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Simple Procedure for Measuring Neutrophil Chemiluminescence

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A simple method is described whereby measurement of neutrophil chemiluminescence was performed at variable reaction temperatures even though the scintillation counting chamber was refrigerated.

Since the description of chemiluminescence (CL) in 1972, the phenomenon has been used to study the nature of the oxidative bactericidal mechanisms of neutrophils (PMN) (5, 8, 10, 20, 21, 25), the intrinsic defects of abnormally functioning PMN (20, 23), and the effects of various agents such as drugs and opsonins on PMN function (1, 3, 6, 9, 11-16, 22, 24, 26). Measuring neutrophil CL, although not technically difficult, has been cumbersome. The need for darkrooms and dark-adopted vials has been obviated by using luminol, a cyclic hydrazide that emits photons when it reacts with oxygen radicals. This raises total counts high enough so that background counts contribute minimally (2). However, temperature variations have been shown to significantly affect PMN CL (7, 18). The 12°C environment in the liquid scintillation counting chamber is far from either the optimal CL temperature (40°C) or physiological temperature (37°C). One solution to this problem was to place the whole counter, or at least a counting chamber, in a 37°C environment. This solution is costly in terms of space and money. We have developed an insulated vial system which allows for temperature control while leaving the scintillation counting chamber refrigerated. Keeping the scintillation counter at 12°C reduces the background counts and allows the instrument to be rapidly changed from measuring radioactive decay to CL.

Leukocytes were harvested from ethylene diaminetetraacetate-anticoagulated peripheral venous blood by dextran sedimentation from paid, informed human volunteers, and contaminating erythrocytes were lysed with buffered NH_4Cl as previously described (19).

Luminol (Sigma Chemical Co., St. Louis, Mo.) was dissolved in Hanks balanced salt solution (HBSS) to make a 10^{-4} M stock solution. Since luminol is relatively insoluble in HBSS overnight stirring at room temperature was required. When the solution was protected from light and stored at 4°C, we found it to be stable for several months. The stock solution was further diluted to 10^{-6} M with Hanks balanced salt solution before each experiment.

The PMN were then washed twice with HBSS containing 10^{-6} M luminol, suspended in 10 ml of HBSS-luminol and stored on ice. Cell viability, checked by trypan blue exclusion, was always greater than 95%. After chamber differentials, the PMN were diluted with HBSS-luminol to the chosen concentration. Contaminating mononuclear cells always represented less than 10% and usually less than 5% of the total cells. PMN were challenged with lyophilized *Staphylococcus aureus* ATCC 25923 (1.7×10^9 bacteria per mg of dry weight). Samples (1 ml) of the PMN preparation were carefully pipetted into 14.5- by 45-mm (4-ml), stoppered, siliconized glass counting vials (Kimble, Chicago, Ill.).

The 4-ml vials containing the PMN were incubated in an aluminum warming block (Scientific Products Div., McGaw Park, Ill.) at 37°C for 1 h. During this period and throughout the experiment each vial was gently agitated by a single inversion of each tube every 4 to 5 min to prevent sedimentation and enhance oxygenation of the cells.

To count a sample, the 4-ml stoppered vial with the bacteria-PMN mixture was placed in a 20-ml glass vial (Kimble) which contained a piece of styrofoam (0.5 cm thick) at the bottom for insulation (Fig. 1). The vials were placed in the instrument and counted for 6 s, and the 4-ml vial was returned immediately to the warming block. Each small vial spent less than 25 s in the refrigerated counting chamber during each counting cycle. The temperature of the reaction mixture declined less than 1°C due to the insulating effect of the styrofoam and the air surrounding the 3-ml vial. Dark adaptation of the vials and darkening of the room were not necessary since background counts contributed only slightly to total counts. The gain on the scintillation counter was adjusted so that the maximum CL of a pilot of the PMN-bacteria mixture

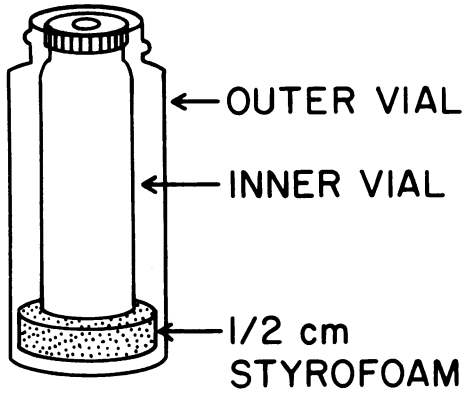

FIG. 1. *Vial-within-a-vial system. The outer vial is a 20-ml, 28- by 58-mm glass counting vial. The inner vial is a 4-ml, 14.5- by 45-mm stoppered glass mini-vial.*

