plus SOD), as well as to the degradation of superoxide. Preliminary experiments looking at the effect of superoxide dismutase on the membrane preparation suggest that the type of CL associated with latex challenge of membranes is not SOD inhibitable.

### *J. Summary*

We have presented a technique for assessing the CL response of PBMC to phagocytizable (latex, zymosan) and non-phagocytizable (dipeptides, fluoride ion) substances. Demonstrated are the effects of some of the technical parameters on the measurement of this response. The equipment and material necessary to conduct this assay are available in most laboratories studying cellular biology.

Although much remains to be elucidated concerning the chemical intricacies and biological pathways involved in the production of CL, it is, nonetheless, clear that there is potential and utility for this assay. We have used it to study monocyte function in psoriasis, a disease where there appears to be a prevalence of "activated" monocytes (Krueger, Jederberg, Ogden, and Reese, 1978; Krueger and Jederberg, 1979). As assessment of inflammatory and immune cell function becomes more of an element in establishing disease states, laboratories may find this assay and modifications thereof useful.

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