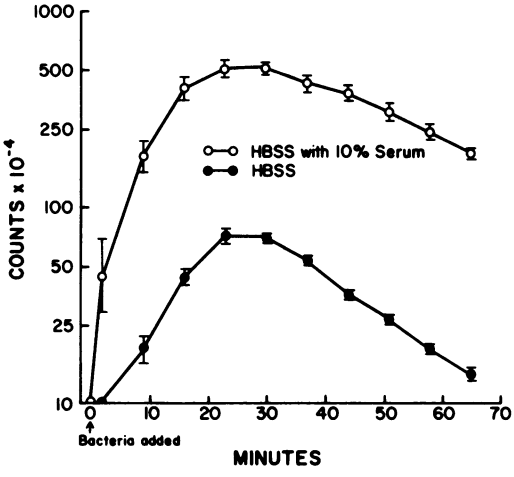

FIG. 2. *Effect of 10% serum on CL. Reaction mixtures containing 10^6 PMN/ml, 0.25 mg of S. aureus per ml, and 10^{-6} M luminol in a total volume of 1 ml of HBSS and incubated at 37°C. The gain on the scintillation counter was set at 7.5%. The effect of 10% serum is compared with reaction mixtures without serum. Mean ± 1 standard deviation.*

expected to give the highest counts in an experiment was less than 600,000 counts per 0.1 min.

This system can conveniently be used to measure the effect of various agents on CL. As an example, Fig. 2 demonstrates the marked enhancement of CL that results when *S. aureus* solution was prepared with 10% serum added. Both peak light intensity and total light emitted were greater in the presence of serum. The importance of opsonization in reaching maximal CL responses has been previously reported (3, 17, 22). Decreasing reaction mixture temperatures from 37 to 31°C decreased maximal intensity of CL, but did not effect the total light emitted (Fig. 3). PMN kept at 4°C never rose above background. These temperature effects are consistent with those found by other investigators (7, 18). Also, warming cells from 4 to 37°C in the absence of bacteria resulted in a burst of CL (Fig. 4). Addition of bacteria during this warming period would lead to variable results. This problem can be avoided by incubating the cells at 37°C for 60 min before challenge.

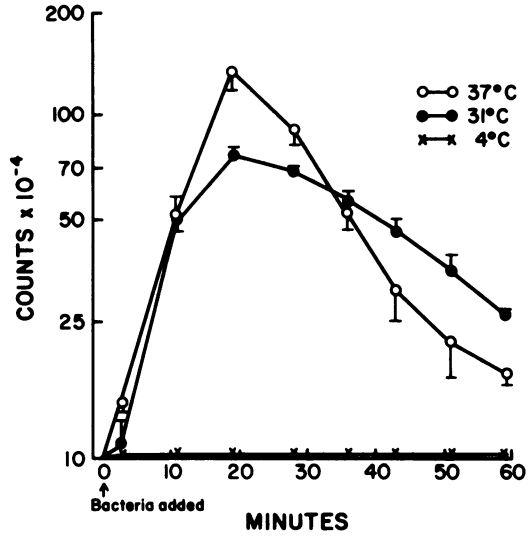

FIG. 3. *Effect of temperature on CL. Reaction conditions were as shown in Fig. 2, except that the gain was set at 100% and the temperature was varied.*

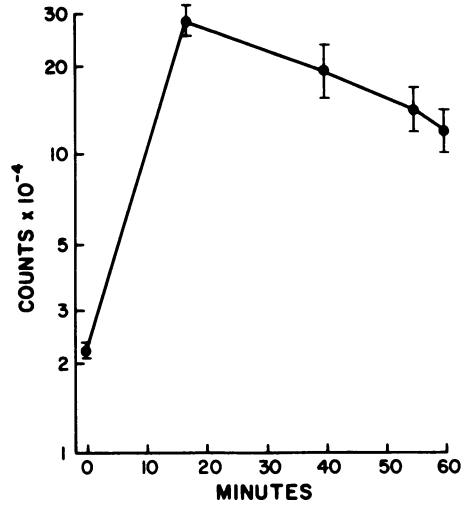

FIG. 4. *CL of PMN alone during warming. Reaction conditions as in Fig. 2, except that 3 × 10^6 PMN/ml were utilized, and the gain was set at 100%.*

The use of a vial-within-a-vial system insu-

lated the reaction mixture from the 12°C environment of the counting chamber. That this system operates like a single-vial system in a 37°C environment is suggested by the similarities of CL kinetics, serum effects, and temperature effects when compared with previous reports (1-5, 7, 17, 18, 22). A disadvantage of the method is that the vials must be handled manually rather than by an automatic vial changer. The loss of automation is balanced by the ease of temperature manipulation and by the cost savings of not purchasing a separate scintillation counter to be used solely for CL measurement. Clearly, the use of a two-vial system greatly simplifies the problem of temperature regulation when measuring CL. This simplification should make accurate CL measurement available to many more laboratories.

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